GSICA
THE ITALIAN SCIENTIFIC GROUP
OF FOOD PACKAGING

DOFATA
UNIVERSITY OF CATANIA

SLIM 2006
Shelf-life International Meeting

Catania 21 – 23 June 2006

Edited by

GIUSEPPE MURATORE & FABIO LICCIARDELLO

Special Issue

ITALIAN JOURNAL
OF
FOOD SCIENCE

CHIRIOTTI EDITORI

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In cooperation with

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This Special Issue of the Italian Journal of Food Science collects the presentations given at the "SLIM 2006, Shelf-life International" congress organised by GSICA, Department DOFATA, University of Catania, held at Catania on 21-23 June 2006.
These papers were reviewed by the Scientific Committee of the congress before their presentation but they did not undergo the conventional reviewing system of the Italian Journal of Food Science.

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INTRODUCTION

This volume collects the contributions to SLIM 2006, the Shelf Life International Meeting, the unique congress dealing with the various aspects connected with the study of the shelf-life of food products.

The congress, organized by the GSICA (Italian Scientific Group of Food Packaging) and the Section of Food Technologies of the University of Catania took place in the Faculty of Agricultural Science of Catania on June 21-23, 2006, being attended by 200 participants coming from all around the world, and especially from Europe, and affiliated to Universities and public and private Research Centres.

The conference gave response to the passionate wish of the participants to deepen their knowledge and broaden their spectrum of interest by meeting colleagues working in various fields. This is on of the main features of SLIM, that is aiming at raising the concept of "Shelf-Life" as a *trait d'union* among scientific fields which are apparently distant.

The volume reports the researches presented as oral and poster presentations, following the scheme of the symposium sessions, addressing, respectively:

- ***Shelf Life modelling***: prediction, by means of mathematical models, of the shelf life and quality decay of foods and beverages; prediction of barrier and protective performance of packaging materials, study of the kinetics of food quality degradation, prediction of sensorial indexes decrease, predictive microbiology, risk assessment.
- ***New technologies for Shelf Life extension***: new materials, active and intelligent packaging, new packaging devices, new food processing technologies, food preservatives alternative to the traditional ones, new techniques for risk reduction.
- ***Shelf Life testing***: non-invasive analytical techniques, sensorial techniques, freshness indicators, selection and validation of reliable quality indexes, evaluation of packaging materials performance; risk management; marketing and logistics aspects of shelf life.

Each session is opened by the report of the main lectures held by international scientists of clear fame.

The researchers, *"professionals engaged in the conception or creation of new knowledge, products, processes, methods and systems"* (from the European Charter of Researchers) are indeed the ones who constantly look for occasions to broaden their horizons: we believe that SLIM 2006 was effective in giving the participants new stimuli for always improving the originality and the foresight of their research.

As a matter of fact, food technologists, microbiologists, engineers, economists, chemists, agronomists, and the authors-all, "made" SLIM, offering their original contribution which is available henceforth and which we hope the reader will find useful.

The GSICA has programmed SLIM 2008, which will be held in Naples on June 2008.

Giuseppe Muratore & Fabio Licciardello

SESSION I

Shelf-life modelling

Chairmen:

Paolo MASI, University of Naples - Italy
Nathalie GONTARD, University of Montpellier - France

MAIN LECTURE

FOOD SHELF LIFE DETERMINATION BASED ON MICROBIAL GROWTH KINETICS

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Mathematical modeling can give a prediction of the food shelf life as a function of intrinsic and external factors of stored or distributed foods. Intrinsic factors such as water activity, pH and compositional factors govern the primary quality attribute determining the deterioration of the food. What is the primary quality attribute may also be determined by external factors such as storage temperature, packaging and other environmental conditions. Intrinsic and extrinsic factors control the deterioration rate of the primary quality attribute to determine the time to reach a limit quality. Growth of spoilage and pathogenic microorganisms is a very important factor determining the shelf life of foods in respect of quality and safety of chilled foods, which constitute a large sector of prepared foods providing convenience and organoleptic preference. Recently, predicted microbiology techniques have been applied to estimate the shelf life and assess the risk of the chilled foods. The growth kinetics of spoilage and pathogenic microorganisms are modeled as function of the intrinsic and external factors. Primary model describes the microbial growth against time, while secondary model gives the growth parameters as function of temperature, water activity, pH, etc. Even though many tools of predicted microbiology are available, practical application of the microbial growth kinetics is limited. This presentation reviews basic principles of describing the microbial growth on foods and then gives the author’s experience in modeling shelf life of Korean prepared chilled foods. Practical aspects of using microbial growth modeling in food shelf life studies are presented in consideration of future intelligent packaging and distribution of foods. The problems of microbial variability in the population distribution and kinetics are also discussed. Two criteria of aerobic bacteria and *Bacillus cereus* are examined as example cases in shelf life evaluation. Future research roadmap is presented for ensuring the safety and quality of chilled foods.

SHELF LIFE MODELLING IN REAL TIME AND REAL CONDITIONS

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ABSTRACT

This topic was presented at the Shelf Life International Meeting, SLIM 2006, in Catania, Italy, 20 – 24 June 2006. This symposium presented the latest in shelf life modelling including means to utilize temperature/humidity sensors or recorders during distribution to measure real world conditions and estimate the impact of abusive conditions on the product life. Such capabilities could improve profitability by accelerating delivery of abused products to compensate for the additional stress caused by abusive conditions, or indicate that the product has become unacceptable and can be pulled before it influences consumers.

Key words: shelf life, modelling, distribution, RFID

SHELF LIFE MODELLING

Packaging is the key to competitiveness. A full packaging evaluation would include eight areas that may be considered in a packaging audit:

1) Packaging at the plant, including an evaluation of production efficiency
2) Transport of the product to market, including the protection functions of distribution packaging
3) Transport through distribution with the traceability required for good manufacturing practices as well as legal requirements
4) Protect the product for desired shelf life (the subject of this paper)
5) Present the product to the consumer – amongst its competition
6) Convince consumer to buy this particular product
7) Provide access of the product by the consumer
8) Provide sustainability, in the current concept of “Cradle-to-cradle”, through which materials and energy are recovered such that the package system has zero or minimum negative impact on future generations.

Shelf life can be defined as the time after production that a food (or other sensitive product, such as pharmaceuticals) product remains acceptable. Many factors affect shelf life, specifically product, processing, packaging, storage environment and company standards. The company standard criteria might not seem important at first, but consider that safety is mandatory (and usually a government requirement), but quality is optional. A company may chose to sell a product on price, whereby cost becomes the prime consideration on the part of the consumer. But if higher profit margins are desired, premium quality may well become a company priority. The definition of acceptability is important, with direct impact on that quality. A good example of this choice involves Meal-Ready-to-Eat, (MRE) military rations. In actuality, the military retort pouch products are prepared with excellent quality. However, the system defines "acceptability" as a food product that supports the life of a soldier, and long shelf lives are considered in which quality degrades with time. The product remains safe, but older products just do not taste as good as the fresher products. The long shelf life has strategic implications which take precedence over quality issues, but the MREs are not designed for sale in a competitive environment.

In addition to the acceptability criteria, the shelf life is influenced by product, production, demand and marketing. Product formulation in terms of preservatives will impact shelf life. Production, demand and marketing influence how much product is made and demand and marketing influence how much product is required. Many companies chose shelf life requirements by committee, that is set a requirement based upon the judgment of individuals or departments, but profitability is enhanced when the shelf life requirement is matched to inventory, *i.e.* is set to allow product to be produced, sold and used, but not overestimated to build up inventory nor underestimated to limit sales.

Once the shelf life duration is determined, the mode of failure must be chosen for modelling purposes. Products may fail for multiple reasons, and these mechanisms may be complex. However, it is often possible to simplify the shelf life model to one mode of degradation if the time factor of one mode is different than the others. For example, a potato chip is sensitive to both moisture and oxidation, but if unacceptable texture occurs through moisture gain before consumer detection of rancidity, the chip can be modeled as a moisture sensitive product. If sufficient moisture barrier is utilized and shelf life suitably extended, the product may then be considered an oxygen sensitive product for modelling purposes. The packaging, therefore influences the choice of the model.

Most shelf life modelling has been designed to accommodate one temperature and one relative humidity, chosen to simulate "standard" conditions, such as 23°C/50%R.H., or an accelerated condition designed to provide estimates for an accelerated storage test confirmation. Single condition modelling provides for easy storage confirmation and provides information to allow product comparisons. However, it does not reflect real world distribution in which conditions change. Products which experience temperature abuse will not perform as predicted in the shelf life model. For example, temperatures can reach 60°C in a truck exposed to the sun in a hot climate. Additional environmental factors will also influence the shelf life, such as pressure changes as products are transported over mountains (lower pressure outside will stress seals inside a hermetically sealed package), humidity changes, shock and vibration experienced in transport (which can effect package integrity (modify permeability). These conditions can be incorporated into a model.

For any predictive shelf life model, one must choose the temperature and humidity or atmosphere conditions for the model. Modelling of distribution adds the additional complexity of providing for changing environmental conditions. It is necessary to choose the level of sampling, that is how often to input temperature conditions. These could be hourly, daily, or monthly data, and can be input from a recorder or sensors in real time. Essentially, the model calculates the effect of the environmental conditions on the product for the duration of the interval and adds that interval time to the shelf life if the product is still acceptable or declares the shelf life value if it has become unacceptable.

The opportunity is to transfer results of real time distribution modelling to distribution scheduling.

MONITORING REAL WORLD CONDITIONS

The difference between distribution shelf life modelling and shelf life prediction is that the varying environmental conditions that the product experiences are required. These environmental conditions may be recorded in the warehouse and fed directly into a computer that includes shelf life modelling software and a data link to the scheduling program. This option is a relatively easy modification of the warehouse system, but restricts the effectiveness to conditions experienced in the warehouse.

Recorders can be incorporated into trucks, rail cars of containers. The container option is especially attractive for intermodal transport systems. In this case the environmental conditions through the distribution environment may be monitored. This monitoring may be recorded for later evaluation or transmitted live in real time.

Additional choices remain even with a choice for container monitoring. The sensors could be placed for the entire container, pallet, shipper, or individual products (or some combination). The choice will depend on the system, the degree of monitoring (*e.g.* will the product be followed into and out of the warehouse after it is unloaded from the container), and the distribution system (how product is shipped from the warehouse). Since sensors can be designed with additional information to facilitate inventory and distribution, individual shipper or product sensors have their application. The system could be designed, of course, to monitor container conditions with a central recorder, and different sensors/computers continue the monitoring from warehouse through retail. In either case, a quick review of the technology available through Radio Frequency Identification (RFID) systems or tags is warranted. Recorders and sensors are available for containers, pallets and shippers, but RFID tags are the only viable option for individual packages. (Note: temperature/humidity sensors are available and have been used for over a decade. However, these devices have no simple means to interface their information with a computer to be used for shelf life analysis).

RFID TAGS

RFID tags are available which include the ability to monitor temperature and relative humidity (RH). This is possible with powered units in shipping containers that can transmit conditions to the crane, warehouse or even a wireless network or cell phone. It is therefore possible to follow shipments in real time. These sensors are called “Active”. They include a battery system which records the information, have the capability of polling the reading device (*i.e.* can initiate transmission), and can be read up to approximately 30 meters distance. These sensors are versatile, and relatively expensive, in the order of $100 USD, so are useful for container or pallet monitoring, but prohibitive for consumer products at and below the shipper level. They can be designed to record temperature, humidity, shock, vibration, and be programmed to include product and company data (as can all RFID tags).

A less costly option is the use of passive sensors. These can also record temperature, humidity, and potentially shock and vibration conditions during distribution to warehouse and/or international transport. Once in the warehouse, information is collected and transmitted to the reader. Passive systems are available in two main types.

Battery Assisted Passive (BAP) devices utilize battery power to collect and store data. The low power battery system is not used to poll reading devices and reading is performed the same as other passive devices. These BAP devices are approximately $5 USD per tag.

Passive RFID tags are inexpensive. The sophistication is in the reader which sends a signal to activate the tag and provides the power for the response. For this reason, passive tags can be small, with a central circuit and an antenna for collecting the signal from the reader.

RFID systems have developed differently in different locations around the world. The EU standard for RFID is 13.56 MHz which is designed for near field applications. Near field tags can be read approximately 2 to 10 cm from the tag, which is ideal for consumer applications such as tickets and passes. It also supplies a security component as the tags cannot be read from distances away from the tags. This feature also limits their warehouse applications.

The United States and Korea, and soon to include Japan have designed tags to work at Ultra High Frequency (UHF) of 900 MHz. A handhold reader can read tags to about 2 meters distance, and industrial readers can read to about 10 meters. If security concerns require limited broadcast distances, the antenna can be designed to limit the read distance.

SHELF LIFE MODELLING IN REAL TIME

With the ability to sample and record temperature and humidity conditions in real time, shelf life prediction software can incorporate the environmental conditions to calculate the actual shelf life, or more accurately, the amount of remaining shelf life. This is accomplished by calculating the impact on the critical determinant for the conditions and duration of each incremental exposure, and then iterate through the exposure conditions. For example, a moisture sensitive product that is polled daily would take the daily condition for 24 hours and calculate the moisture content. If the product remains within acceptable limits the process is repeated for the next day’s conditions. This can identify when a product has become unacceptable in the warehouse, but this is not the advantage of this approach. Predictive shelf life modelling can estimate the remaining shelf life as the time required to take current moisture level to the critical value under a specified condition. It then becomes easy to choose a moisture content that allows sufficient time to distribute and sell the product. Warehoused products can then be ranked on the basis of available shelf life (or moisture content) and shipped on this basis. Therefore the monitoring and computation is used to modify shipping schedules as necessary to optimize product shipments and quality.

MODIFYING THE DISTRIBUTION SYSTEM

Most food and pharmaceutical, as well as other companies making sensitive products, typically employ a First In – First Out (FIFO) distribution philosophy. FIFO systems ship products out in the order they are received. This system is valid as long as the distribution environment remains relatively constant (as evident in controlled distribution systems such as refrigerated or frozen). The oldest product gets used first, which is logical, normal, and universal. However, the difficulty becomes evident when abusive conditions are experienced for some shipments. An example will illustrate.

Consider four shipments for a sensitive product with the following profile:

Shipment 1 - normal distribution (ambient temperature and humidity close to "standard" conditions)
Shipment 2 - normal distribution
Shipment 3 - abused, overheated such as the truck sitting in the sun on a hot day
Shipment 4 - minor temperature abuse.

With a FIFO distribution system, the above shipments would be warehoused and distributed in the order received, with the following results:

Shipment 1 - good condition
Shipment 2 - good condition
Shipment 3 - abused-sub-standard
Shipment 4 - marginal condition.

Let us now utilize a "PROPOSAL" system, specifically, Products Released On Properties Obviously Selected for Additional Life. Environmental conditions were recorded during distribution and this information was used to calculate available shelf life remaining. Products were then shipped on the basis of shortest available shelf life. For normal shipments, this system defaults to FIFO schedules, but for abused shipments, it would add profits as shown below. PROPOSAL Distribution for this scenario would result:

Shipment 1 - former Shipment 3 - good condition
Shipment 2 - former Shipment 4 - good condition
Shipment 3 - former Shipment 1 - good condition
Shipment 4 - former Shipment 2 - good condition.

With distribution based on available remaining shelf life, quality of products is optimized. Furthermore, products that have been temperature abused to the point of unacceptability can be flagged in the system for disposal or reprocessing such that unacceptable product does not get shipped to retail stores. Unacceptable products can impair the company's reputation, but also can incur disproportional expense if product is returned to the company and replaced. Replacement costs then include cost of both products (original and replacement), transportation costs for both (typically more expensive than standard distribution), plus administrative expense.

CONCLUSIONS

When distribution environmental conditions remain within relatively narrow ranges, sensitive products will perform as anticipated and be well served with a standard first in-first out distribution system. The technologies available through temperature and humidity sensors and RFID tags allows companies to determine impact of variable environmental conditions on product quality during distribution and optimize the distribution system on the basis of available remaining shelf life. Optimized distribution will enhance product quality, improve consumer acceptance and reduce product returns, and thereby improve profitability.

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RAPID PREDICTIVE MODELLING FOR PRODUCT DEVELOPMENT

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ABSTRACT

Predictive microbiology models can be used to design good quality shelf-life challenge tests. They can reduce the number of studies required by describing which conditions always lead to either growth or no growth. Literature models are, however, generally limited to a few microbial species (mainly pathogens) and to a specific number of environmental conditions – principally temperature, pH and water activity. The methods used to obtain a predictive model usually involve large experimental designs and labour intensive plate counting.

Using the method of time to detection (TTD), predictive models can be obtained within a short period of time (typically 2- 5 days) and that predictions can be made on the basis of combining functions found for individual inhibitory effects. We have developed a model based on the hypothesis that multiple inhibitory factors combine independently (known as the Gamma hypothesis).

The inhibitory effect of salt, pH, acetate and sorbic on the TTD of *Salmonella poona* were examined individually and in combination. The results of this study support the Gamma hypothesis suggesting that there are no synergistic interactions between the inhibitory factors and that growth can be predicted on the basis of a library of known effects. More importantly to the Food Industry, the results can be used to design challenge studies and can give shelf-life estimates.

Key words: predictive modelling, preservative, hurdle, synergy

INTRODUCTION

The hurdle concept developed by Leistner (1995) requires the aggregation of various preservation processes – chemical, physical and biological, to control the growth of spoilage or pathogenic organisms in foods. The hypothetical basis of hurdle technology is that the combination of several inhibitory processes or events (hurdles) is better, or can achieve equivalent results, than a single inhibitory action. The goal of an appropriate hurdle technology is to increase the acceptability of a product to the consumer whilst ensuring a maximum shelf-life with respect to safety and spoilage.

Predictive microbiology, or “the quantitative microbial ecology of foods” (McMeekin & Ross 2002) attempts to provide mathematical models of microbial growth under a variety of environmental conditions – e.g. temperature, pH, a_w and the effect of preservatives. Predictive modelling can be seen, therefore, as the quantification of hurdle technology. Extensive research in recent years has focused on food safety, whilst spoilage issues have received less attention (McMeekin *et al.,* 1997).

Two principal mathematical constructs are used in predictive modelling: curve fitting and mechanistic, or pseudo-mechanistic modelling. The former methodology can only be used with a full data set - it has no ability to predict anything other than from the interpolation of data under identical conditions to that used to produce the curve fit. Prediction outside the concentration, temperature or a_w ranges used is not mathematically allowed, nor is it justified to use the model obtained as part of an overall examination of a completely different system. The latter modelling constructs suffer from a different problem – how to quantify or model interactions between different environmental effects. Both forms of models, however, lead to the same conclusion: any system under consideration is always a special case.

For polynomial fitting the latter conclusion is unambiguous, for mechanistic/or pseudo mechanistic modelling the problem arises from the basis of the definition under which interactions are quantified. If, under commonly used definitions, combined hurdles give a greater effect than expected, then any novel combination can give patentable opportunities. But this also leads to a major problem: if synergy occurs on combination of different hurdles then the synergy discovered, by its very definition, precludes the ability to model or predict successfully combined inhibitory systems. This is because synergy is seen as a serendipitous effect and it is the unusual and surprising ‘synergistic effect’ that is at the heart of the granting of a patent. If, however, we could calculate the apparent synergistic effect then it is no longer surprising, nor an unusual occurrence.

That the description of interaction within predictive microbiology is a contested issue can be seen in many of the publications in this field. Polynomial fits are used to suggest synergy between factors, but since polynomials have no physical basis nor do the ‘interaction’ terms. The Gamma concept (Zwietering *et al.,* 1992) states that different antimicrobial effects (or hurdles) combine together independently. More recently, however, the Gamma concept has been expanded to take account of apparent synergistic or interactive effects, for example, between temperature, pH and water activity (Augustin and Carlier 2000), temperature, pH and weak acids (Le Marc *et al.,* 2002), and mixed weak acids (Coroller *et al.,* 2005).

We argue that the original Gamma concept for the quantification of hurdle technology is the more appropriate hypothesis and that apparent interactions are incorrectly defined as synergy. Herein is reported an examination of the effect of combined hurdles on the growth of *Salmonella poona.*

MATERIALS AND METHODS

Culture Preparation

Salmonella enterica serovar *poona* (ATCC 4840) was grown overnight in a flask containing 80 ml tryptone soya broth (TSB; Oxoid CM 129) shaking at 30°C. The cells were harvested, centrifuged to a pellet, washed and re-suspended in distilled water. A standard inoculum was produced by diluting the culture to an OD of 0.5 at 600 nm.

Inhibitor analysis

All analyses were performed in a Bioscreen Microbiological Analyser (Labsystems Helsinki, Finland). For individual inhibitors, the method of Lambert & Pearson (2000) was used, whereas for combined inhibitors, a chequerboard arrangement using the method of Lambert and Lambert (2003) was used. Chequerboards of combined weak acids were prepared at constant pH.

pH analysis

The pH of thirty TSB solutions was adjusted with HCl to give a pH range from 7 to 3. These solutions were placed into a Bioscreen plate in triplicate. Diluted standard inoculum (pH adjusted) was added (50 µl) to all wells except the negative control wells. The plate was then incubated for 5 days at 30°C.

Model Fitting

Data obtained from the Bioscreen (tables of optical density (OD) and time) were transformed to the reciprocal and made relative to the shortest time to detection, giving the Relative Rate to Detection (RRD). The reciprocal transformation also stabilised the data variance. Wells which showed no growth during the period of the experiment were set to an RRD = 0.

In all analyses Equation 1 was used to fit the data:

$$RRD = \exp\left\{-\left[\left(\frac{H^+}{10^{-P_1}}\right)^{P_2} + \sum_{i=2}^{n+1}\left(\frac{inhibitor_i}{P_{2i-1}}\right)^{P_{2i}}\right]\right\} \qquad (1)$$

Where H^+ is the hydrogen ion concentration, the parameters P_1 and P_2 define the inhibition due to pH alone, the summation term gives the function for the inhibitory effect of *n* inhibitors (not including pH), each of which is defined by two parameters. The concentration of weak acid at a specific pH was calculated using the Henderson-Hasselbalch equation (2).

$$pH = pK_a + \log\left(\frac{Anion}{Acid}\right) \qquad (2)$$

When present, the second inhibitor was either a second weak acid or salt. For pH independent studies the pH term was removed. The equation was fitted to the data using non-linear regression with the minimised sum of squares as the search criterion. Analyses were done using the JMP Statistical Software (SAS Institute Cary NC USA).

Table 1. Reciprocal model parameters for the individual and combined effects of pH, salt and weak acids on *Salmonella poona.*

	Inhibitor	Parameter	Value	95% CI	RMSE (Bias/Accuracy)*
Single effects	pH	P1	4.478	4.452-4.503	0.0389 (1.016/1.047)
		P2	0.637	0.609-0.667	
	Salt	P3	34245	329936-35580	0.0376 (0.997/1.076)
		P4	1.554	1.450-1.668	
pH & salt	pH	P1	4.387	4.365-4.409	0.0339 (0.986/1.0744)
		P2	0.738	0.700-0.778	
	Salt	P3	40380	38801-42040	
		P4	1.799	1.635-1.993	
Na acetate & pH	pH	P1	4.300	4.263-4.335	0.0377 (1.004/1.070)
		P2	0.841	0.768-0.925	
	Acetic	P3	215.4	200.7-231.5	
		P4	0.911	0.839-0.993	
K sorbate & pH	pH	P1	4.549	4.495-4.594	0.0351 (1.012 /1.073)
		P2	0.697	0.650-0.752	
	Sorbic	P3	97.2	89.1-105.9	
		P4	1.37	1.161-1.692	
	Sorbate	P5	4791	3948-6346	
		P6	1.400	1.006-2.038	
K sorbate, Na acetate & pH	pH	P1	4.796	4.761-4.834	0.0261 (1.000/1.058)
		P2	2.173	1.855-2.691	
	Acetic	P3	234.1	227.4-241.0	
		P4	0.892	0.863-0.923	
	Sorbic	P5	131.7	128.0-135.6	
		P6	1.546	1.448-1.652	
	Sorbate	P7	5811	5301-6439	
		P8	0.747	0.706-0.791	

* Bias and accuracy are as defined by Ross 1996.

RESULTS AND DISCUSSION

Inhibition by pH, salt and combined pH and salt

Time to detection (TTD) data from the growth of *S.* poona over a range of pH were transformed to relative rates and analysed using (1) with n = 0. The minimum time to detection observed was 315 minutes at pH = 7.1. The parameter values are given in Table 1. The minimum pH was calculated from the intercept of the tangent of maximum slope with the pH axis (Lambert & Pearson 2000); pH$_{\text{min}}$ = 3.79 +/-0.056.

The data from the salt inhibition were analysed using (1), with n = 1, but with the pH term removed; the parameters obtained are given in Table 1, the MIC$_{\text{calc}}$ = 6.52%.

As an initial test of the Gamma hypothesis, the inhibitory effect of combinations of salt and pH were studied. A micro-titre plate of all combinations of 10 pH and 10 salt concentrations was prepared. The data were modelled using (1) with n =1, the parameters found are given in Table 1. The correlation matrix showed no indication of any significant correlations between the parameters. Since the inhibitory functions for the individual effects of salt and pH were known, a prediction of the RRD contour map was made and compared with the RRD contour map modelled from the experimental data, Figure 1. There was close agreement between the two suggesting that salt and pH in combination affect the growth of *S. poon*a independently.

Inhibition by pH, Na acetate, K sorbate and their combinations.

The data obtained from a chequerboard of Na acetate and pH were initially examined using (1) with n = 2, with the anion of the weak acid being the second inhibitor. The result, however, of the non-linear regression analysis was consistent with acetate having no effect on the inhibition observed. Figure 2 gives a plot of the total acetate concentration against the observed and modelled RRD over the pH range 4.06 to 6.52. Parameters obtained are given in Table 1.

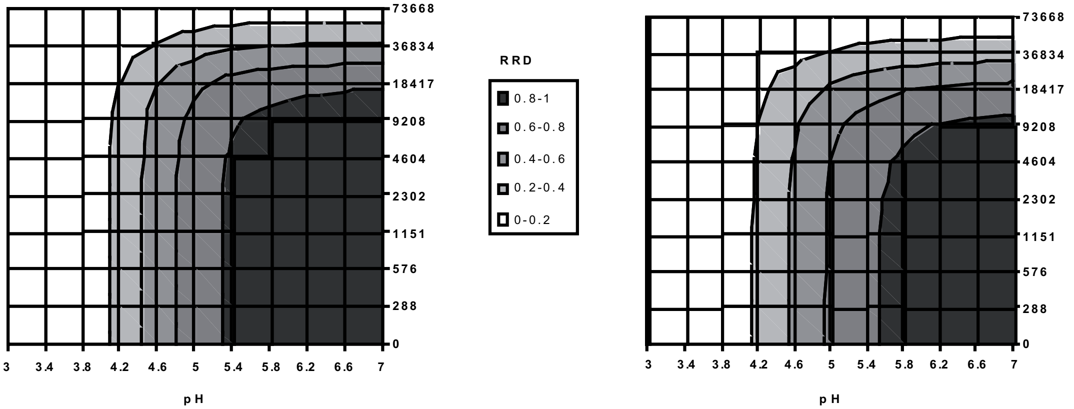

Fig. 1. Comparison of the predicted (left) and modelled (right) RRD contour maps for the relative rate to detection of pH and salt inhibition of *S. poon*a. The minimum time to detection = 356 mins.

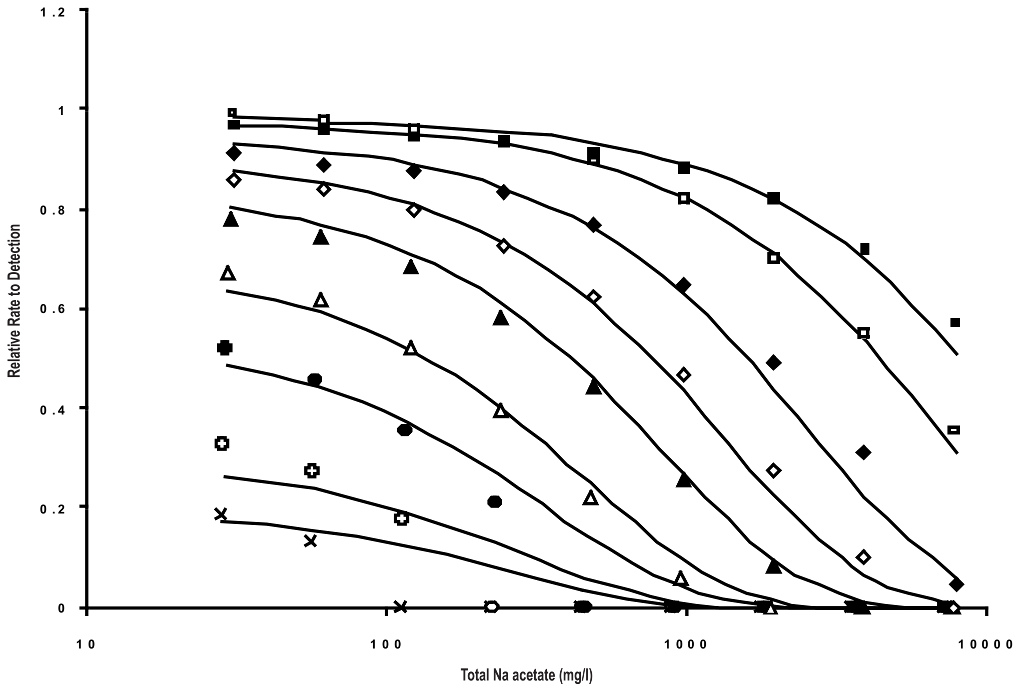

Fig. 2. A plot of the total Na acetate against the observed (symbols) and calculated RRD (solid lines); pH 6.52 ■; 6.25 □; 5.81 ◆; 5.49 ◇; 5.22 ▲; 4.82 ❐; 4.55 ●; 4.21 ○; 4.06 ×.

The value for P_3 (215 mg l^{-1} of acetic acid) is stating that at this concentration in solution, governed by the Henderson-Hasselbalch equation, there will be a specific level of inhibition (RRD = 1/e) attributable to the acetic acid concentration alone. Since the pH also has an independent inhibitory effect, as the pH is lowered there is a summed inhibitory effect from both the pH and the acetic acid.

For K sorbate the addition of the anion to the model (i.e. n =2) gave a better fit to the data with respect to the RMSE over the acid only model (n=1), the F ratio = 42.7, giving a p-value <<0.001. Parameters obtained are given in Table 1. From the table, it would appear that sorbic acid is approximately 50 times more inhibitory than sorbate. However, this simple figure hides the importance of the pKa. Equipotency is obtained at approximately pH 6.35, below this pH sorbic acid is more potent for a given amount of total K sorbate added, e.g. at pH 4.67, 500 mg l^{-1} of total K sorbate will produce equal amounts of sorbic and sorbate anion in solution, but the sorbic acid will increase the time to detection of *S. poona* from approximately 332 minutes to over 12000 minutes, whereas the concentration of sorbate will increase the TTD only by five minutes.

Combinations of sorbate and acetate ion at various fixed pH were examined to see if the observations of the inhibitory effect against *S. poona* were also consistent with the Gamma hypothesis (i.e. could be modelled by Equation 1) and whether a prediction could be made based on a composite equation built from the individual functions already found for the weak acids and pH (Table 1).

For a constant pH the relative rate to detection depends only on the amount of acetic acid and sorbic/sorbate present. At a constant pH, however, the ratio of sorbic/sorbate and also of acetic/acetate is constant. This means that there is a simple relationship between the weak acid and its anion, which would lead to high correlations in the modelling and possible non-convergence. Therefore (1) was reformulated as

$$RRD_{pH=const.} = \exp\left\{-\left[\left(\frac{Total\ acetate}{P_3}\right)^{P_4} + \left(\frac{Total\ sorbate}{P_5}\right)^{P_6}\right]\right\} \quad (3)$$

Essentially we are stating that the function $\left(\frac{Total\ acetate}{P_3}\right)^{P_4}$ is a composite function of the effect of acetic and acetate ion, similarly for $\left(\frac{Total\ sorbate}{P_5}\right)^{P_6}$, at constant pH.

If the hypothesis of independent action is valid, then the observed contour plots of RRD against total acetate and total sorbate will be identical to those modelled using (3), and also to the predicted RRD contour map produced using (1) and the values from Table 1 for constant pH.

Figure 3 compares the observed RRD contour maps with those predicted using the parameters in Table 1. The parameters obtained from the fit of (3) to the iso-pH data are given in Table 2. The combined data were further analysed using (1) with n=3 the (see Table 1).In all cases the predicted RRD were in close agreement with the observed and also to the values modelled from the above composite equation.

The studies performed suggest the validity of the Gamma hypothesis – that combined antimicrobial hurdles act independently. In no case were data obtained that suggested the presence of synergy. In other areas of biological science what constitutes synergy is also hotly debated (e.g. Greco *et al.*, 1995; Odds 2003).

These studies further show that predictive models can be used in a truly predictive sense (other than a simple interpolation of a fixed model). By establishing a library of parameters, novel combinations of inhibitory effects can be combined to predict regions of growth and no-growth. This alone would make challenge studies more focused, since it allows the product developer to quickly eliminate hurdle combinations which do not substantially inhibit growth, and also those that lead

Table 2. Modelled parameters (Equation 3) for Na acetate/K sorbate inhibition of *S. poona* at constant pH.

Inhibitory effect	Parameter	pH 6.5	pH 6.0	pH 5.5	pH 5.0
Na acetate	P_1	14092	5004	1637	599
	P_2	0.876	0.843	0.862	0.958
K sorbate	P_3	3740	2038	973	300
	P_4	0.966	1.134	1.368	1.326
	RMSE	0.0071	0.018	0.027	0.027

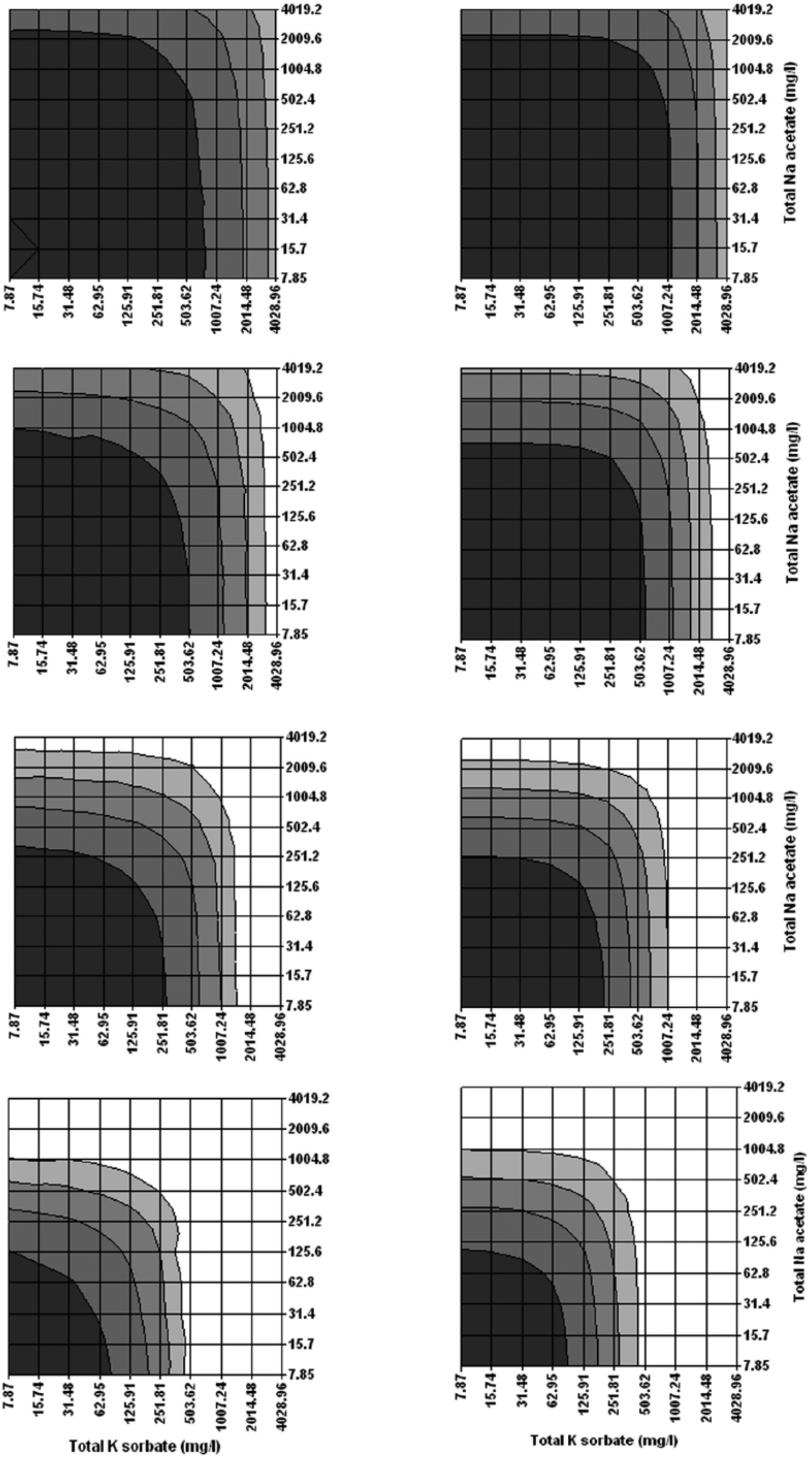

Fig. 3. Comparison of the observed (left) and predicted (right) relative rates to detection of *S. poona* for combinations of Na acetate and K sorbate at pH 6.5, 6.0, 5.5. and 5.0 (top to bottom respectively). Minimum times to detection were 322, 335, 335 and 433 minutes at pH 6.5, 6.0, 5.5 and 5.0 respectively. Contours are in steps of 0.2 RRD. The darkest shading represents full growth (RRD between 1 and 0.8).

to an ‘over-preserved’ product. Since the models are based on time to detection, they can also be used to suggest a likely-shelf life, although the variance structure of microbial growth data needs to be thoroughly examined to ensure reasonable confidence intervals.

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PREDICTION OF CONSUMER ACCEPTANCE LIMITS OF BAKERY PRODUCTS FROM OXIDATION INDEXES

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ABSTRACT

The aim of the present research was to evaluate the relationship between consumer acceptance and development of oxidation in lipid containing bakery products. To this purpose, biscuits were considered. The oxidation kinetics and the acceptance limits were evaluated as a function of temperature from 20 to 45°C. The oxidative stability was measured following the changes of peroxides, whereas consumer acceptance limits were detected by applying survival analysis concepts.

Results indicate that the oxidative indexes can be related to the evolution of consumer acceptance. In fact, the shelf-life, determined by consumer acceptability, was associated to the same peroxide values limits independently on storage temperature.

Key words: bakery products, consumer acceptance, oxidation, shelf-life

INTRODUCTION

Nowadays, the shelf-life prediction of shelf-stable foods is still a time spending process which difficulty fit industrial needs. The possibility to apply a rapid procedure for shelf-life prediction would be of enormous benefit for quality control programs. The set up of this procedure requires the possibility to follow the evolution of a quality index by using a simple and quick analytical method. The working assumption to successfully evaluate the shelf-life is that the selected index should be related to the consumer acceptability. In fact, the shelf-life of microbiologically stable foods is mainly attributable to the depletion of their sensorial properties (Hough *et al.*, 2003). Consequently a consumer panel would be the most appropriate tool to determine when a food reaches the end of its life, but this approach finds limited applications being highly expensive.

Unfortunately, it is not easy to find out an index, representative of the sensory decay, which could be measured in economically acceptable lengths of time.

The aim of the present work was to evaluate the relationship between consumer acceptance and development of oxidation in lipid containing bakery products. To this purpose biscuits with 20 % (w/w) fat content were considered. The oxidation kinetics and the acceptance limits were evaluated as a function of temperature from 30 to 45°C. The oxidative stability was measured following the changes of peroxides, whereas consumer acceptance limits were detected by applying survival analysis concepts. The latter is a branch of statistics extensively used in clinical and epidemiological studies, which was also applied in food shelf-life studies (Hough *Et al.,* 2003; Cardelli and Labuza, 2001; Gambaro *et al.,* 2004; Anese *et al.,* 2006).

MATERIAL AND METHODS

Sample preparation

Fresh made commercial biscuits containing 20 % (w/w) of fat were purchased at an Italian factory. Original packages containing 250 g of biscuits were stored in oven at 30, 37 and 45°C.

ANALYTICAL DETERMINATION

Fat separation. Biscuit fat was obtained by solid-liquid extraction using diethyl ether– petroleum ether (Carlo Erba, Italy) mixtures (1:1 v/v). In particular, ground biscuits and solvent mixture in the ratio 1:2.5 w/v were stirred at room temperature for 1 h. After filtration through filter paper (Whatman n. 1), the fat was separated from the solvent by evaporation (Heidolf Instruments, mod. 4001, Germany).

Peroxide value analysis. The peroxide values (PV) of the fat samples were carried out according to *AOAC* (1995).

Sensory analysis. The end of shelf-life of biscuits stored at different temperatures was determined through sensory analysis by applying the survival analysis (Gomez *et al.,* 2001; Hough *et al.,* 2003, 2004). This method has been developed to evaluate times until an event of interest, often called survival time, taking into account the presence of censored data (Gomez *et al.,* 2001). A group of 70 consumers was considered. Consumers were screened according to the criterion that they usually consumed biscuits. Aliquots of 10 g of biscuits were placed into 100 mL-capacity odourless plastic containers and sealed with a pressure cap. Before sensory test, samples were let to equilibrate at room temperature for 1 hour. Each consumer received 6 samples corresponding to 6 randomly distributed storage times. Panelists were asked to sniff the samples and report whether the product was acceptable or unacceptable. The CensorReg procedures from S-PLUS (Insightful Corporation, Seattle, USA, ver. 7) were used according to Hough *et al.* (2003, 2004). Censoring was defined as follows: at a given storage time t two possible answers could be given by the panelists: a) the sample was perceived as acceptable, indicating that it would be rejected beyond time t, thus the data is right censored; b) the sample was perceived as unacceptable, indicating that panelist would start rejecting the product before time t, thus the data is left censored. Data were fitted by the Weibull distribution model and the storage time corresponding to 10, 30, 50 and 70 % of consumers rejecting the product (F(%): 10, 30, 50 and 70 %) were estimated by maximizing the likelihood function. This is a mathematical expression, which describes the joint probability of obtaining the data actually observed on the subjects on the study as a function of the unknown parameters of the model being considered.

Data analysis. The results reported in this work are the average of at least three determinations and the coefficients of variation, calculated as the percentage ratio between the standard deviation and the mean value, were less than 5% for peroxide value determinations.

Linear regression analysis by least squares regression was performed by Windows Excel and the goodness of fitting evaluated on the basis of statistical parameters of fitting (R^2, p, standard error). Fitting by maximizing likelihood function was performed by S-PLUS (Insightful Corporation, Seattle, USA, ver. 7).

RESULTS AND CONCLUSION

Instrumental analyses. Figure 1 shows the increase in peroxide value of biscuits as a function of storage time at 30, 37 and 45°C. From the increasing part of the curves, the pseudo zero rate constants of peroxide formation were calculated by linear regression analysis. Results are reported in Table 1. As expected the rate constant enhanced as temperature increases. The values of peroxide formation rate (k_{PV}) were reported as a function of the reciprocal of temperature and the Arrhenius

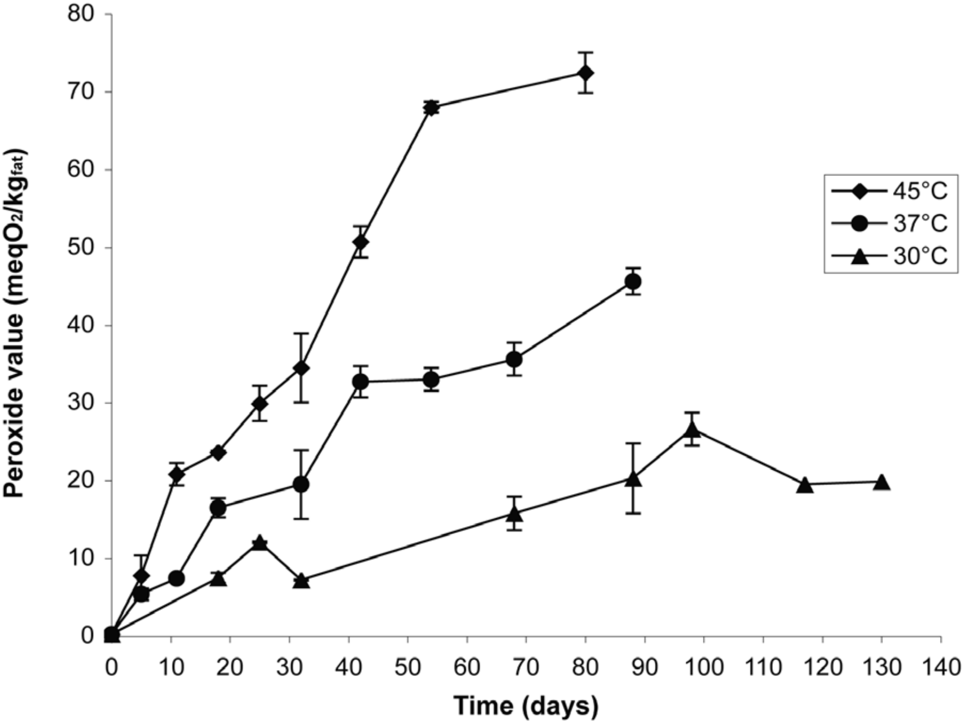

Fig. 1. Changes in peroxide value of biscuits stored at different temperatures.

Table 1. Pseudo zero rate constant of peroxide formation of biscuits stored at different temperatures and corresponding regression parameters.

Temperature (°C)	Interval time (days)	k_{PV} (meqO_2kg$^{-1}_{fat}$ days^{-1})	SE	p	R2
30	0-98	0.23	0.03	<10-4	0.916
37	0-88	0.51	0.04	<10-5	0.946
45	0-54	1.10	0.09	<10-5	0.965

equation was calculated by linear regression analysis. A very good linearity was achieved from 30 to 45°C (R^2=0.99, $p<0.05$):

$$\ln k_{PV} = 10569\frac{1}{T} + 33.38 \qquad (1)$$

Sensory analysis. Biscuit samples stored at different temperatures were analysed for sensory acceptability and data elaborated by survival analysis. The Weibull distribution was chosen because of its simplicity and good fit to the data. Storage times corresponding to 50 % of consumers rejecting the biscuits were calculated accordingly and reported in Table 2.

From the pseudo zero rate constants it was possible to determine the peroxide value in correspondence of the time at which 50 % of the consumers rejects the samples (Table 3). Statistical analysis demonstrates that these values are not significantly different (p>0.05).

Similar elaboration was done considering different percentages of consumers rejecting the sample (F(%): 10%, 30%, 70%). Results evidenced that PV limits at any F (%) is not temperature dependent. Therefore, for each critical probability level, a mean value of PV was calculated (Table 4). These values can be used as

Table 2. Shelf-life of biscuits, defined as storage time corresponding to 50% of consumers rejecting the sample, as a function of temperature.

Temperature (°C)	Time at 50 % consumers rejecting the sample (days)		
	95 % lower limit	Estimated	95 % upper limit
30	46.6	57.1	70.0
37	21.6	26.3	32.2
45	7.9	10.7	14.4

Table 3. Peroxide value corresponding to 50 % consumers rejecting the sample, as a function of temperature.

Temperature (°C)	Peroxide value at 50 % consumers rejecting the sample (meqO_2kg$^{-1}_{fat}$)		
	95 % lower limit	Estimated	95 % upper limit
30	12.7	15.1	18.1
37	14.8	17.2	20.2
45	11.3	14.8	19.4

Table 4. Mean values of PV limit at different percentages of consumers rejecting the sample.

Consumers rejecting the sample (%)	Peroxide value ($meqO_2kg^{-1}{}_{fat}$)		
	95 % lower limit	estimated	95 % upper limit
10	4.9	6.4	8.9
30	8.6	10.9	14.4
50	12.9	15.6	18.1
70	18.4	21.7	25.7

peroxide limits to determine when 10, 30, 50, and 70 % of consumers reject the biscuits and finally, on the basis of industrial policy, to estimate the shelf-life of the product.

Reporting the PV limits as a function of the failure probability, a linear relationship was found ($R^2=0.99$, $p<0.05$):

$$PV_{\lim} = 0.254\,(F\,\%) + 3.505 \tag{2}$$

where $PV_{\lim}$ is the peroxide value to be used as shelf-life limits and F(%) the percentage of unsatisfied consumers by the industry to define the shelf-life. This equation allows to define the peroxide value that indicated the end of the product shelf-life defined on the basis of industry needs.

Since the formation of peroxides follows a pseudo zero order kinetics, the shelf-life of biscuits can be finally predicted accordingly:

$$SL = \frac{PV_{\lim} - PV_i}{k_{PV,T}} \tag{3}$$

where SL is the shelf-life, expressed as days, $PV_{\lim}$ is the peroxide value corresponding to the limit of biscuit sensory acceptability chosen on the basis of equation 1, PV_i is the peroxide value at zero storage time, $k_{PV,T}$ represents the pseudo zero rate constant at the selected temperature. Since k_{PV} values can be extrapolated from the Arrhenius equation, this equation can be used to evaluate the shelf-life of biscuits at any temperature of interest between 30 and 45°C.

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LECTURE

SHELF LIFE OF PET BOTTLES ESTIMATED VIA A FINITE ELEMENTS METHOD SIMULATION OF CARBON DIOXIDE AND OXYGEN PERMEABILITY

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ABSTRACT

Shelf life estimation is a very important issue in the beverage industry. Depending on the packaging application, estimation of the carbon dioxide loss or of the oxygen ingress through the plastic bottle walls has always been desirable and challenging at the same time.

Up to now, most of this work is being carried out experimentally measuring the O_2 or the CO_2 transport through the package. In this work an FEM approach is presented, which is shown to be able to replace time consuming, expensive lab tests.

Gas permeability through the package is treated as a transient state diffusion problem in a 2D axisymmetric geometry.

As required inputs, the exact bottle drawing is needed with the estimated or the measured thicknesses along the various bottle sections. Cap geometry and initial fill level must also be input. Data of strain induced crystallinity as obtained by density for the main bottle areas (unoriented areas, shoulder, base and body) have to be fed into the software as well.

For each plastic material involved, data of gas (O_2 or CO_2) solubility and diffusivity are entered as a function of temperature and crystallinity of the material.

The model was able to fit the experimental measurements of CO_2 loss and O_2 ingress with an average error lower than 5%.

Key words: carbon dioxide, FEM, oxygen, permeability, PET bottles, shelf life

INTRODUCTION

Very often the shelf life of a packaged food is heavily dependent on the resistance that the polymeric container walls are able to offer to various compounds diffusing through them.

Examples of this condition are carbonated mineral waters, soft drinks and beer. The carbonation level in these products decreases with time as a result of permeation mechanisms through the bottle walls.

Moreover, their organoleptic properties are mostly determined by their gas content in such a way that a carbon dioxide reduction of only 15% is generally enough for the drink to taste flat.

The beverage industry is therefore much involved in testing for CO_2 loss or O_2 ingress in order to check that each modification imparted to the packaging (bottle weight reduction, cap change, new bottle shape, etc.) does not significantly decrease the required shelf life.

This is normally achieved through labour and especially time intensive experimental measurements, which require the containers to be filled and repeatedly measured over time up to several months.

A modelling tool able to reduce this stage to no more than a few hours would speed up the process and would rationally optimise many features of the packaging (especially the thickness, i.e. the final weight).

As a matter of fact, models very often tend to use oversimplified assumptions, like the reduction of the container to a mono-dimensional film (just radial diffusion is taken into account) and the disregard of local geometry variations in terms of thickness distribution of the bottle walls and of the local physical properties (density/crystallinity). This features noticeable difficulties in properly defining what is the average effective diffusivity and solubility, when material properties do vary on a variable geometry itself.

In this work these restraints could be relaxed by using a 2D radial symmetric model, which therefore has all the characteristics of a 3D model, in which the real bottle geometry is used as the integration domain.

MATERIALS AND METHODS

Bottles

All the containers used for the experimental measurements of permeability were manufactured in PET (Poly(ethylene terephthalate)). PET from various suppliers was used, but it was always of ‘water grade’ type (modified with about 2% isophthalic acid and solid stated to a final intrinsic viscosity of 0.74-0.80 dl/g).

The bottles were produced by a two stage technique consisting first of injecting the preforms on dedicated injection molding machines (Husky Injection Molding Systems Ltd., Bolton, Ontario, Canada) and then stretch blow molding these preforms on blowing machines (Sidel, Le Havre, France).

Closures

Bottles were closed with various caps, which were mainly of the one-piece type (high density polyethylene mono-material) or of the two-piece type (polypropylene outer shell + ethylene vinyl acetate seal).

Chemical carbonation

All the carbon dioxide (CO_2) permeability measurements were done via chemically carbonating the bottles with sodium bicarbonate (VWR International) and hydrochloric acid (standard solution 6 mol/l from Riedel-de Haen).

Stoichiometric amounts of acid and base were added to deionised water to develop the desired initial CO_2 levels in the bottles.

Permeability

CO_2 levels were measured via pressure and temperature readings. Pressure in the bottles was measured using an aphrometer (similar to the Zahm&Nagel piercing devices) equipped with an MBS 3000 pressure transducer (Danfoss A/S, measuring range: 0-10 bar, accuracy: ±0.5% FS). Temperature readings were performed with a Checktemp digital thermometer, supplied by Hanna Instruments Inc. (accuracy: ±0.3°C).

Knowing pressure, temperature and volume of the headspace of the bottle (which can be easily measured), the ideal gas law can be applied to calculate the moles of CO_2 in the gas phase. By Henry's law and knowing the Henry's constant for the CO_2/water system, the concentration of CO_2 in water can then be determined.

By measuring the CO_2 amount repeatedly at various storage times a carbonation loss curve can be built.

Five bottles for each storage time were measured. Bottles were stored at 22±1°C or at 40±1°C.

Dissolved oxygen (O_2) measurements were carried out by means of a YSI 550 dissolved oxygen meter (YSI Inc.)

For O_2 ingress measurements, deionised water was previously deaerated by Nitrogen bubbling through the water under vacuum; Argon flushing of the headspace ended the procedure before capping the bottle. In this manner reproducible values of dissolved O_2 could be attained (around 0.2 mg/l).

The bottles were then stored for various times at 22±1°C and analysed for dissolved O_2 in water. Five bottles for each time batch were used. In this way an O_2 ingress curve over time can be generated.

Bottle thickness

A Gawis OD9500 device from AGRTopWave LLC was used for automatic thickness measurement of the bottle walls along various heights.

Density

Various bottle parts were measured for density using an AG 204 DeltaRange balance from Mettler Toledo International Inc., equipped with the density determination kit for determining the density of solids.

Percent crystallinity values (X) were computed from density measurements using the following formula:

$$X = \frac{\rho - 1.335}{1.455 - 1.335} \quad (1)$$

where ρ is the density of the sample. The density of completely crystalline PET is assumed to be 1.455 g/cm^3 while that of completely amorphous PET is 1.335 g/cm^3.

Software

AutoCAD 2006 from Autodesk Inc. was used to work on the bottle technical drawings before importing them into FEMLAB 3.1 (COMSOL Inc.), which was used to run the FEM simulations.

The process of mass transport through a package is complex, very often not allowing an analytical mathematical solution, so that use of numerical methods is required.

In fact, the solutions are further complicated by practical problems, such as migration with media partitioning, dependence of diffusion coefficient D on sorbed penetrant concentration C, such that D = D(C), deviations from Fickian behaviour in diffusion, which are related to different penetrant sorption modes (Comyn J., 1985) (see for instance dual mode sorption in glassy polymers) and the case of binary gas mixtures, in which the effects of competition between both components must be taken into account (Chern R.T *et al.*, 1983). In such cases, analytical solutions are rarely available, if not feasible at all.

Actual geometries (thicknesses) and physical properties (density) of the bottles are also varying locally from zone to zone, following complex features, whose approximation by the regular plane sheet concept (Crank J., 1975) is rather questionable in the real case. Therefore, the finite element method was selected in this work to approach some of these complicated problems in a simpler and more efficient way.

The basics assumptions used to carry out the modelling work have been (depending on the case of CO_2 exit or O_2 ingress through the package):

- Initially, the migrant is distributed uniformly in the packaging matrix and/or in the food.
- Migration occurs from one side of the packaging to a liquid food or vice versa.
- The liquid food is well mixed so that there is no migrant concentration gradient in the food.
- The surface mass transfer coefficient is much larger than the diffusion coefficient, implying the migration is controlled by Fickian diffusion in the packaging and the effect of mixing is negligible.
- Diffusion coefficient and partition coefficient are constant during migration and depend only on temperature.
- Equilibrium exists all the time during migration at the interface of packaging and food.
- No significant swelling of the polymer is assumed to occur when a gas permeates through the polymer itself.
- When modelling CO_2 or O_2 mass transfer, the effects of competition on the sorption of the pure components studied and other possible components (e.g. N_2) are neglected.

All this assumptions lead to a Fickian diffusion of the gases through the PET bottle walls, in which the transfer amount and rate are controlled by the diffusion coefficient and the partition factor.

Generally PET, being a glassy polymer below 70°C, fits poorly into a pure Fickian diffusion mechanism.

Other models are therefore preferred to predict the mass transfer through PET, like the dual-mode sorption model (Barrer R.M. *et al.*, 1958) and the gas-polymer matrix model (Raucher D. and Sefcik M.D., 1983). The latter seems to be slightly favoured in the PET case (Brolly J.B. *et al.*, 1996).

By this model, it is assumed that D increases with C and S decreases with C and thus the permeability $P = D \cdot S$ can turn out to be practically unaffected by C, as can be verified in the case of the data provided by Brolly J.B. *et al.* (1996).

So, the approach of this work has been to try keeping the simpler Fickian model

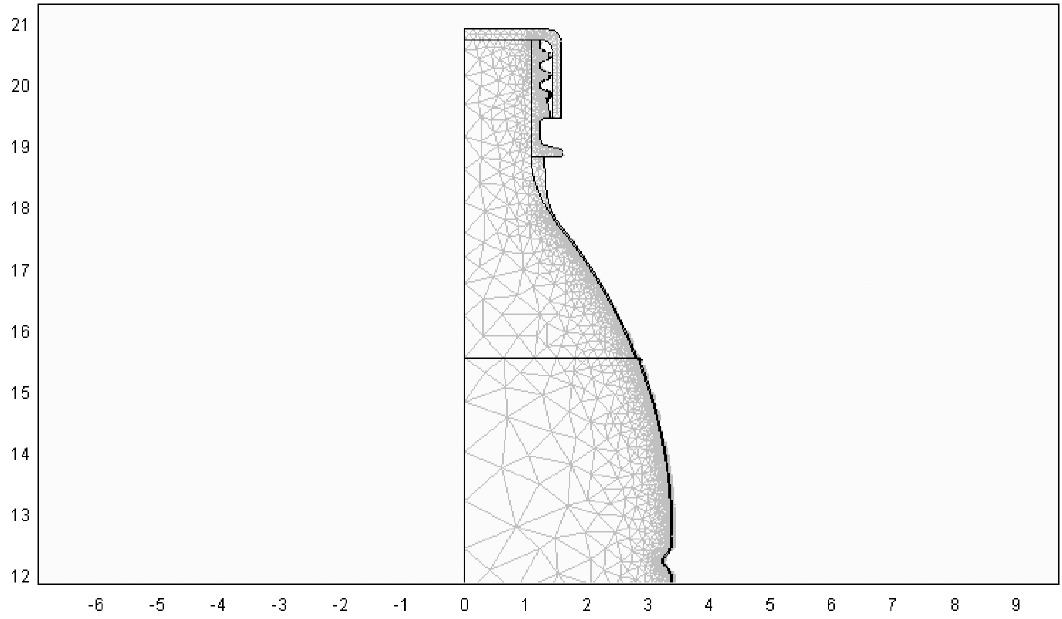

Fig. 1. Bottle drawing representing the level of details.

and test it against the experimental observations of gas permeability through actual PET bottles. This Fickian approach has also been followed by others (Del Nobile M.A. *et al.*, 2003).

The simulations were run on a 2D axisymmetric domain, exploiting radial symmetry. The advantage over other models, which try to predict the product shelf life in plastic bottles (Del Nobile M.A. *et al.,* 1997), is that the domain is not approximated to a plane sheet, having the surface of the real bottle and a thickness equal to the average thickness of the bottle itself; rather, the actual 2D drawing was used with the further advantage of using the actual thickness and crystallinity distribution (see details in Fig. 1), the latter not being usually modelled (Del Nobile M.A. *et al.*, 2003).

The partial differential equation to solve for was:

$$\frac{\partial C}{\partial t} = \nabla(D\nabla C) \tag{2}$$

to be solved in non-steady state conditions, where C is the concentration of the gas and D the diffusion coefficient.

The boundary conditions were:

Constant gas concentration on all the outer boundaries (CO_2=0; O_2=external concentration in the environment).

Flux continuity at each domain boundary between different phases (e.g. headspace-water, etc.), but different concentrations from domain to domain due to partitioning.

The initial conditions were:

CO_2 concentration = 0 in all domains except in the water domain, in which it was set at the concentration obtained by the chemical carbonation; O_2 concentration = initial known concentration in the water and in the headspace.

Diffusion coefficient for CO_2 and O_2 in the polymer was considered a function of temperature, T, and of crystallinity, X, being introduced into the model in the following form:

$$D_{eff} = ae^{-\frac{b+cX}{T}} \qquad (3)$$

where D_{eff} stands for the 'effective' Fickian diffusion coefficient *a* is a pre-exponential factor, *b* and *c* are positive constants, *X* is the percent crystallinity, *T* is the temperature in K.

Solubility was similarly expressed as per the following form:

$$S = a(b - cX)e^{\frac{d}{T}} \qquad (4)$$

in which *a*, *b*, *c* and *d* are positive constants.

The constants were directly taken or fitted to permeability, diffusivity and solubility values found in the literature for the CO_2/PET and O_2/PET systems (Brolly

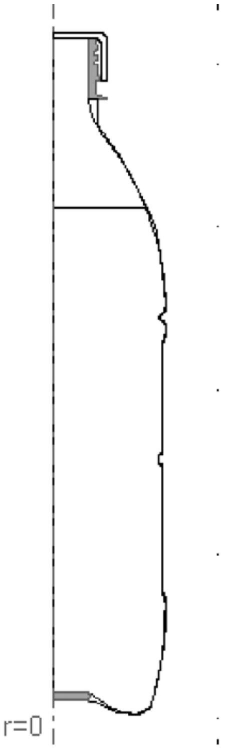

Fig. 2. Non-oriented or amorphous areas of the bottle as assumed in the model (coloured areas).

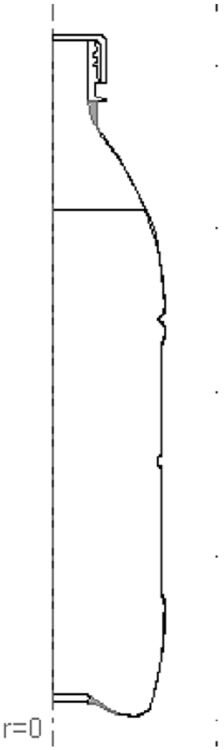

Fig. 3. Partially oriented areas of the bottle as assumed in the model (coloured areas).

J.B. *et al.*, 1996, McGonigle E.A. *et al.*, 2001, Lewis E.L.V. *et al.*, 2003, Lin J. *et al.*, 2001, Koros W.J. and Paul D.R., 1978, Michaels A.S. *et al.*, 1963, Natu A.A. *et al.*, 2005).

By filling in the right value for X, each main zone of the bottle could be properly taken into account without extreme approximations, as shown in the following figures (Fig. 2 and Fig. 3).

Fig. 2 shows the practically 'pure' amorphous areas of the bottle, while Fig. 3 depicts the partially oriented zones in the neck and in the base. All the remaining sectors (body and shoulder) are fully oriented and described by an average strain induced crystallinity value. All the crystallinity values were experimentally measured via density, as described in the previous section.

Solubility and diffusivity values for CO_2/ O_2 in the cap were also introduced using expressions of the same type as n. 3 and 4, with the proper constants.

Solubility and diffusivity values for CO_2/ O_2 in water were calculated respectively by using Henry's law and experimental data available in the literature.

Temperature could be treated as a constant or a variable, with the possibility of studying different thermal cycles.

RESULTS

The main purpose of this work was to run various models implying different bottle geometries, different storage conditions, etc. and assessing their predictive ability by comparing the theoretical results with the experimental measurements.

The following graphs show how the predicted permeability curves (solid lines in the figures) matched with the respective experimental observations (points in the figures). Permeability is graphed in terms of CO_2 mass loss from the container or O_2 concentration increase in water.

Fig. 4 compares room temperature versus 40°C storage for a half litre bottle, all other things being equal.

Fig. 5 shows how 3 grams difference in the bottle weight affects the permeability in a half litre bottle stored at 40°C.

Fig. 6 illustrates the same comparison as Fig. 4 but for a 1 litre bottle.

Fig. 7 highlights how, for a complex temperature variation pattern (white curve) actually input into the model, the model itself is able to capture the real permeability trend (black curve); if the average temperature were used instead, then the model would predict less reliably as per the grey curve.

Fig. 8 illustrates the O_2 ingress curves for a half litre bottle comparing two different weights (19.5 g vs. 16.5 g).

The single highest deviations observed between experimental points and theoretical curves were in the range of ±10%, being on average below ±5%.

CONCLUSIONS

A computer model, which accurately reflects the physics of the permeability process of a gas through a plastic bottle, was developed using the finite element method. The accuracy of the model in predicting permeability was successfully demonstrated by comparing the simulation results to experimental data, both for

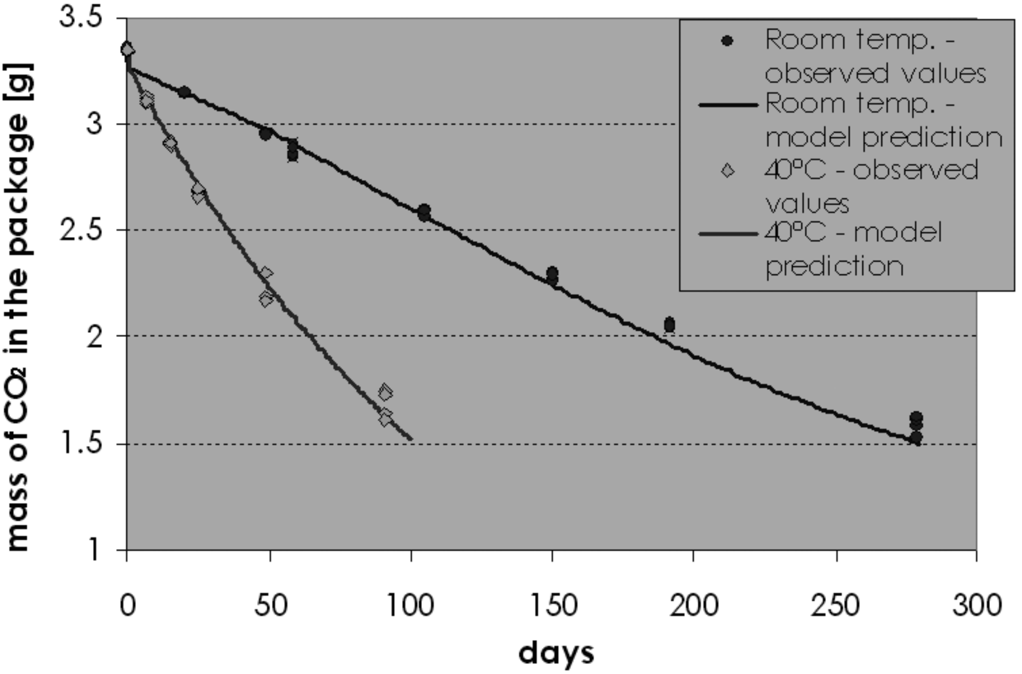

Fig. 4. CO_2 loss for a 0.5 l bottle – 26 g – room temperature vs 40°C. Initial product carbonation: 3.3 CO_2 vol (STP).

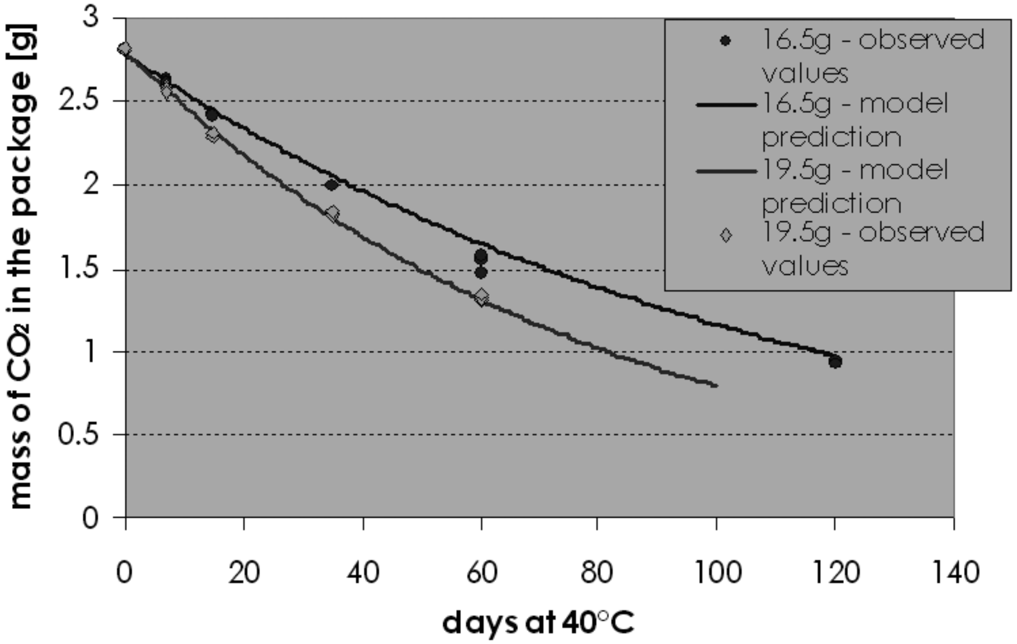

Fig. 5. CO_2 loss for a 0.5 l bottle – 40°C – 19.5 g vs 16.5 g. Initial product carbonation: 2.8 CO_2 vol (STP).

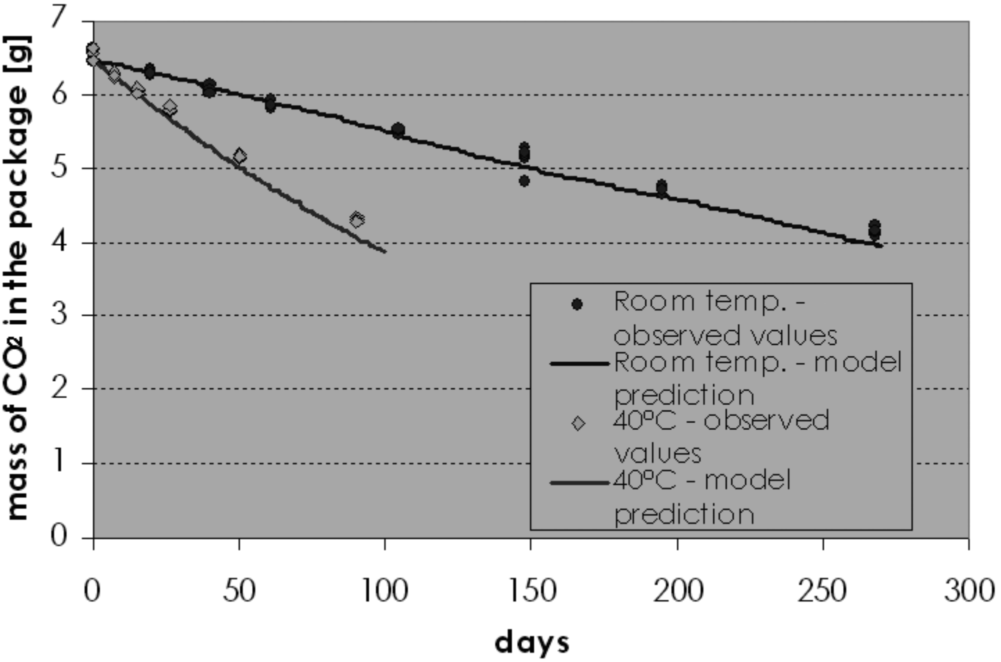

Fig. 6. CO_2 loss for a 1 l bottle – 44 g – room temperature vs 40°C. Initial product carbonation: 3.3 CO_2 vol (STP).

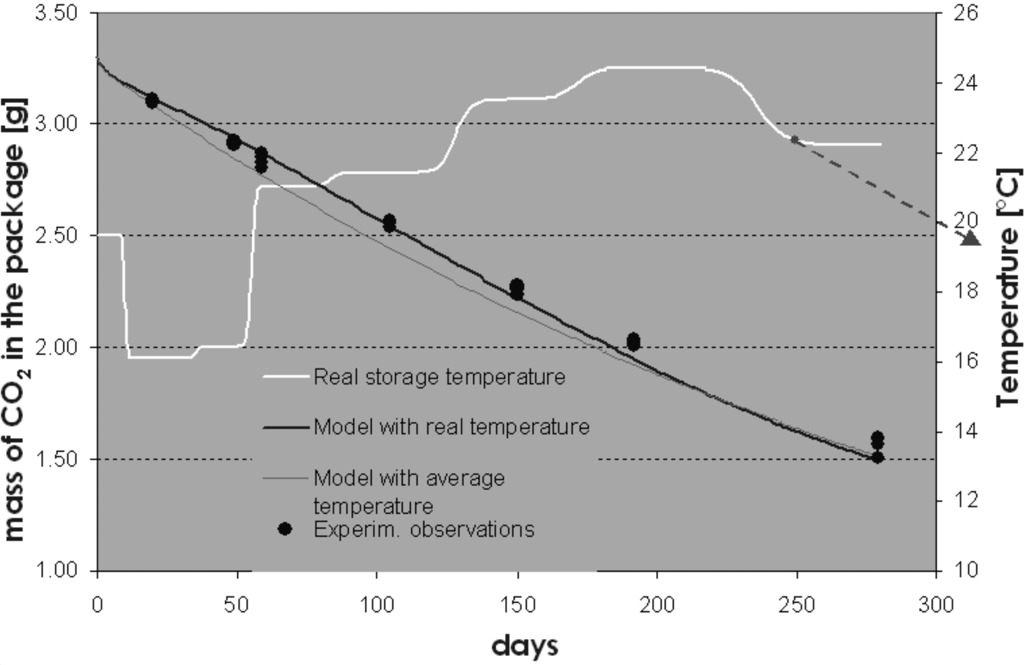

Fig. 7. CO_2 loss for a 0.5 l bottle – 26 g – effect of random temperature cycling. Initial product carbonation: 3.3 CO_2 vol (STP).

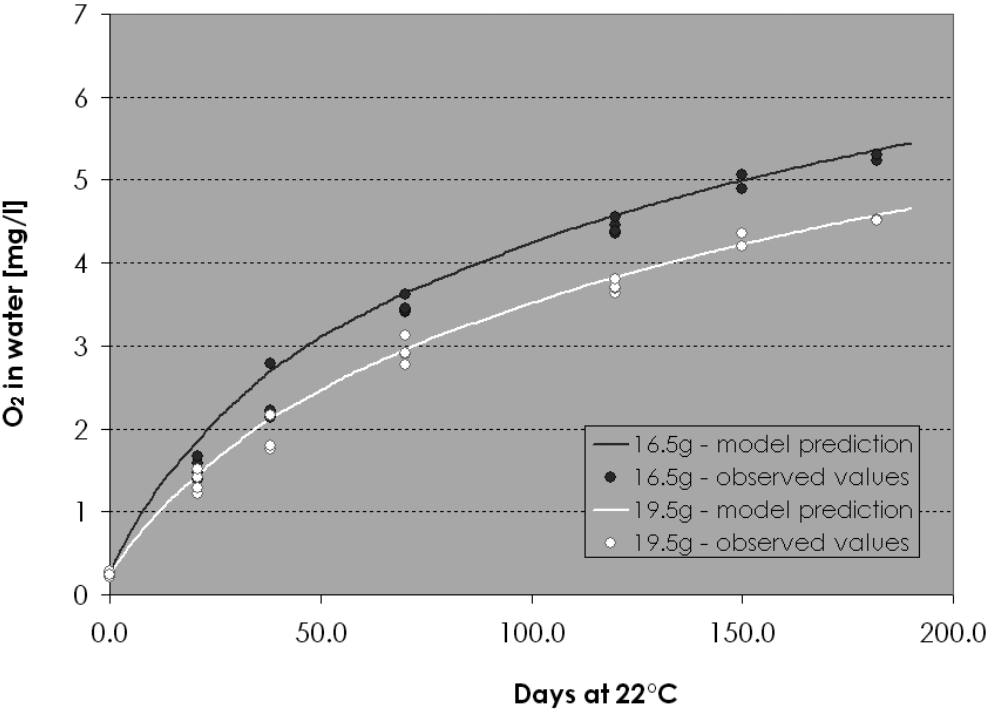

Fig. 8. O_2 ingress for a 0.5 l bottle – room temperature – 19.5 vs 16.5 g. Initial oxygen concentration in water: 0.25 ppm.

CO_2 and O_2 permeation. Maximum differences between predicted and observed values were below ±10%, while average differences were below ±5%.

Although a simpler Fickian diffusion assumption was used, very good fitting to the experimental measurements was achieved. Therefore, no further theoretical refinement of the model, especially to include D and S dependence on the penetrant concentration, was deemed necessary.

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QUALITY OF ORANGE JUICE CONSIDERING THE MATRIX AND THE PACKAGING MATERIAL: AN INTEGRATED APPROACH

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ABSTRACT

The vitamin C content and the colour of an orange juice made from concentrate were measured during 9 months of storage at 20°C in glass, standard monolayer polyethylene terephtalate (PET) and multilayer PET (PET/Nylon and oxygen scavenger/PET) containers. In plastic packaging materials, the loss of quality was related to the oxygen permeability and glass enabled the best preservation of ascorbic acid and colour. The losses of aroma compounds by permeation through the bottle (PET) and the stopper (HDPE high density polyethylene) have also been investigated. The results showed that permeation was mainly done through the stopper. The use of a multilayer stopper (HDPE with internal joint LDPE/EvOH/LDPE) enabled to limit the permeation, whatever the PET bottle used.

Key words: aroma compounds, ascorbic acid, colour, high density polyethylene, orange juice made from concentrate, polyethylene terephtalate

INTRODUCTION

The quality of orange juice is mainly related to its vitamin C content, its colour and its flavour. The major changes which occur in the juice are losses in vitamin C, development of off-flavours and non-enzymatic browning (Sizer *et al.*, 1988). Currently, food companies are looking for suitable raw material and packaging to maintain quality and to promote the increase of shelf life. Plastic packaging materials are more and more convenient for economic reasons and among these, PET (polyethylene terephtalate) and HDPE (polyethylene high density) are widely used, respectively for the bottle and the stopper of a large amount of food drinks. However, plastic packaging materials can interact with the food itself causing sorption and permeation which can lead to an unbalanced flavour profile. Moreover, the oxygen permeability of the packaging material is an important factor controlling the shelf life of the product. New technologies, such as oxygen scavengers or barrier films, tend to decrease the oxygen crossing over, to extend the shelf life and to limit oxidation reactions occurring in the product (Zerdin *et al.*, 2003; Baiano *et al.*, 2004).

In a previous study, Berlinet *et al.*, 2005 showed that changes in aroma compounds during storage where strongly correlated to the duration of storage, but not to the packaging material (bottle) and its corresponding oxygen permeability. In the present work, the vitamin C content and colour have been measured in the juice in function of the packaging material used. The losses of aroma compounds by permeation through the bottle (PET) and the stopper (PEHD) have also been investigated.

MATERIALS AND METHODS

Preparation of orange juice from concentrate. Orange juice was prepared from concentrate as previously published (Berlinet *et al.*, 2005). The reconstituted juice (pH 3.9) was flash-pasteurized for 20 s at 92 °C and bottled in different packaging materials under cold aseptic conditions.

Packaging materials.

Table 1. Principal characteristics of the PET bottles used.

Bottle	Code	Composition	Oxygen permeability in U ($1U = 10^{-20}$ kg m^{-1} s^{-1} Pa^{-1})	Density kg.m^{-3}
Monolayer	PET 1	PET	6.79	1350-1360
MULTILAYER BINDOX®	PET 2	PET/NYLON-OXYGEN SCAVENGER/PET	0.62	1200-1250

Two different PET packaging materials (0.33 l) from Amcor were used (Table 1). Glass bottles (0.5 l) were used as the reference packaging material. All bottles were sealed after filling with aluminium foil in order to avoid a cap effect and for the measurement of permeation, they were sealed with 2 different HDPE stoppers (Table 2). The bottles were stored at 20°C under artificial light or darkness.

Vitamin C analysis. Vitamin C contents were measured using 2,6-dichloroindophenol titrimetric method (AOAC, 1984). Each analysis was performed in triplicate on 3 different bottles from the same batch after 3 months and 9 months, either under artificial light (AL) or in the darkness (D).

Colour. L*, a*, and b* values were measured with an X-Rite 3200 colorimeter. Each measure was performed in triplicate on 3 different bottles from the same batch.

Permeation. The PET bottle filled with juice and closed with one HDPE stopper was placed just after manufacture in a permeation cell equipped with a Mininert®

Table 2. Principal characteristics of the HDPE stoppers used.

Code	Composition	Internal joint	Oxygen permeability in U (1U = 10^{-20} kg m^{-1} s^{-1} Pa^{-1})
A	HDPE	No joint (septum ?)	1959
B	HDPE	LDPE/EVOH/LDPE	1161

valve allowing the entry of a SPME Carboxen/PDMS fiber (Supelco, Bellefonte, PA, USA). After 2 months of ambient storage, SPME extraction of the permeation cell headspace was performed with 40 min of fiber exposure time. After extraction, the fiber was retracted then inserted into the GC injector port for thermal desorption at 280°C (GC 8000 Top, Fisons Instruments, Arcueil, France). Temperature of detector was 250°C. The oven temperature was programmed to rise from 35°C to 240°C at 5°C.min^{-1} and the column was a Supelcowax 10 (Supelco, Bellefonte, PA, USA) 30 m x 0.32 mm i.d. x 1 µm film thickness). Compounds were identified from their EI mass spectra or based on authentic standard compounds by using a FISONS GC-8000 gas chromatograph coupled to a FISONS MD-800 quadrupole mass spectrometer with an electron ionisation mode (EI).

RESULTS

Vitamin C

At zero time, juice made from concentrate had a vitamin C content of 383 mg.L^{-1}. After 3 months of storage in glass, vitamin C decreased by ~19% (Table 3). Under same storage conditions, losses observed in (PET 1) and (PET 2) were ~42% and ~29%. These data show that losses in vitamin C were partly related to oxygen permeabilities of the different packaging materials : (PET 1), having the highest oxygen permeability, showed the highest losses while (PET 2) with a permeability 10 times lower exhibited less losses for vitamin C. This effect was emphasized after 9 months of storage (Table 3). Losses observed in glass bottles (no oxygen permeability) were attributed to oxygen present in the bottle headspace and in the reconstituted juice.

No statistical difference (ANOVA, $p<0.05$) was revealed between the vitamin C content in the juice stored under AL or in D, whatever the packaging material used, as well after 3 months than after 9 months of storage. The AL used was emitted by 16 fluorescent tubes with an average intensity of light of 750 lux (close to that used in the rays of supermarket) which did not cause any additional losses of vitamin C compared to D. Conrad *et al.* (2005), with monolayer PET, also found no positive

Table 3. Vitamin C (mg.L^{-1}) contents of the juice (n=3).

Bottle	3 months (AL)	3 months (D)	9 months (AL)	9 months (D)
glass	310 +/- 25	304 +/- 13	310 +/- 3	300 +/- 8
PET1	220 +/- 9	224 +/- 5	132 +/- 2	125 +/- 3
PET2	270 +/- 5	259 +/- 12	255 +/- 8	275 +/- 2

effect of storage in D when compared with storage under AL, losses of vitamin C being almost identical.

Colour

L*, an indicator of lightness, showed no significant changes during the first 3 months of storage whereas b* tended to decrease indicating that the juice was less yellow (Table 4). The more characteristic evolutions were an a* increase (Figure 1). After 6 months of storage, this increase was higher with PET1 than with PET2. Glass showed the lowest a* increase. Positive Δa* indicates that the juice was redder than green. These results were in agreement with the formation of brown pigments during storage. Thus the modification of colour during long-term storage was partly related to the oxygen permeability of the packaging material used.

Table 4. L* and b* values of the juice under AL (n=3, SD= +/- 0.1).

	L*		b*	
Bottle	t0	3 months	t0	3 months
glass	67.3	68.7	52.2	49.9
PET1	67.3	67.8	52.2	50.2
PET2	67.3	68.0	52.2	49.6

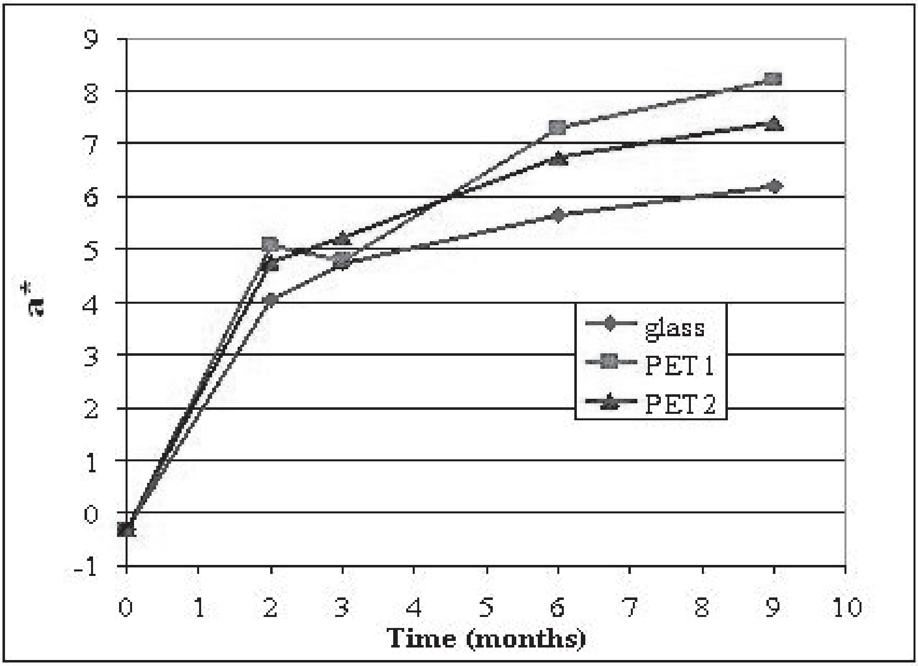

Fig. 1. Evolution of a* in the orange juice made from concentrate in glass, PET1 (monolayer) and PET2 (Bindox) during storage.

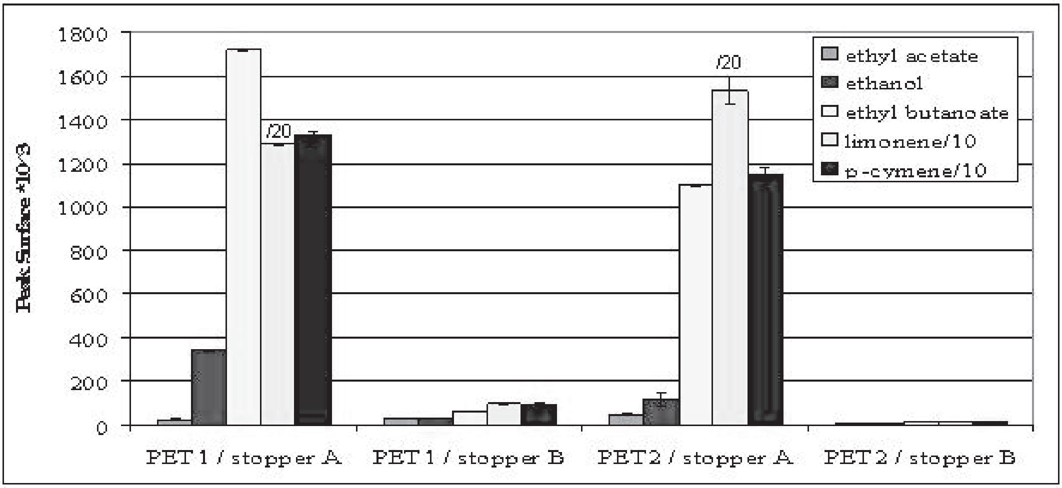

Fig. 2. GC area of aroma compounds after 2 months of storage in the permeation cells (n=3).

Permeation of aroma compounds

The results of permeation through PET bottle and HDPE stopper are shown in Figure 2. We made four different groups out of 2 PET bottles (PET1 and PET2) and 2 HDPE stoppers (A and B). Whatever the PET bottle used, the permeation of aroma compounds was much higher with stopper A (HDPE) than with stopper B (HDPE with LDPE/EvOH/LDPE joint). For instance, the permeation of ethyl butanoate, one major flavour compound, which participates in the freshness notes of the juice, was 30 times lower with the multilayer stopper B than with the monolayer stopper A. Stopper B, due to its EvOH layer, has an oxygen permeability 1.6 times lower than stopper A (Table 2), which may permit to decrease the oxygen crossing over. This EvOH layer could also have the advantage to limit the permeation of several aroma compounds considerably, particularly those which are hydrophobic such as limonene.

CONCLUSIONS

In comparison with monolayer PET, multilayer PET with improved oxygen barrier properties showed better vitamin C contents and colour during 9 months of storage. Permeation of aroma compounds was mainly due to the monolayer HDPE stopper. Future studies will quantify the permeation in order to make a mass balance of the aroma compounds during storage and to know the importance of their transfer with the packaging material in the global degradation.

ACKNOWLEDGMENTS

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NON–DIMENSIONAL MODEL OF BACTERIAL GROWTH UNDER NON–ISOTHERMAL CONDITION FOR THE PREDICTION OF FISH SHELF–LIFE

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ABSTRACT

A new dimensionless model of bacterial growth, built on the base of a four-parameter logistic model, is proposed. Different temperature series were tested in order to simulate bacterial growth under non-isothermal conditions. Usefulness of the model to preliminary prediction of shelf-life was confirmed by comparison of results obtained from proposed dimensionless model, that were easily converted into known dimensional units, with results presented in the literature.

Key words: bacterial growth, non-dimensional model, non-isothermal conditions, shelf-life prediction

INTRODUCTION

Storage of fish is related with transformations that accompany chemical, biochemical and microbiological changes, which take place in the stored food [1]. Both needs of safety and a short expiration time of fresh seafood cause that application of techniques, which ensure freshness of products oriented to commerce, is required. Often, cooling or freezing are applied for that purpose. In parallel, in order to protect food against spoilage during its storage, modified atmosphere could be used [2]. The development of new and more effective means to extend fish shelf-life requires a better understanding of the various factors which have impact on the freshness of fish. In order to meet this need, laboratory tests are extensively used to investigate experimentally, and mathematical models of microbial growth are developed/adapted to the specific problem in order to improve prediction of the impact of various factors on fish shelf-life. Due to the intrinsic complexity of food spoilage processes, physical and chemical factors which play a role in food storage are many and not all fully known, and the wide variety of bacterial species involved makes this task very difficult. Unfortunately, also criteria of food quality are not unequivocal. For instance, common challenge tests of maximum acceptable presence of the microbe of concern are commonly used, based on the measurement and/or mathematical modeling of bacterial growth rate [3]. Results of such tests are often expressed in numbers of 10^5–10^6 cells per gram, thus determining “a margin of safety”, which is independent of the nature of microbes and, hence, not representative of bacterial pathogens [4]. For this purpose, a concept of specific spoilage organisms (SSO) could be employed to predict fish shelf-life [5]. A wide variety of equations describing bacterial growth rate can be found in the literature, e.g. in [6, 7], although range of applicability of these models is limited [5]. There is no proper mathematical model that contains all physical factors, like temperature, water activity, pH, oxygen or NaCl concentration on bacterial rate growth. Moreover, response of different bacterial species to physical factors and type of feed can also be very different. For instance, while low temperature blocks growth of mesophile bacteria, growth of Pseudomonas is not restricted by temperature lower than -8°C [1].Correct determination of shelf–life prediction requires to specify a set of parameters, which are necessary to conduct numerical computations. Thus, a proper mathematical model has to describe how many bacteria are present at current time and what growth rate under specified conditions is expected. Current problems related to modeling of prediction of shelf-life are discussed in [3]. Models are based upon the observation that all bacterial cultures are characterized by periods as lag phase, logarithmic growth, stationary phase and a last phase during which the bacterial colony dies. For food safety it is always necessary to determine cells concentration at which food is useless/dangerous for consumption, and the time during which such concentration is met. Generally, a lag time, a kinetic of exponential growth and initial cells number are indispensably in every predictive mathematical model. Other parameters depend on the type of equation chosen for numerical computation. Up to now, good-practice recommends to determine growth rate under sets of constant conditions, in order to obtain kinetic parameters, which can then be used in computations under variable conditions [8]. Belehradek power function is given as a proper model of the influence of temperature on biological processes:

$$k = b(T - T_{ref})^n \tag{1.1}$$

where k is the specific rate, b and n are constants depending on the type of organism, T is the test temperature whereas T_{ref} is the reference temperature such that k=0. A modified form of Belehradek equation is the half-empirical equation proposed by Ratkowsky and Olley, known as square root model [9]:

$$\sqrt{\mu} = b(T - T_{\min}) \tag{1.2}$$

In this case, μ is used as the specific growth rate, T_{min} is fixed as the point of cross-cut of regression line (μ)^1/2 vs. T with y-axis, whereas b corresponds with slope of the regression line. These kinetic parameters may be evaluated on the basis of experiments and their values have a direct physical interpretation. Consequently, the growth rate changes with temperature, whereas $T_{\min}$ is the temperature below which the microbial growth stops. The lag phase corresponds to a period of slow increment of microbes, during which cells adjust to environmental conditions that surround them. All these parameters depend on the species of microbe as well as on the type of substrate. Models taking into account the influence of temperature on the growth rate, including Ratkowsky's model, are presented and discussed in [7,10] More complex models contain influence of other factors, like the model proposed by Neumeyer *et al.* [11] which takes into account the relationship between the growth rate of pseudomonads and both temperature and water activity.

MATHEMATICAL MODELING OF BACTERIAL GROWTH AND CALCULATION OF SHELF-LIFE

Among the available models, a four–parameter logistic model is used in the present work in order to estimate the increment of bacterial concentration with time (Eq. 2.1), as proposed by [12], in which prediction of shelf life of gilt-head seabream was tested on the basis of this model with success.

$$\log N(t) = \log\left(N_{\min} + \frac{N_{\max} - N_{\min}}{1 + \exp\left(-\mu_{\max}(t - t_i)\right)} \right), \tag{2.1}$$

where $N_{\min}$ and $N_{\max}$ are initial and final asymptotic values of bacterial cell concentration respectively (CFU/g) (CFU=Colony Forming Unit), t is time expressed in hours, t_i is the time required for a concentration of half of the maximum concentration be attained, whereas $\mu_{\max}$ is the maximum specific growth rate. The kinetics of the process determines its rate of growth (Eq. 2.2) and a lag phase (Eq. 2.3), which are expressed by using the Belehradek-type equation [9, 12].

$$\sqrt{\mu_{\max}} = b_\mu (T - T_{\min\mu}) \tag{2.2}$$

$$\sqrt{\frac{1}{\tau_{lag}}} = b_l (T - T_{\min l}) \tag{2.3}$$

Here, T is the temperature in degrees Celsius, $T_{\min\mu}$ is a minimum temperature for growth estimated by extrapolation of the regression line $(\mu)^{\wedge}1/2$ vs. T and b_μ represents the slope of this regression line.

As stated above, time-dependent logistic model for constant temperature was employed to write Eq. 2.1. The idea is to incorporate temperature dependence into this equation. To this aim, (Eq. 2.2) is combined with Eq. 2.1 to give the following explicit relationship between cells number, temperature and time:

$$N(t) = N_{\min} + \frac{N_{\max} - N_{\min}}{1 + \exp\left[-b_\mu^{\,2}(T - T_{\min\mu})^2 (t - t_i)\right]} \tag{2.4}$$

In order to obtain a non-dimensional expression, trivial rearrangements produce:

$$\frac{N(t) - N_{\min}}{N_{\max} - N_{\min}} = \frac{1}{1 + \exp\left[-b_\mu^{\,2}(T - T_{\min\mu})^2 (t - t_i)\right]} \tag{2.5}$$

The following dimensionless parameters are introduced:

$$n = \frac{N(t) - N_{\min}}{N_{\max} - N_{\min}}; \quad \tau = b_{\mu}^{2}(T_{\min\mu} + 273)^{2} t; \tag{2.6 a,b}$$

$$\tau_i = b_{\mu}^{2}(T_{\min\mu} + 273)^{2} t_i; \quad \theta = \frac{T - T_{\min\mu}}{T_{\min\mu} + 273} \tag{2.6 c,d}$$

where temperatures are expressed in degrees Kelvin, to yield the simple form:

$$n(\tau) = \frac{1}{1 + \exp\left[-\theta^{2}(\tau - \tau_i)\right]} \tag{2.7}$$

We now introduce the following auxiliary variable:

$$x = \frac{1-n}{n} \tag{2.8}$$

to yield

$$x(\tau) = \exp\left[-\theta^{2}(\tau - \tau_i)\right] \tag{2.9}$$

Eq. 2.1 is valid for constant temperature. When temperature fluctuates with time, Eq. 2.1 will still be valid for short time periods, across which a constant (average) temperature is considered. This will correspond to $\Delta\tau < \tau_i$. Under this assumption, incremental expressions can be derived as follows:

$$\ln x = -\theta^{2}(\tau - \tau_i) \tag{2.10}$$

$$\tau = -\frac{1}{\theta^{2}} \ln x + \tau_i \tag{2.11}$$

Time step $\Delta\tau$ is equal

$$\Delta\tau = \tau_{j+1} - \tau_j \tag{2.12}$$

thus

$$\Delta\tau = -\frac{1}{\theta^{2}} \ln x_{j+1} + \tau_i + \frac{1}{\theta^{2}} \ln x_j - \tau_i \tag{2.13}$$

$$\Delta\tau = -\frac{1}{\theta^{2}} \ln \frac{x_{j+1}}{x_j} \tag{2.14}$$

At last, relationship between dimensionless cells number in next time steps equals

$$-\theta^{2}\Delta\tau = \ln \frac{x_{j+1}}{x_j} \tag{2.15}$$

$$\frac{x_{j+1}}{x_j} = \exp(-\theta^2 \Delta\tau) \tag{2.16}$$

$$x_{j+1} = x_j \exp(-\theta^2 \Delta\tau) \tag{2.17}$$

If temperature increase/decrease, then value of dimensionless temperature respectively change. Thus, final form of equation depends on current value of θ, and:

$$x_{j+1} = x_j \exp(-\theta_{j+1}^2 \Delta\tau) \tag{2.18}$$

or, equivalently:

$$n_{j+1} = \frac{n_j}{n_j + (1 - n_j)\exp\left(-\theta_{j+1}^2 \Delta\tau\right)} \tag{2.19}$$

RESULTS

Main scope of protection and storage of fish is the prolongation of the lag phase as well as maintaining conditions that ensure limited microbial growth. In order to compare changes of growth rate with time, different temperature profiles were studied. Results from numerical computations, in the form of plots, are shown. The following system of symbols was used on figures: lines (dashed, dotted or solid) with symbols (asterisk, cross and plus) represent growth rates, whilst corresponding temperature profiles are reported as lines (dashed, dotted or solid) with no symbols. On the plots two scales on the Y-axis were used. On the left side of each plot dimensionless cells number is shown, while on the right side the scale of dimensionless temperature is given.

As first effect of "unfreeze" was tested, i.e. only one rapid jump from low to high temperature takes place. As seen in fig. 1, this type of change distinctly reduces time when bacterial concentration is low. Moreover, the time delay between the sudden temperature increase and the visible cell growth gets shorter as we wait longer before we raise the temperature. This is a consequence of the fact that, even at low temperature, cell concentration grows such as to start from a larger concentration when

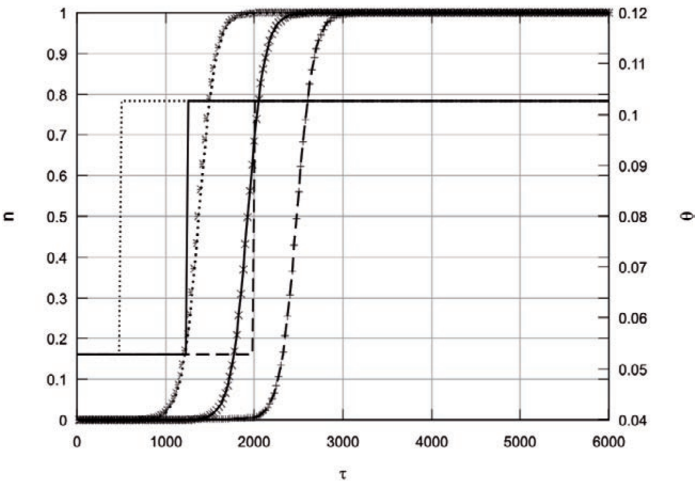

Fig. 1. Rapid increase of temperature shift in time. Initial concentration $n_1 = 2.65 \cdot 10^{-5}$ (temperature profiles: 1 ·······, 2 ━━━, 3 ▬ ▬, growth curves: 1 ✱··✱ , 2 ✱—✱, 3 +—+).

temperature is increased at later times. However, the shape of growth curves does not change, because all significant increase of cell concentration takes place at the high temperature level. As next we considered a temperature history obtained by alternating low and high temperature (fig. 2), whereas the initial value of cell concentration was the same, like in the case described before. It could be observed that, depending on temperature, the slope of growth rate line varied. Moreover, three different subdivision ratios in period were studied. According to expectations, when the system is maintained at high temperature longer that at low temperature, then the growth of cell concentration is faster (fig. 2).

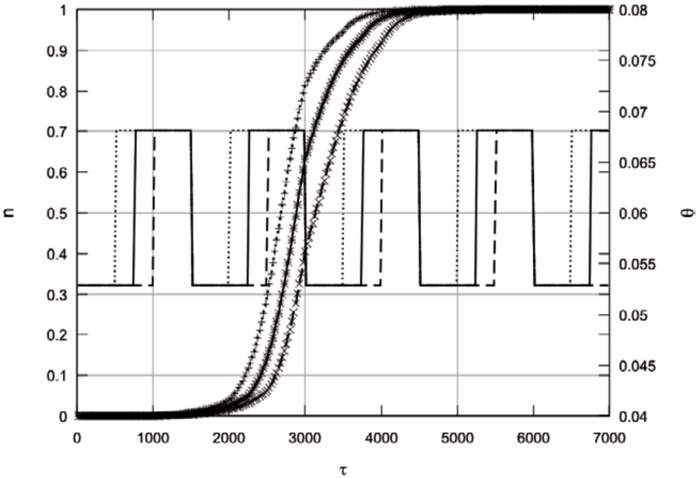

Fig. 2. Jumps between lower and higher temperature. Initial concentration n_1= 2.65*10^{-5} (temperature profiles: 1 ········, 2 ——, 3 ‒ ‒, growth curves: 1 ✱··✱· , 2 ✱—✱, 3 +—+).

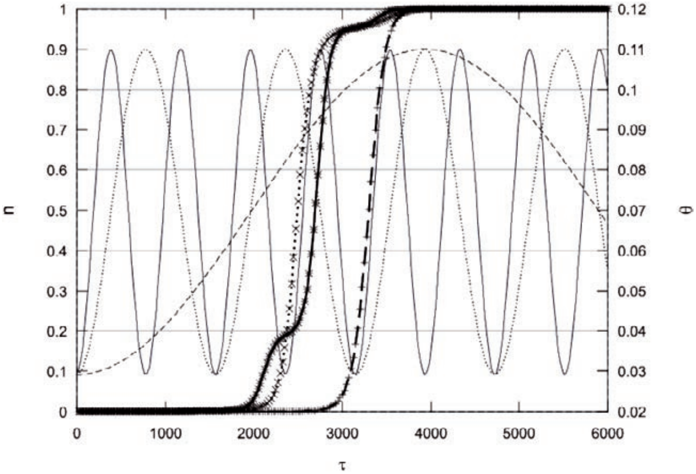

Fig. 3. Sinusoidal fluctuations of temperature as a function of period. n_1=3.5*10^{-7} (temperature profiles: 1 ········, 2 ——, 3 ‒ ‒, growth curves: 1 ✱··✱· , 2 ✱—✱, 3 +—+).

Also sinusoidal temperature history were considered. In fig. 3 it can be observed how length of period impact on bacterial growth. As seen, series with short periods and rapid fluctuations of temperature cause that increment of bacteria is faster than for series with long period. Further study contains comparison of three constant and three time dependent temperature profiles. Computed dimensionless microbial number is reported as a function of dimensionless time in the different cases, and logarithmic curves reconstructed from the dimensionless variables are reported for reference, as they constitute the usual way of reporting bacterial growth in the literature. In all cases an initial value of microbial number n_1= 3.23*10^{-07} is considered. Fig. 4 presents results obtained for three different constant temperatures, whereas Fig. 5 shows two sinusoidal and one sharp profiles of temperature with relevant growth curves.

The figures reports the dimensional values (in Celsius) of the tempera-

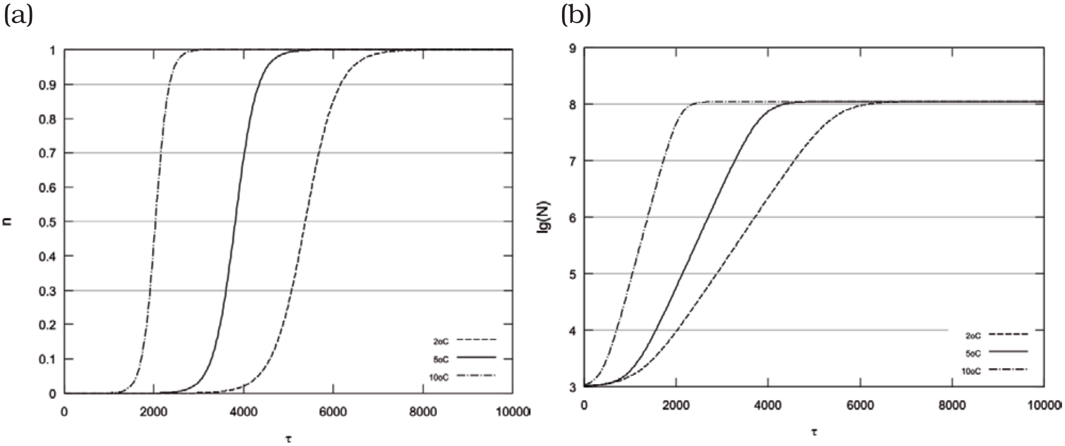

Fig. 4. Dimensionless microbial number vs. dimensionless time (a) and reconstruction of corresponding to them logarithmic curves (b).

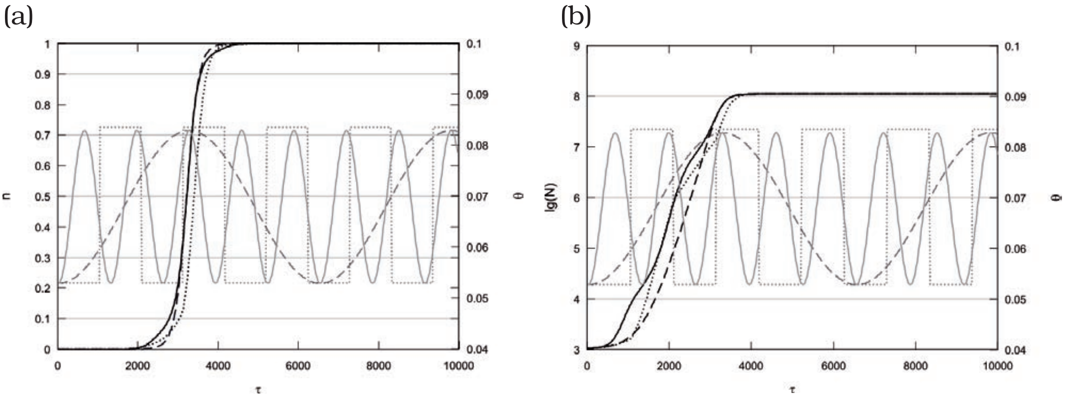

Fig. 5. Dimensionless microbial number vs. dimensionless time (a) and reconstruction of corresponding to them logarithmic curves (b). (temperature profiles: 1⋯⋯, 2——, 3— —, growth curves: 1⋯⋯, 2——, 3— —).

ture that would correspond to a case in which the other kinetic parameters are chosen to be, respectively, b_{μ}=0.0193 and $T_{\mu min}$= –11.80°C, while initial and final microbial amount equal N_{min}=$10^{3.01}$ and N_{max}=$10^{8.05}$ CFU/gram. The margin of safety was contained in the n-range between $8.82*10^{-4}$ to $8.90*10^{-3}$, what corresponds with assumption range of microbial concentration from 10^5 to 10^6 CFU/gram, respectively. Additionally, in this case 500 units of dimensionless time are equivalent of 24 h. For such chosen values, results of our model favourably compare with data found in the literature [12]. Fig. 6 a margin of safety for dimensionless model is shown. As seen, the range of lower branch of growth-curves corresponds to chosen margin of safety, what facilitates to isolate the period of time when fish-product maintains commercial properties.

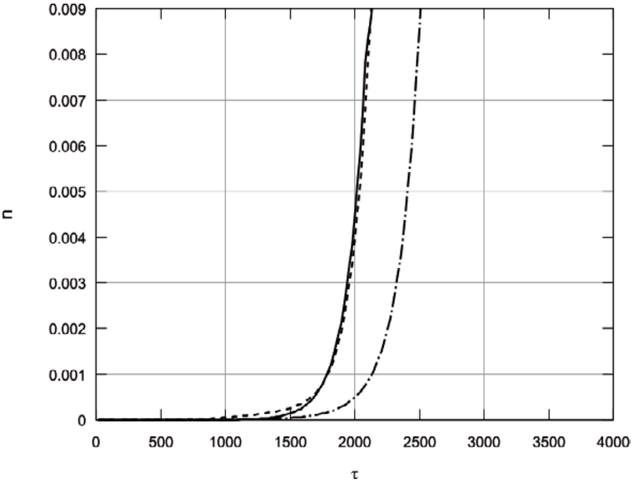

Fig. 6. Margin go safety corresponds with temperatures profiles studied above (Fig. 5).

CONCLUSIONS

An innovative dimensionless model of bacterial growth rate was presented (Eq. 2.19). Correctness of this model was confirmed by numerical simulations. It was shown that the model reproduces a distinct sensitivity on temperature fluctuations. Moreover, the dimensionless model is constructed in such a way as to be completely independent of time time steps taken in simulations can be arbitrarily small. This is an evident advantage of the proposed model in comparison with "traditional" ones, because it makes it possible to simulate continuous changes of temperature. This model enables also easier determination of model parameters from experiments and is flexible, in the meaning that different kinetic equations might be used for computations. For example, Belehradek-type equation (2.2) used for computations could be simply replaced by any other in order to make comparison of different types of kinetics or to identify the best kinetic equation for a given set of data. Additionally, it was shown that 'real' run of the growth process, i.e. described by dimensional units, is easy to extract from the dimensionless model.

Index of symbols

b_μ–coefficient related to rate of growth; N–bacterial cell concentration (CFU/g); n–dimensionless bacterial cell concentration; T–temperature,°C; $T_{\min\mu}$–minimum temperature for growth estimated by extrapolation, °C; t–time hr; t_i–time required for a concentration of half of the maximum concentration be attained,hr; $\mu_{\max}$–maximum specific growth rate, 1/hr; θ–dimensionless temperature; τ–dimensionless time; τ_i–dimensionless time required for a concentration of half of the maximum concentration be attained.

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LECTURE

PREDICTION OF STABILITY IN EXTRA VIRGIN OLIVE OIL

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ABSTRACT

In a previous investigation a phenomenological model, based on combined stability/instability indices, was set up to predict stability of extra virgin olive oil (EVOO). According to the model proposed, degradation of EVOO can be monitored by measuring parameters (degradation parameters) which have to be sensitive to oil degradation. From this phenomenological model the following predictive hypothesis was set up to develop a mathematical model for virgin olive oil degradation prediction: the same combination of stability/instability index values corresponded to the same degradation rate, or rather different stability/instability combinations corresponded to different degradation rates.

The aim of this study was to confirm the validity of this phenomenological model, to study in depth and to confirm the predictive hypothesis described. The degradation level of a sample series, optimized by a central composite design, was analyzed. Statistical data processing, carried out by partial least squares (PLS), allowed us to confirm the validity of phenomenological model and to improve it. Subsequently, a shelf-life test was carried out. Statistical data processing, carried out by principal component analysis (PCA), showed that degradation parameters significantly explained the degradation rate. In particular, K_{270} value and tyrosol and hydroxytyrosol content had a regular, non-asymptotical behaviour, and, as a result, a kinetic study for these parameters could be obtained. These results showed that the predictive hypothesis was likely to be true and suggested that some new shelf-life tests would be required to further investigate our predictive stability model.

Key words: extra virgin olive oil, stability indices, degradation parameters, predictive modelling

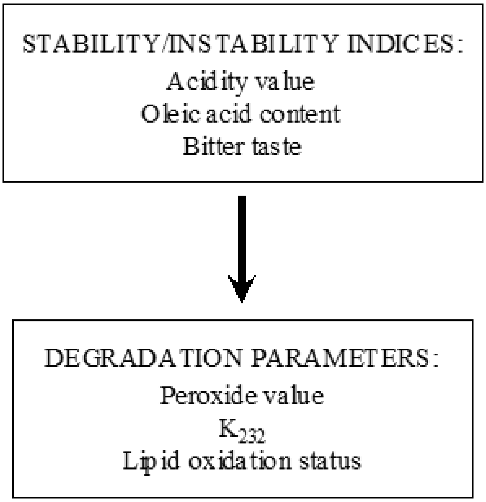

Fig. 1. Phenomenological model of EVOO stability.

INTRODUCTION

Degradation may occur during storage and shelf-life of extra virgin olive oil (EVOO) because of both autoxidation and photo-oxidation of triglycerides and degradation phenomena against phenolic compounds. Predicting degradation may be of interest for oil companies to allow them to improve oil selection and blending.

In previous investigations (Zanoni *et al.*, 2005; Siliani *et al.*, 2006) a phenomenological model, based on combined stability/instability indices, namely acidity, oleic acid content and bitter taste, has been set up to predict stability of EVOO (fig. 1). The above indices have been used to determine sensitivity to degradation during oil shelf-life.

According to the model proposed, degradation of EVOO can be monitored by measuring parameters, designated as degradation parameters, which have to be sensitive to oil degradation. Their value and kinetic variation depend on both stability/instability indices and operating conditions of oil packaging and commercial activities. Moreover, from the above studies linear relationships between oleic acid content and palmitic acid, linoleic acid and stearic acid contents of EVOO (Zanoni *et al.*, 2005) have been obtained; an exponential relationship between oleuropein aglycon and bitterness (Siliani *et al.*, 2006) of EVOO has also been obtained.

Based on the above phenomenological model a predictive hypothesis was set up to develop a mathematical model for EVOO degradation prediction: the same combination of stability/instability index values would corresponds to the same degradation rate, or rather different stability/instability combinations would corresponds to different degradation rates.

The aim of this study was to confirm the validity of the phenomenological model, to study in depth and to confirm the predictive hypothesis described.

MATERIALS AND METHODS

The degradation level of seventeen EVOO samples from 2003 olive oil season was evaluated to confirm the validity of the phenomenological model. The samples differed for their stability/instability index contents and their composition was planned by a Central Composite Design (CCD) in order to optimize the analytical effort (table 1). EVOO lots from Italy, Spain and Greece were blended to obtain samples having stability/instability indices responding to CCD and degradation parameters were then evaluated.

A shelf-life test was carried out to demonstrate our predictive hypothesis. Three EVOO lots (A, B, C) were obtained by blending virgin oil from Italy, Spain and

Table 1. Extra virgin olive oil samples composition planned by Central Composite Design.

Samples	Acidity (% oleic acid)	Oleic acid (%)	Bitter intensity
1	0.4	67.0	1.0
2	0.8	67.0	1.0
3	0.4	78.0	1.0
4	0.8	78.0	1.0
5	0.4	67.0	3.0
6	0.8	67.0	3.0
7	0.4	78.0	3.0
8	0.8	78.0	3.0
9	0.3	72.5	2.0
10	0.9	72.5	2.0
11	0.6	63.3	2.0
12	0.6	81.8	2.0
13	0.6	72.5	0.3
14	0.6	72.5	3.7
15	0.6	72.5	2.0
16	0.6	72.5	2.0
17	0.6	72.5	2.0

Table 2. Composition of extra virgin olive oil lots for the shelf-life test.

Samples	Acidity	Oleic acid	Bitter intensity
B	0.48	78.46	3.0
A	0.49	77.89	2.5
C	0.52	78.39	3.0

Greece. They had a similar stability/instability index value (table 2), but a different degradation parameter value. Samples were kept in transparent glass bottles exposure under light at room temperature in order to simulate usual shelf-life conditions. On each lot, samples were taken after 7, 14, 21, 28, 42, 70, 77 and 84 days of storage, and degradation parameters were measured.

Measurement of EVOO stability/instability indices

Acidity (% oleic acid), fatty acid content (%) and bitter taste intensity were measured by EU official methods (Anonymous 2002). Sensory evaluation of bitterness was performed by five judges, who were familiar with oil sensory quality. Judges were requested to evaluate bitter taste intensity by assigning a score between 0 (absence of attribute) and 5 (extreme intensity of attribute).

Measurement of EVOO degradation parameters

Peroxide value (meqO_2kg^{-1}) and spectroscopic parameters, i.e. K_{232} and K_{270}, were measured by EU official methods (Anonymous 2002).

Secoiridoid and lignan concentrations were determined by HPLC following the method described by Cortesi *et al.* (2002). Phenolic and lignan compounds were identified and quantified (ppm) as a tyrosol equivalent. The total phenolic compound content (ppm) was determined by the combination of individual, either identified or non identified, chromatographic peaks.

Lipid oxidation status was evaluated by HPLC according to Rovellini *et al.* (1999).

Data processing

The central composite design was carried out by Modde 7.0 software package (Umetrics, Umea, Sweden). Principal component analysis (PCA) and partial least square (PLS) regressions were used to classify samples by Unscrambler 8.0 software package (Camo As, Oslo, Norway).

RESULTS AND DISCUSSION

The degradation level of the sample series was analyzed and the combination of their stability/instability indices was optimized by a Central Composite Design. Statistical data processing, carried out by PLS, allowed us to confirm the validity of our phenomenological model and to improve it (fig. 2). In particular, in addition to previous degradation parameters, K_{270} tyrosol and hydroxytyrosol contents were also shown to be significantly correlated to stability/instability indices. Significance of these new parameters was in agreement with the work by Mattei *et al.* (2005), where K_{270} and tyrosol and hydroxytyrosol contents have been shown to result from oxidation of triglycerides and degradation of phenolic compounds, respectively.

Subsequently, in order to study in depth and to confirm the predictive hypothesis reported, a shelf-life test was carried out. Statistical data processing, carried out by PCA, showed that degradation parameters significantly explained the degradation rate. In particular, K_{270} and tyrosol and hydroxytiyrosol contents had a regular, non-asymptotical behaviour, and, as a result, a kinetic study for these parameters could be obtained. Their behaviour can be described under the storage conditions applied by pseudo-zero order kinetics. In addition, from a comparison between the samples, it can be noted that every parameter increased as a function of time at a similar rate for all three lots. Figure 3 shows the increase rate of hydroxytyrosol in the three lots, which can be described by the following kinetics:

$$\text{OH-Tir}_A = 0{,}10t + 32{,}80 \qquad (r = 0{,}95)$$
$$\text{OH-Tir}_B = 0{,}10t + 23{,}81 \qquad (r = 0{,}95)$$
$$\text{OH-Tir}_C = 0{,}08t + 22{,}45 \qquad (r = 0{,}96)$$

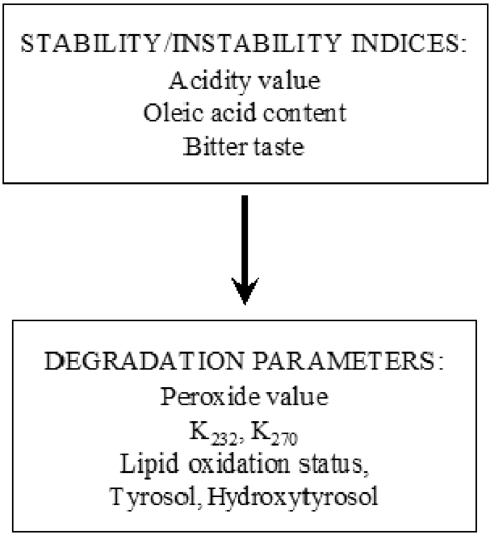

Fig. 2. Validated and improved phenomenological model of EVOO stability.

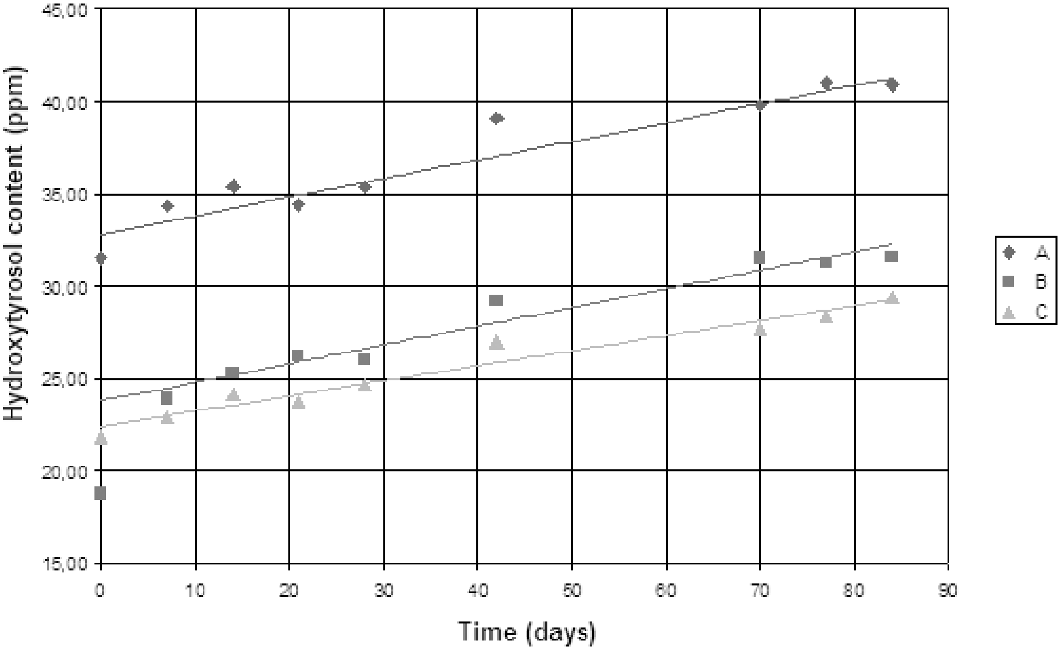

Fig. 3. Kinetics of hydroxytyrosol behaviour in the oil lots.

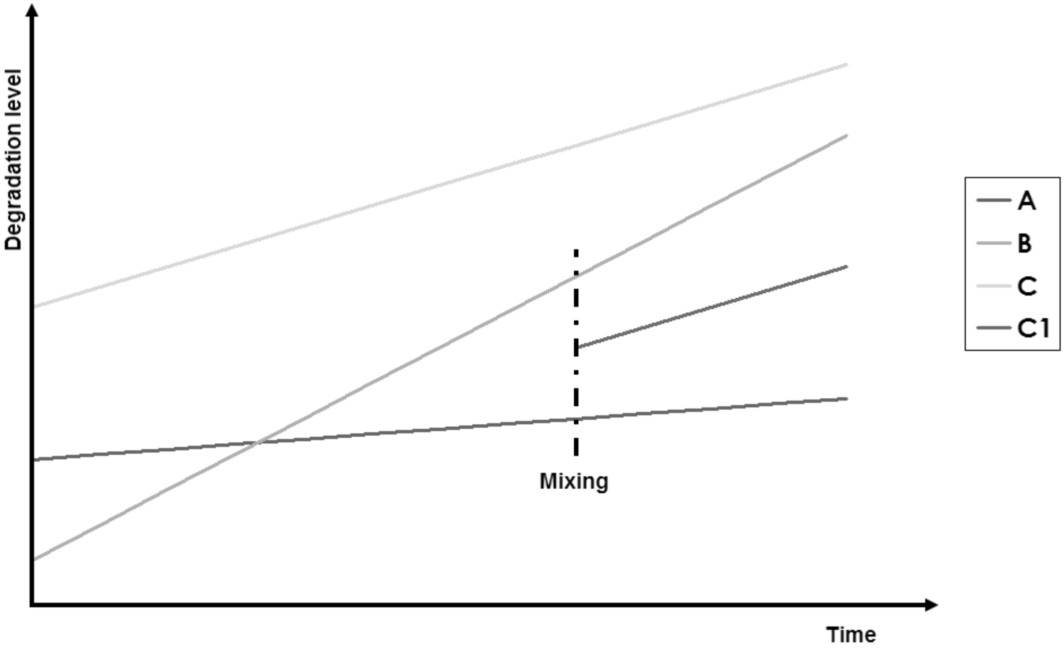

Fig. 4. Shelf-life test development.

The kinetics have a similar slope, so it can be noted that, under the same conditions of acidity, oleic acid content and bitter taste, the hydroxytyrosol content increased at a similar rate for all three lots. Analogous results were also obtained for K_{270} and tyrosol content.

These results showed that the predictive hypothesis was likely to be true, but they suggested that some new shelf-life tests were required to further investigate our predictive stability model. In particular, shelf-life tests are being carried out, which were developed as shown in figure 4. They were, for instance, designed to show that, assuming a sample A having a higher initial degradation level than sample B, sample A should have a lower degradation rate than B. Another aim would also be to show that sample C_1, which was obtained by suitably mixing A and B, should have a similar degradation rate to that of sample C, which has the same combination of stability/instability indices.

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INFLUENCE OF OXYGEN ON THE RESPIRATION RATE OF A FRESH-CUT PRODUCT: MATHEMATICAL MODEL AND MODIFIED ATMOSPHERE PACKAGE DESIGN

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ABSTRACT

The aim of this work was to study the influence of oxygen on the respiration rate of annurca apples in order to design a modified atmosphere package suitable for improving the product shelf life. The respiration rate of fresh-cut apples was determined in a modified closed system at 15°C using a gas mixture with 2, 5, 10, 15 and 21% of oxygen and nitrogen as balance. The respiration rate of fresh cut annurca apples increased with oxygen concentration and it was reasonably well described by Michaelis-Menten non competitive model. The maximum respiration rate estimated at 15°C was 8.5±0.5 and the model parameter K_m, which account for oxygen concentration corresponding to the half of the maximum respiration rate, was 1.08±0.04 (R^2=0.99). To validate the model, the product was packed with a permeable film. The mass/volume ratio in was selected in order to obtain at equilibrium a gas composition of 5% O_2 and 15% CO_2. Then, a mathematical model based on mass balance was solved to predict the O_2 and CO_2 variation on time in the package head space. Results confirm the capability of the model used to assist in designing the modified atmosphere package for fresh-cut annurca apples.

Key words: fresh-cut annurca apple, modified atmosphere package, modelling, respiration rate

INTRODUCTION

Modified atmosphere packaging (MAP) can potentially reduce the problems associated with processed fresh product, allowing to commercial suitable shelf-life. Nevertheless, if MAP is improperly designed, it may be detrimental for the shelf-life of fresh-cut products (Torrieri, 2002). MAP technology for fresh product is based on the modification of the atmosphere inside the package which results as the consequence of two kinetic processes: the transfer of gases through the packaging materials and the respiration of the product (Emond *et al.,* 1991). The respiration rate of fruit and vegetables is affected by many intrinsic and extrinsic factors (Andrich *et al.,* 1998; Gorny *et al.,* 2000), hence, to properly design MAP, suitable predictive mathematical models are required to be able to take into account respiration rate as a function of varying temperature and gas conditions (Fonseca *et al.,* 2002b; Hertog & Banks, 2003). Mathematical models of respiration rate of fresh fruits and vegetables have been recently reviewed by Fonseca *et al.* (2002a). In agreement to them, the Michaelis-Menten non competitive model may be usefully used to quantify the effect oxygen on the respiration rate of fresh-cut annurca apples. Due to the large variation of data from one vegetable produce to another, it is not possible to extrapolate the behaviour of annurca apples from available data in literature. Thus, the objectives of this study was (i) analyse the influence of O_2 concentration on respiration rate of fresh cut annurca apples; (ii) develop a predictive model, relating respiration rate to O_2, that may be used in the design of MAP for this product (iii) validate the model in real condition.

MATERIALS AND METHODS

Mela annurca apples were collected in S.Agata dei Goti (BN) and sent to the traditional redness process. Then, the apples were processed (cleaned, peeled, cored, and sliced by means of a sharp knife) and their respiration rate measured in a modified closed system (Torrieri, 2003). The apple slices were inserted in the system and hermetically closed. Next, through two valves, the gas was flushed into the system at constant flow rate (100 ml/min) for a time necessary to let the samples equilibrate. After this time, the valves were closed and started the gas sampling procedure to monitor the O_2 and CO_2 change with time in the jar headspace. 3 ml of gas mixture was drawn from the jars at regular time intervals (Δt) and analysed using a gas analyzer for O_2 and CO_2 % (PBI Dansensor, Danmark). The respiration rate was analysed at 15°C by using gas mixtures containing 2, 5, 10, 15 and 21% of oxygen with the balance N_2. The rate at which O_2 was consumed (RR_{O_2}) and CO_2 was produced (RR_{CO_2}) in the course of the experiment was estimated as:

$$RR_{O_2} = -\frac{Vf}{W\,100} \cdot \frac{dO_2}{dt} \qquad 1$$

$$RR_{CO_2} = -\frac{Vf}{W\,100} \cdot \frac{dCO_2}{dt} \qquad 2$$

Where W is the mass of the sample (kg) and V_f (ml) is the jar headspace given by:

$$Vf = V - \frac{W}{\rho} \qquad 3$$

Where V is the volume (ml) of the jar at each sampling time and ρ the apparent density of the annurca apples (8.1×10^{-4} kg ml^{-1}). The respiration quotient, RQ, was calculated as:

$$RQ = \frac{RR_{O2}}{RR_{O2}} \qquad 4$$

The dependence of respiration rate (RR_{O_2}) on oxygen concentrations was modelled by a Michaelis-Menten model:

$$RR_{O2} = \frac{RR_{max}\ Y_{O2}}{K_m\ Y_{O2}} \qquad 5$$

Where Y_{O_2} is the O_2% (v/v) in the initial gas mixture, RR_{max} is the maximal respiration rate (ml/kgh) and K_m is the O_2% corresponding to $\frac{RR_{max}}{2}$. The model constants were estimated by fitting the model to the experimental data by non-linear regression using the Table curve 3D software (Jandel Scientific). The RR_{CO_2} was calculated as:

$$RR_{CO_2} = RQ\ RR_{O_2} \qquad 6$$

The evolution of the oxygen and carbon dioxide inside the headspace of the package was simulated by using the simula software, using as input parameter (i) the constant of the Michaelis-Menten model and the RQ to calculate the respiration rate, (ii) the permeability of the film, (iii) the volume of the package, and (iv) the mass and (v) density of the sample. To validate the model, the fresh-cut annurca apples were packed in a polistirene tray and sealed with a polymeric film (OPP). The mass/volume ratio in was selected in order to obtain at equilibrium a gas composition of 5% O_2 and 15% CO_2.

RESULTS AND CONCLUSIONS

Figure 1 shows O_2 consumption rate (RR_{O_2}) of fresh slices of Annurca apples stored at 15°C as a function of the oxygen percentage concentration (%). The respiration rate increase with 16% by increasing the O_2 concentration from 2% to 21%. The dots represent the investigated respiration rate and the solid lines represent the respiration rate as predicted by Michaelis-Menten model (eq.5). The agreement is very satisfactory ($R^2_{adj}<0.99$). The values of the RR_{max} and K_m were 8.5±0.5 ml/kgh and 1.08 ±0.04%, respectively. The value of K_m for fresh cut slices of Annurca apple is comparable to that of other cut fruits and vegetables but it is relatively

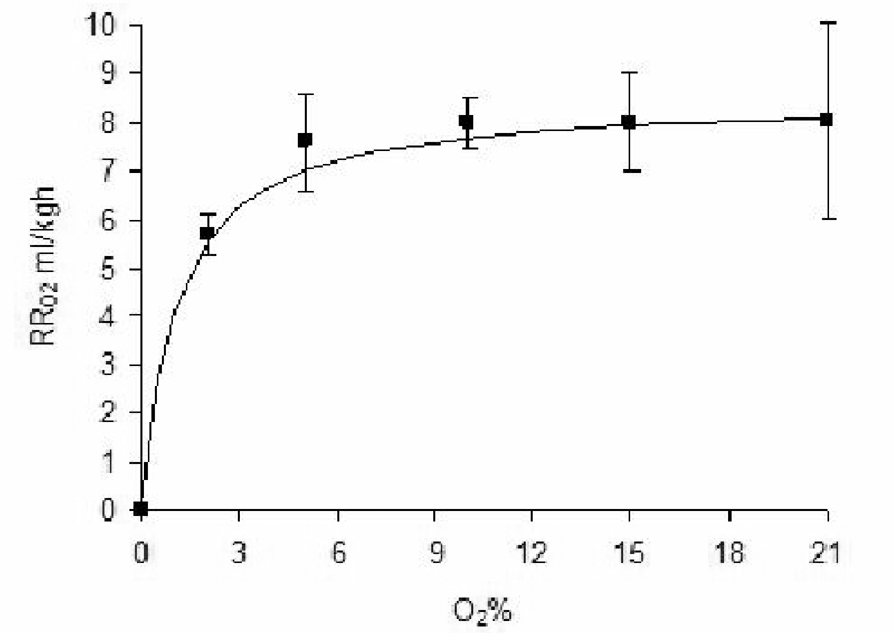

Fig. 1. O_2 consumption rate (RR_{O_2}) of fresh cut annurca apples stored at 15°C vs. O_2 concentrations (■). The line represent the respiration rate as predicted by the Michealis and Menten model.

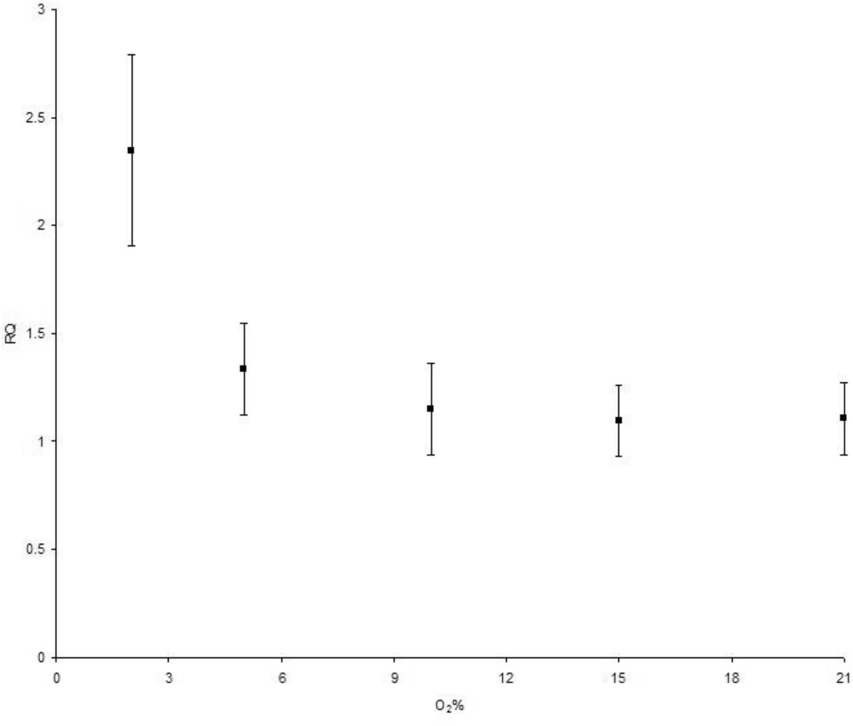

Fig. 2. Effect of O_2 concentration on the respiratory quotient (RQ) of fresh cut annurca apples.

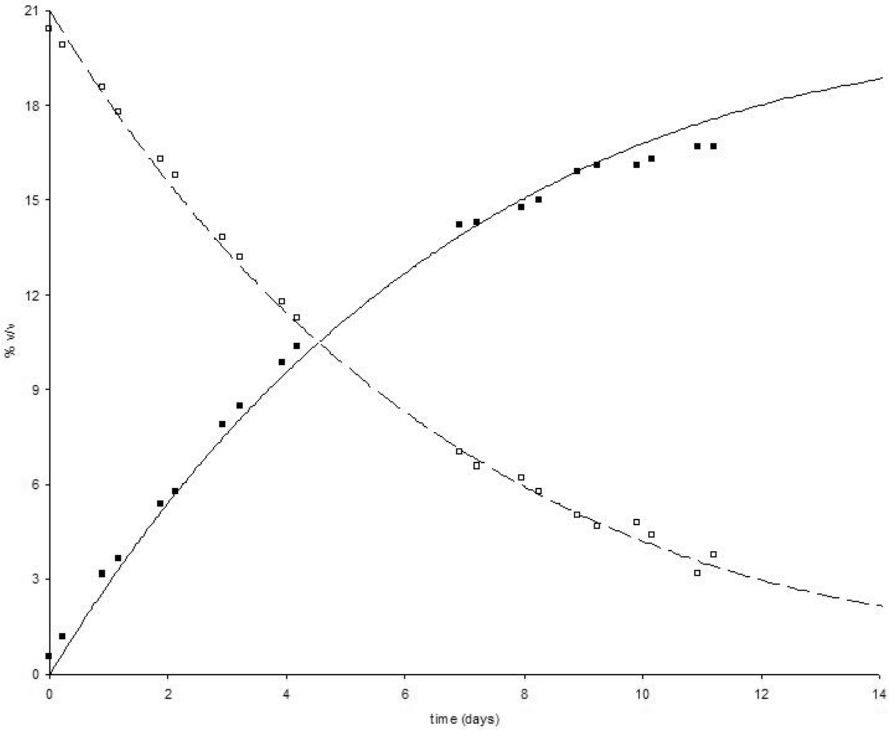

Fig. 3. Evolution of oxygen (■) and carbon dioxide (□) on time in a package with annurca apple sliced. Dots represent experimental data, the line represent the model prediction.

low as compared to K_m value estimated for whole apples: values of K_m for shredded coleslaw, cut carrots, and cut broccoli, reported in literature, are 1.083, 1.15 and 1.4%, respectively, whereas the one for whole apples is 6.96% (Haggar, Lee, & Yam, 1992). Concerning the respiratory quotient (Figure 2), it varies from 0.8 to 1.4 in the range of 5-21% of oxygen. Anaerobic respiration at 2% of oxygen was observed (RQ>1.3), that can be assumed to be the RQ breakpoint for fermentative process of annurca apples. On the basis of this results, the gas composition inside the package was predicted. In figure 3 is shown the oxygen and carbon dioxide evolution inside the package with 200g of apples stored a 15°C. The dots represent the experimental data and the line the simulation solved by using the estimated model parameter. The agreement between the predicted data and the experimental data is very good.

In conclusion the results of this work pointed out that a Michaelis-Menten non competitive model well describe the influence of oxygen on the respiration rate of fresh cut annurca apples. The estimated parameter at 15°C were 8.5±0.5 ml/kgh and 1.08 ±0.04% respectively.

ACKNOWLEDGMENTS

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DEGRADATION OF β-CAROTENE IN AN AQUEOUS MEDIUM EXPOSED TO FLUORESCENT AND HALOGEN LIGHTS UNDER DIFFERENT OXYGEN PARTIAL PRESSURES

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ABSTRACT

During distribution and at retail level, transparent packages are exposed to natural and artificial light which may impair food quality and marketability, due to the photosensitivity of the product. The aim of this work was to study the influence of different light sources and various oxygen partial pressures on the degradation rate of β-carotene in aqueous medium. Firstly, a survey of the light sources used in commercial display areas was performed in order to investigate the most common conditions of illumination. As a function of the obtained results, three types of light (a cool white, a warm white and a halogen lamp) were chosen for the experimental phase. An aqueous solution of β-carotene was closed in clear glass vials and stored under various conditions of lighting and oxygen partial pressure. The pigment photodegradation was monitored in terms of β-carotene concentration and colour evolution. The pigment degradation was correlated to illuminance and UVA irradiance values of the selected lamps. In particular, the fluorescent lamps caused a degradation higher than the halogen lamp because of the emission on the blue region of the spectrum, where β-carotene absorbs. Lighting was a critical variable also in the presence of a low oxygen partial pressure (5 kPa) and even if more slowly than at 20 kPa of O_2, the β-carotene degradated completely during storage.

Key words: light effects, oxygen partial pressures, β-carotene, kinetic

INTRODUCTION

During distribution and retail, foods are displayed in stores where artificial light is used for illumination and this exposure may cause deterioration of lipids, vitamins, proteins and natural pigments (Bekbölet, 1990). The effects of light on the degradation of packaged foods have been widely investigated especially when photosensitizing nutrients (e.g. riboflavin, chlorophyll) and lipidic substrates are involved. Photodegradative reactions may also take place in an aqueous system that does not contain lipids but, in this case, not much information is available on the specific effects of light (Henry *et al.*, 2000). Moreover, a large number of studies are focused on the effects of monochromatic lights and only few of them evaluate exposure under real light sources. In this work, attention was focused on the degradation of β-carotene which is one of the most important carotenoids because it gives colour to foods and beverages and it has an important role both as nutrient and antioxidant. The aims of this work were (1) the selection of the most widespread artificial lights used by different commercial retails; (2) the study of the influence of light sources with different spectral emissions and the oxygen partial pressures on the reaction kinetics of β-carotene in an aqueous medium; (3) the comparison between the rates and the mechanisms of reaction in the dark and under illumination.

MATERIALS AND METHODS

In the first part of the work, a survey of the light sources was carried out in 9 different large-scale retail trades around Milan, noting the typology of the lamps used in different sections. For each light source the irradiance in the UVA region (Wm^{-2}) and illuminance (lux) were measured with a portable digital photoradiometer (Delta Ohm HD2102.2) at the level of different foods (vegetables, meat, dairy products, pasteurized beverages, fish, gastronomy products). In the second part of the study, aqueous samples containing β-carotene (Roha Caleb, UK) at a concentration of 18 mg ml^{-1} were prepared and buffered at pH 7.2. Samples were put into clear glass vials, inside of which two oxygen partial pressures were created: 20.9 kPa of oxygen was used to simulate the packaging in air, and 5 kPa to simulate the oxygen residual after modified atmosphere packaging. The vials were then hermetically closed and placed on turning tables inside dark and thermostatted cabinets. The samples were exposed to a constant illumination for 10 days at 33 ± 2°C. The same experiments were also carried out in the dark. For each selected light, illuminance and irradiance in the UVA region were recorded in the real conditions of storage of the samples. The degradation of β-carotene was determined by measuring absorbance at 479 nm in time with a spectrophotometer (Perkin Elmer Lambda 650). The colour changes were monitored in the transmittance mode with the same spectrophotometer and the CIE L*, a* and b* values were calculated by a software package (Color version 3.00 Perkin Elmer Instruments).

RESULTS AND DISCUSSION

The survey showed that 75% of the total commercial light sources was represented by fluorescent lamps and 18% by halogen lamps. The remaining 7% was represented by a combination of both. In particular, among fluorescent lamps it was possible to distinguish the light sources as a function of the colour temperature of the lamps. The cool white lights (4000 K) represented 40% of the total fluorescent lamps, the warm white lights (3000 K) totalled 25%, while 33% of the total was represented by the lamp called Nature (3300 K) which is characterized by an emission in the red region of the spectrum. On the basis of the data obtained by the survey in the retail shops, three commercial lights were selected: a fluorescent warm white (Osram Dulux T 26W/830), a fluorescent cool white (Osram Dulux El Longlife 30W/840) and a halogen light (Osram Decostar® 51S Standard 20W 36°). During the light exposure, the curves describing the changes in β-carotene concentration, lightness and redness parameters had a sigmoidal shape, so a modified Gompertz equation (1) was used to describe this behaviour and to obtain useful information to compare the effects both of light exposure and the oxygen partial pressures.

$$Y= \pm a * \exp(-\exp((b*e/a)*(c-x)+1)) \qquad (1)$$

where: a is the asymptotic level, b is the specific rate (h^{-1}) and c is the induction time (h).

The goodness of fit was assessed using the R^2 and the mean relative error. The correspondence between the predicted and the experimental data, both at 5 and 20 kPa of oxygen, suggested that the phenomena could be described in terms of the sigmoidal model. As an example, Figure 1 and 2 show the evolution of β-carotene and the changes in lightness values obtained with cool white light. For the same oxygen partial pressure, a ratio between the induction time estimated under illumination and that estimated in the dark (L_L/L_D) was calculated. The same calculus was performed on the rate values (R_L/R_D) (Table 1). At 20 kPa of oxygen, the induction time was higher in the dark than under illumination (the ratios were lower than 1) while the light exposure accelerated the decay reaction rates (the ratios were higher than 1). On the contrary, at 5 kPa of O_2, the reaction rates in the dark

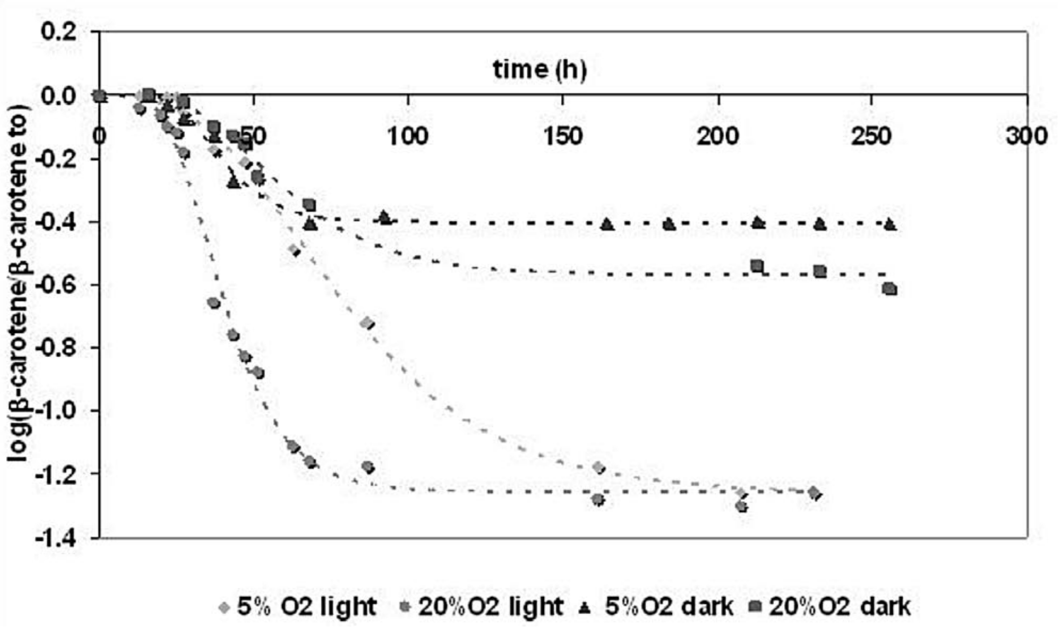

Fig. 1. Evolution of β-carotene concentration in different conditions of lighting and oxygen partial pressure.

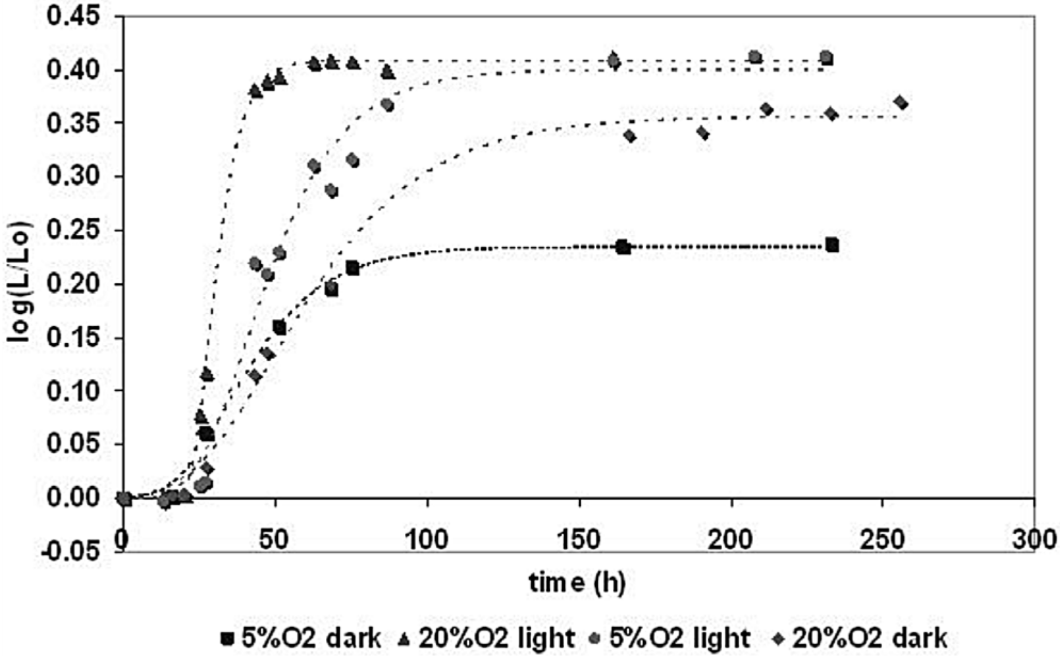

Fig. 2. Evolution of lightness (L*) in different conditions of lighting and oxygen partial pressure.

and under light were quite similar (the ratios were close to 1). If only the rate of reaction is considered, it seem that at the lowest oxygen partial pressure the light exposition made no difference. But, considering the second derivative of the curves, it was possible to check when the reaction tended to the asymptotic level. It was evident that at 5 kPa of oxygen the reaction decelerated earlier in the dark than under light, but in the dark the reaction did not get exhausted completely (Figure 1 and 2). Moreover, the rate of decrease of β-carotene concentration seemed to be correlated with the rate of increase of the light, as the similar accelerate factors demonstrated (both at 5 and 20 kPa). The comparison among different lamps in

Table 1. Comparison among the estimated rates of β-carotene concentration decay and lightness changes in different conditions of lighting and oxygen partial pressure.

Lamp		β-carotene		Lightness (L*)	
		20 kPa O_2	5 kPa O_2	20 kPa O_2	5 kPa O_2
Cool white	Lag (h)	LL/LD = 0.8	LL/LD = 1.3	LL/LD = 0.9	LL/LD = 1.4
	Rate (h-1)	RL/RD = 3.9	RL/RD = 1.0	RL/RD = 4.0	RL/RD = 1.1
Warm white	Lag (h)	LL/LD = 0.3	LL/LD = 0.6	LL/LD = 0.1	LL/LD = 0.2
	Rate (h-1)	RL/RD = 2.9	RL/RD = 0.9	RL/RD = 2.8	RL/RD = 0.9
Halogen	Lag (h)	LL/LD = 0.7	LL/LD = 0.5	LL/LD = 0.3	LL/LD = 0.2
	Rate (h-1)	RL/RD = 2.3	RL/RD = 0.9	RL/RD = 1.8	RL/RD = 0.8

Table 2. Comparison among the degradation rates in different conditions of lighting and oxygen partial pressure.

Rate (h^{-1})	Cool white	Warm white	Halogen
Illuminance (lux)	3300	2700	1800
UVA Irradiance (Wm^{-2})	0.065	0.052	0.017
β-carotene 20 kPa O_2	0.035 ^{a}	0.026 ^{b}	0.021 ^{c}
β-carotene 5 kPa O_2	0.014 ^{a}	0.012 ^{a}	0.006 ^{b}
L* 20 kPa O_2	0.020 ^{a}	0.014 ^{b}	0.009 ^{c}
L* 5 kPa O_2	0.008 ^{a}	0.005 ^{b}	0.004 ^{b}

Different superscripted letters indicate statistically different groups ($p<0.05$).

the β-carotene degradation in terms of estimated rates of β-carotene concentration decay and lightness changes was performed (Table 2). The highest degradation rate was found for the cool white lamp, while the lowest for the halogen lamp. These data were well correlated with the illuminance and UVA irradiance data measured during storage experiments (3300, 2700 and 1800 lux, and 0.0065, 0.0052 and 0.0017 Wm^{-2} respectively for the cool white, the warm white and the halogen lamps). In other words, the higher the illuminance and irradiance data, the higher the rate of degradation. Different reaction rates were also found between the two fluorescent lamps which were more similar in terms of UVA irradiance and illu-

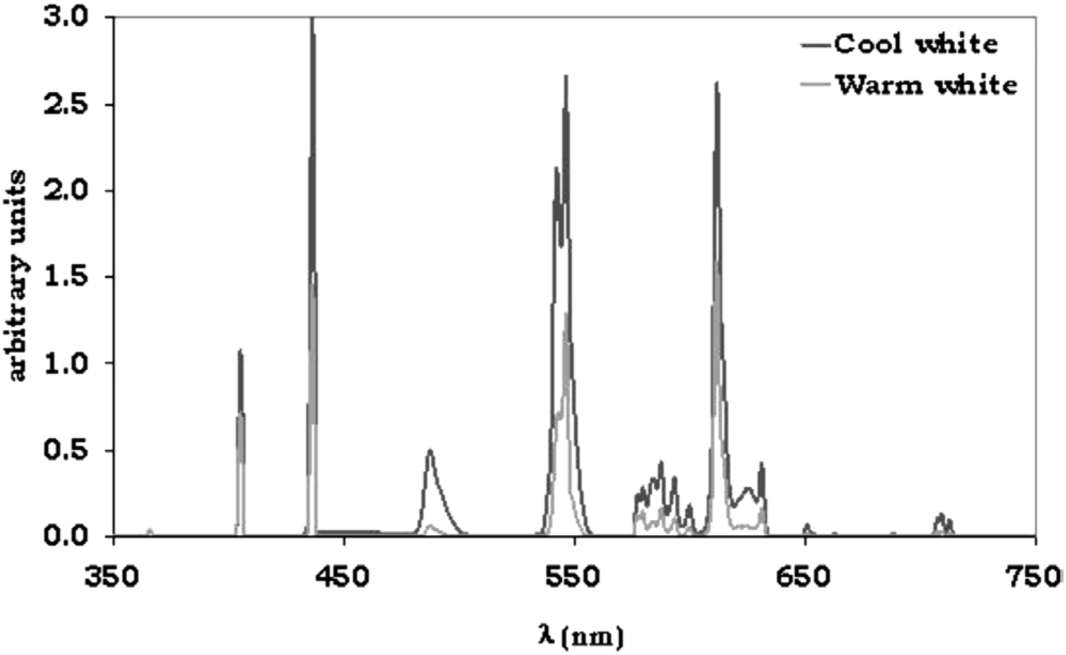

Fig. 3. Spectra of the two halogen lamps in the real conditions of the exposure.

minance. To better investigate these results, the relative spectral distributions of the energy were acquired with an optical probe connected to a detector in order to simulate the real illumination geometry of the cabinets. It was evident that the cool white lamp had a higher emission in the blue region of the spectrum where the β-carotene absorbs (Figure 3).

CONCLUSIONS

Lighting proved to be a critical variable in the presence of a low oxygen partial pressure (5 kPa) and, even if more slowly than at 20 kPa of O_2, the β-carotene degradated completely during storage. The pigment degradation was correlated to the illuminance and UVA irradiance values of the selected lamps. Moreover, the differences in the blue region of the emission spectra contributed to explaining the different decay rates of the fluorescent lamps. Halogen lamps gave the minor negative effects on β-carotene degradation: its use should be recommended for the lighting of foods rich in carotenoids. In conclusion, packaging materials performances and modified atmosphere technique should be optimised in order to reduce the oxygen content in foods. Furthermore, the light source has to be chosen with care and the packaging material should reduce not only the UV radiation but also some visible wavelengths.

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DEVELOPMENT OF A SOFTWARE TO PREDICT THE SHELF LIFE OF FRESH FISH ACCORDING TO SENSORY AND MICROBIAL PARAMETERS

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ABSTRACT

The objective of this work was to develop a user-friendly software able to predict the shelf life of farmed fresh fish according to the microbial growth but also to the sensory evaluation. Besides, the efficiency of various Time Temperature Integrator (TTI) devices to predict shelf life has been evaluated.

Farmed turbot *(Psetta maxima)* samples were packaged in PVC film and stored at 0, 5, 10 and 15°C. Microbial growth and sensory attributes were monitored at regular time intervals. The model of Baranyi and Roberts was fitted to the bacterial growth curves. Furthermore, the response of three types of ttis Monitor Mark®, Fresh Check® and Check Point®) were measured at the same temperatures. The rate of change of the colour parameters was estimated by fitting empirical models to each set of TTI colour measurements in time.

Sensory scores were analysed by principal components analysis. The models developed were implemented in a user-friendly excel add-in ("Fish Shelf Life Prediction Program (FSLP)"). This software tool allows predicting sensory acceptability, growth of spoilage bacteria in fish products and the response of TTIs for the temperature profiles input by the user. Utilities regarding graphical options and tools to compare observations and predictions are also implemented.

Key words: modelling, seafood, shelf life prediction, software

INTRODUCTION

Fresh and lightly preserved seafood products are supposed to be distributed at a constant chilling temperature. However, during commercialisation, they are usually exposed to higher temperatures. The storage temperature is the main factor influencing the quality and safety of fish products.

Fish trade in general is paying increasing attention to aquaculture products as a source of fish and other seafood products (FAO, 2003; Josupeit *et al.* 2001). Such is the case of turbot *(Psetta maxima)* a highly valued flat fish species appreciated for its firm, white and flavourful flesh. Recently, increasing production of this species as an aquaculture product has raised its availability.

Development of models to predict growth, survival or inactivation of microorganisms in foods has been a most active area of food microbiology within the last 20 years. Numerous mathematical models have been generated within the field of predictive food microbiology but relatively few of these models are included in available application software. For food safety evaluation, Pathogen Modelling Programme (Buchanan, 1993) and Growth Predictor have been developed. Moreover, Seafood Spoilage Predictor (SSP) (Dalgaard *et al.* 2002) software has been developed to include both kinetic models for growth of specific spoilage microorganisms and empirical relative rates of spoilage models. However, this software does not consider sensory acceptability attributes. However, taking into account the consumers perception is important because in some cases, the consumers reject the product before bacterial growth have reached the level indicator of spoilage. The objective of the present work was to develop an user-friendly software able to predict the shelf life of farmed fresh fish according to the microbial growth but also to the sensory evaluation. Besides, the efficiency of various time temperature integrator (TTI) devices to predict fish shelf life was also studied.

MATERIALS AND METHODS

Data were generated in experiments with turbot samples *(Psetta maxima)* obtained directly from aquaculture, and packaged in extended PVC film. Microbial growth and sensorial characteristics were monitored in samples stored at controlled isothermal conditions 0, 5, 10 and 15°C in incubators (Ibercex V-450). Dynamic storage experiments were also conducted by storing the turbot samples, at controlled non-isothermal conditions. The response of three types of TTIs, Monitor Mark® (3M, USA), Fresh Check® (TempTime, USA) and Check Point® (Vitsab, Sweden), were measured at the same temperatures. TTIs were adequately activated just before introducing them in the incubator chambers, and responses of 10 TTIs of each type were measured for replicates at each time.

The model of Baranyi and Roberts (1994) was fitted to the bacterial growth curves. The rate of change of the colour parameters was estimated by fitting empirical models to each set of TTI colour measurements in time. Sensory scores were analysed by principal components analysis. The first principal component represented 97% of the total variability and was used as a global sensory indicator. The maximum specific bacterial growth rate, the rate of change of the colour parameters and the rate of change of the sensory indicator were modelled as a function of temperature by fitting the Arrhenius model.

Models were validated in dynamic temperature conditions comparing the predicted and the measured values for all microbial, sensory and TTI response parameters.

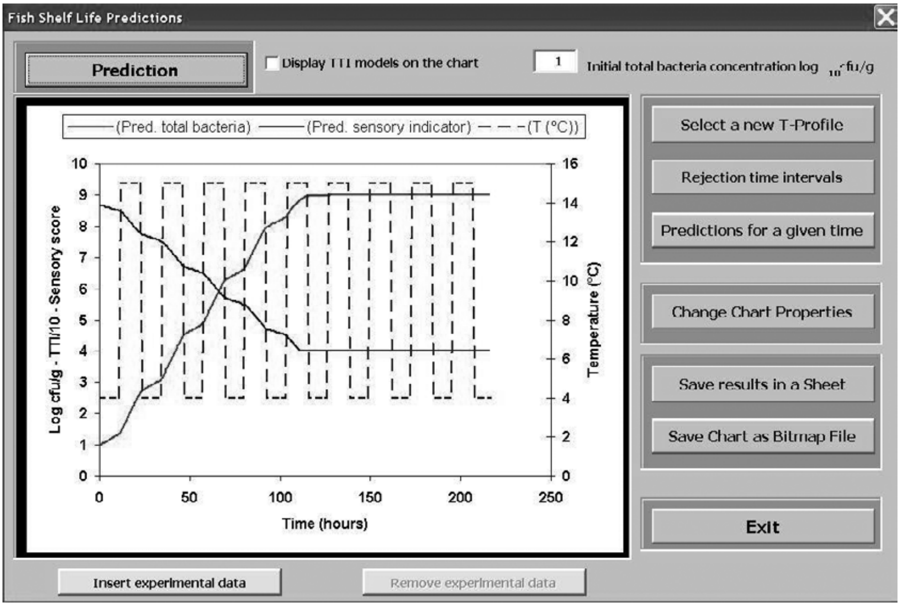
The Fish Shelf Life Prediction Program (FSLP) is an Excel Add-In implemented in Visual Basic for Applications.

RESULTS AND CONCLUSIONS

The software developped (Fish Shelf Life Prediction Program (FSLP)) allows the prediction and the visualization of sensory acceptability, growth of spoilage bacteria in fish products and the response of TTIs at constant and fluctuating temperature conditions. Moreover, the software allows the graphical comparison of experimental microbiological or sensory raw data with the respective model at either constant or fluctuating temperature. Optionally, the predictions and plots can be saved as an Excel workbook.

Fig.1 shows an example where Fish Shelf Life Prediction Program (FSLP) software was used to predict the growth of total bacteria and the response of a sensory panel for turbot samples packaged in PVC film. In this example, the first step was to enter a time (h) – temperature (°C) profile in an Excel sheet. In the case shown, the storage temperature fluctuated between 4 and 15°C. The initial level of total bacteria was 10^2 CFU g^{-1}. Fig. 2, shows the shelf life of the product taken into account microbiological limits and sensory acceptability after pressing the "rejection time interval" dialog box. The example indicated that according to the model for the sensory perception, the rejection limit is reached at 64 hours. The total bacteria load predicted at those time was 6,7 log 10 CFU g^{-1}. Moreover, the Fish Shelf Life Prediction Program (FSLP) indicated the rejection limit according to the model for the TTIs indicators (Fresh Check® and Monitor Mark®).

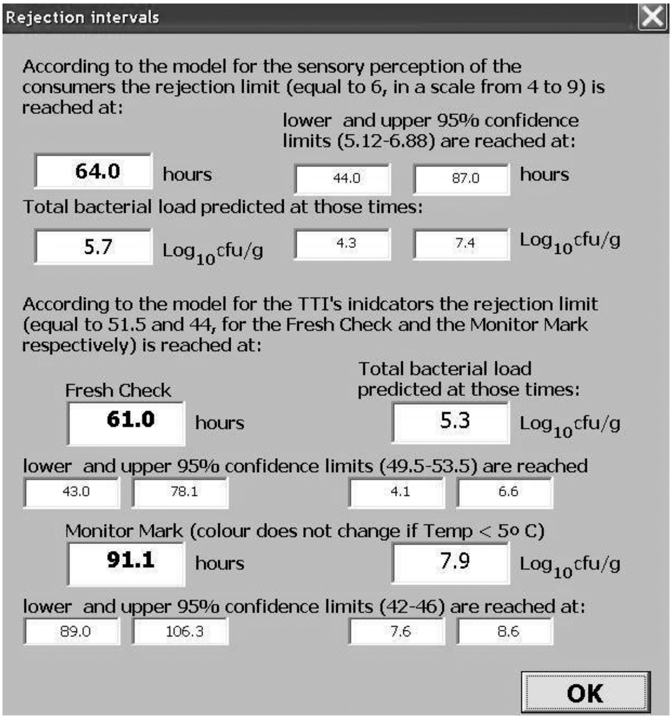
At present, these models and software are being validated for other kind of



☐ Display TTI models on the chart

Initial total bacteria concentration log $_{10}$ cfu/g: 1

Fig. 1. Output from "Fish Shelf Life Prediction Program (FSLP)" software showing the effect of a temperature profile on growth of bacteria total and the response of a sensory panel of turbot samples.



According to the model for the sensory perception of the consumers the rejection limit (equal to 6, in a scale from 4 to 9) is reached at:

64.0 hours

lower and upper 95% confidence limits (5.12-6.88) are reached at:

44.0 87.0 hours

Total bacterial load predicted at those times:

5.7 Log_{10}cfu/g

4.3 7.4 Log_{10}cfu/g

According to the model for the TTI's inidcators the rejection limit (equal to 51.5 and 44, for the Fresh Check and the Monitor Mark respectively) is reached at:

Fresh Check

61.0 hours

Total bacterial load predicted at those times:

5.3 Log_{10}cfu/g

lower and upper 95% confidence limits (49.5-53.5) are reached

43.0 78.1 4.1 6.6

Monitor Mark (colour does not change if Temp < 5º C)

91.1 hours

7.9 Log_{10}cfu/g

lower and upper 95% confidence limits (42-46) are reached at:

89.0 106.3 7.6 8.6

Fig. 2. Screenshot from "Fish Shelf Life Prediction Program (FSLP)" showing the microbiological, sensory and TTI rejection times for turbot samples stored at the entered fluctuating temperatures (between 4 and 15°C) according to the prediction models developed.

seafood products. The simulation can be valuable to a wide variety of companies in fish industry for quality and safety. The software would be also exploited for educational purposes.

In the future, the software developed in this work to predict shelf-life will be applied to the assessment and management of seafood safety and quality and may be combined with seafood traceability systems.

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A VIEW ON THE MODELLING OF THE LOSS OF SHELF-LIFE OF THE PRODUCTS PACKAGED IN MODIFIED ATMOSPHERE (MAP)

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ABSTRACT

The purpose of the author's own research was to propose a model which would allow to estimate the probability of the loss of shelf-life in MAP packaged products, as a function of separated forecasting factors including: input quality level, type of packaging and the input mix of gases in the packaging. For that purpose, the Cox proportional hazard model used in the analysis of survivals was applied. The examined material consisted of salted peanuts packaged in a mix of gases in bags of metalized laminate and in metal cans, with a changing original content of oxygen in the packaging.

The loss of shelf-life model thus constructed can be used to estimate the loss of durability of products packaged in modified atmosphere model, taking at the same time into account all the tested criteria.

Key words: shelf-life lost, modified atmosphere packaging, survival analysis

INTRODUCTION

In solving the issue of durability of the packaged products, an important source of data is the possibility of estimating the probability of durability loss taking into account different forecasting factors. For that reason, in order to analyse the process of the loss of shelf life of MAP packaged peanuts, an attempt was made to develop a model of durability loss in products, named with a symbol: SLL (Shelf Life Loss) (BLOOSFELD *et al.*, 2002; PORRETTA, 2004).

The examined material consisted of salted peanuts packaged in a mix of gases in bags of metalized laminate and in metal cans, with a changing original content of oxygen in the packaging. The packaged peanuts were stored in standardised conditions and evaluated as to the changes of sensorial quality and PV in 4-weeks' intervals, until the moment of shelf life loss (UCHEREK, 2004).

THE LOSS OF SHELF-LIFE MODEL

The model was developed on the basis of a function reflecting the preservation of shelf life called the survival function - S(t).

In order to construct the S(t) function, one needs to define such concepts as: event (loss of durability) – the probability of its occurrence is the object of the analysis, and period of durability, that is the period from the start of the observation to its completion as a result of the occurrence of an event. In the case of the analysed peanuts, the loss of shelf life occurs at the moment when ITSQ reaches a critical value, i.e. 3,0 point (BLOSSFELD *et al.,* 1989).

The S(t) function describing the distribution of the probability of the random variable T in fact determines in general the model of shelf life loss of a product. For the purpose of the development of the shelf life loss model (SLL), the Cox proportional hazards model was used. It is one of the most general and most commonly used models of regression in the survival analysis. For the estimation of that model, the partial likelihood method was used, proposed also by D. R. Cox (1992).

In order to determine the influence of a given factor on the preservation of product shelf life, the tested material was divided into subgroups, including: initial level of quality of the product, kind of packaging and initial composition of the mixture of gases in the packaging. Then, for each of those sub groups independently, a function of durability preservation was set. After estimating the durability preservation function for the subgroups, using the method of L. Kaplan and P. Meier, the researchers compared the above functions using suitable statistical tests, including: long-rank test and F-Cox test (BLOSSFELD *et al.,* 2002; COX, 1992; KAPLAN *et al.,* 1998).

On the basis of the above reflections, using the long-rank and F-Cox tests, the potential forecasting factors were identified, so as to determine durability periods available in the tested material. The variables from a preliminary selection were verified in the Cox's model of proportional hazards (COX, 1992).

In the development of the shelf life loss model (SLL) using the Cox's model of proportional hazards, the predetermined forecasting factors were taken into account, to wit: input quality level of product, type of packaging and input mix of gases in the packaging.

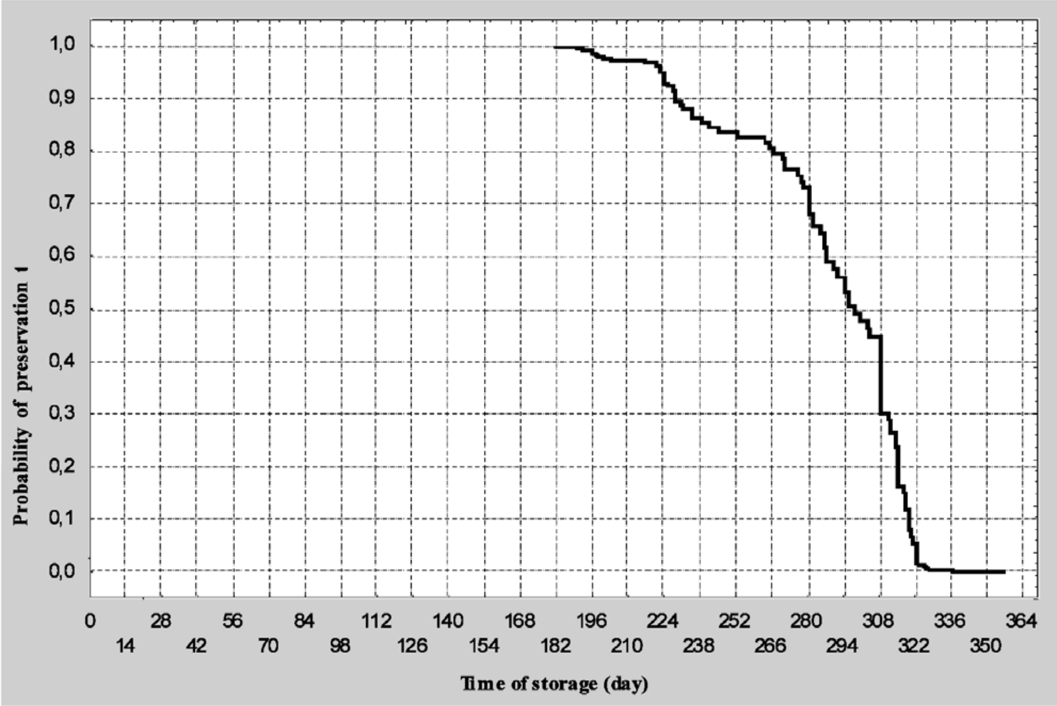
The results of the estimation of the Cox's model of proportional hazards for the above independent variables are shown in table 1. On the basis of the description in that table one can affirm that the entire model as well as the individual variables are characterised by a very high significance (the level of significance p falls in the range 0,00000 - 0,000551). The developed SLL model can therefore be applied to estimate the loss of shelf life of MAP packaged products, taking into account all the following criteria (UCHEREK, 2004):

– input quality level of product,
– type of packaging,
– input mix of gases in the packaging.

Table 1. The results of the estimation of the Cox's model of proportional hazards for the independent variables for peanuts in modified atmosphere packaging (MAP).

The forecasting factors	Dependent variable: time of storage (days) λ^2= 184.474, df = 5, p = 0.0000					
	Beta	Standard Error	t	Index beta	Wald Statyst	p
Input quality Peroxide level of product Value [PV]	12,5099	3,62057	3,45523	271009,0	11,9386	0,0005
Type of packaging	1,1881	0,19176	-6,19585	0,3	38,3885	0,0000
Input mix of gases in the packaging	1,9641	0,18607	10,55555	7,1	111,4198	0,0000

Source: own's work.



* program Statistica6.0 don't draw the curve of shelf life that probability of preservation the adequate quality=1.

Fig. 1. The shelf life loss reflected by the estimated shelf life loss model SLL, based on mean values of all the independent variables. Source: own's work.

Thus, while characterising the MAP packaged peanuts by means of each of the above factors, it is possible to estimate for them individual curves of expected loss of durability.

An example of durability loss reflected by the estimated durability loss model SLL, based on mean values of all the independent variables, is shown in fig. 1. From the results obtained it can be inferred that peanuts retain very good quality during more than 180 days of storage, with a 50% drop of quality occurring after the 294 day, while the period of durability of MAP packaged peanuts on average totals 330 days, i.e. 11 months.

CONCLUSION

Recapitulating the above considerations, we can state that the proposed model SLL for the MAP packaged products, developed using the Cox's model of proportional hazards, can be an efficient tool used to estimate the loss of durability of the packaged products. While analysing the obtained results of durability loss probability, the manufacturers, striving to maintain a high level of quality of the products supplied on the market can shorten the period of durability recommended in standardisation documents so as to avoid 100% loss of quality (for peanuts at the moment of crossing the critical value ITSQ=3,0 point (UCHEREK, 2004; 2006).

The proposed SLL model can also be developed for other food products taking into account the forecasting factors separated for the given kind of product and using the presented statistical tools. The possibility of evaluating the loss of durability obtained thanks to the model can be applied in comparative research at the moment of developing the quality parameters of the product, packaging, and selecting the parameters of the MAP packaging system.

Certainly, the information resulting from the considerations of the loss of durability in packaged products using statistical methods applied in the survival analysis, such as L. Kaplan and P. Meier method, and the Cox's model of proportional hazards, can contribute to the selection of optimum values of the quality parameters, including the combination product-packaging-environment, so as to obtain a suitable durability of the product (COX *et al.*, 1984; KAPLAN *et al.*, 1998).

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APPROACHING THE DISTRIBUTION COSTS FOR MINIMALLY PROCESSED PRODUCTS

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ABSTRACT

Within the new model of food consumption, such as the spreading of ready-to-use food, more and more importance are gaining minimally processed. These are farm products that firms involved in commercialisation introduce into retail points of sale after manufacturing process them into small containers to keep unaltered their organoleptic characteristics and integrity (*shelf life*). Even though these companies adopt processing methods based on simple schemes, they have an interrupted working schedule throughout the year.

In Italy, the companies involved in "prepared" vegetable produce production are locate, above all, near the fresh product production areas, even if some have appeared in the neighbouring territories; that one are, however, still remain privileged to stock raw materials.

According to what said above, it sounded interesting to carry out a survey on the structural characteristics of the firms involved in commercialisation dealing with minimally processed, as well as on the distribution costs that such companies bear from the stocking of raw materials to the retail sale, in view of the fact that the placement onto the retail counter is direct. This passage, in fact, is very important because products are high perishable, and many passages from hand to hand, which means through intermediates, are to be avoided. Firms involved in commercialisation, therefore, intercept most of the trade phases that bring products from the farm to the retail counter, so making products more usable in terms of time and space. That's because products, thanks to them, reach final consumers at the right time and place.

Given that, in the province of Catania, minimally processed firms involved in commercialisation, which have appeared only recently, however in involving process, it was seem interesting start up a economic research that we are set.

The facilities for prepared vegetable produce processing, in province of above, really, have born thanks to some companies already involved in the agricultural product trade, such as vegetable and citrus fruit, which have destined part of their investments and/or sector of organization and management to this new activity. This is possible in connection with shorter space that facilities and instrumental needed and to worker easy adaptation to various production stage of manufacturing process. This last facilities get their supplies past the Straits of Messina, in central-south Italy, following stocking calendars based on the ripening seasons of vegetable.

In this work we have aimed at individualizing the distribution costs concerning the expenses borne by distribution companies to make products available, usable and sellable.

Our task has not been easy at all, but we deem to have fulfilled it to our best covering all main aspects even if not all, being this such a subject that will always deserve further study. However, we are sure to have given a good contribution to this new field of research.

Key words: distribution, costs minimally processed products

INTRODUCTION

In the past few years the fresh vegetable product sector has undergone a deep transformation process trying to meet consumers' tastes and preferences that have changed and still change especially towards quality and timesaving. Fresh agricultural products tend loosing their characteristic of undifferentiated *commodities* to acquire those that allow meeting final consumers' demand, which has become more and more specific, and the segmentation strategies of the processing industry. On the market at present, agricultural products, thanks to the preservation technologies are very differentiated as types and subtypes[1], in order to meet the different needs of final consumers of different categories in relation to their social-economical status, which often pushes to have new life styles and food habits. Among the multiform goods scenario, from food consumption point of view, *minimally processed products* bear the characteristic of being like a link between the traditional orthofruit products and those intentionally processed, because they keep their freshness thanks to the refrigeration process they undergo[2].

The interest towards *minimally processed products* arose in Italy at the beginning of the 80s spreading during the following years at the expense of fresh orthofruit ones, which have been affected by a decrease in consumption especially in the last five years, as shown in a recent research carried out by ISMEA. From the year 2000

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[1] In fact, together with traditional orthofruit products, which according to an acknowledged goods classification are referred to as "*Fresh food products*", there are vegetable preserves and semi-preserves ("*Trasformed food products*"), frozen vegetables ("*Frozen food products*"), processed vegetable that still are fresh with no preservatives added ("*Minimally processed products*") and pre-cooked ones, grilled or little steamed, with no preservatives or condiments added ("*Underdone food products*") (De Muguruzza, 1990).

[2] They, in fact, simply undergo a mechanical processing consisting in removing those parts that are not good, cutting, washing, drying, weighting, and packaging, so that they can be consumed raw with no further processing required. Besides, being them products that differentiate for typology and composition (single-dose or mixture) and colour (chlorophyll, carotene, lycopene, anthocyanins), under a good control during the production and distribution process their shelf-life quality can compete with that of fresh products, above all if put into the great distribution, where the maintenance of low temperature during all post-harvest phases is guaranteed. Moreover, to qualify their offer of orthofruit products, the unbranded sector, several great distribution chains, even for *minimally processed products*, tend to create their own *private label* in order to assure consumers about the healthiness of the products purchased.

to 2004 consumption of some of the main fresh vegetable products reduced of 18%, while minimally processed products one increased of 8-9%. These latter are even being introduced into institutional catering, such as restaurants, hotels, canteens, etc. having realized fresh vegetables have intrinsic quality properties like their aspect, consistency, taste, aroma, nutritional value, guarantee and hygiene; as well as extrinsic quality properties including packaging, labelling, short processing required to be ready to eat, and their 7-8-day-lasting shelf-life. The preparation process of *minimally processed* vegetables is carried out by companies that, independently from the location of their facilities at national level, get raw products from the main production areas of Centre-South Italy, including the agricultural production centres of Apulia, Campania, Abruzzi and low Latium, and transport them by means of refrigerated trucks and more functional logistic systems, all based on the newest preservation techniques. Sicily has not been left out of the phenomenon at all. In the last five years about ten firms involved in commercialisation dealing with fresh and preserved orthofruit and citrus fruit products, half of which located in the territory of Catania, have individualized in producing ready-to-eat vegetables the possibility of amplifying and diversifying their activities, so creating a linked stock organization and management. For this end they have acquired technologically-advanced processing lines, have employed qualified personnel and arranged an appropriate trade organization considering that such companies, as far as the ready-to-eat products is concerned, take care of introducing them into the market by themselves. The market we are dealing with consists of retailers exclusively, enough spread throughout the area. The economic-agrarian research in the past few years has more and more taken into consideration the role played by *minimally processed products* within the food habits aimed at choosing the very *convenience food*. The price ratio of ready-to-eat salads compared to traditional ones is on average 6:1 and 8:1 (Casati, 2005). Facing such a quick dynamics it was deemed interesting to start a survey to highlight the distribution costs for *ready-to-eat* vegetables assimilable to the *fresh-like* ones.

MATERIALS E METHODS

Our research was carried out in the province of Catania, starting by individualizing all firms involved in commercialisation in *minimally processed products* trade to know about their organization and management, and to define the representative "study case" object of our research field. To this end a detailed survey was organized with widespread territorial investigation focusing on the information provided by field experts. Data gathered then were opportunely cross-compared with that provided by the ISO quality system certified, no official data source existing. The acquisition of information about the consistency, structure and trade organization of the company located in the area we focused our survey on, allowed us to assign an high significance to the "sample" company even for the availability shown by the person in charge of the collaboration. The aim of our research was that of determining the medium distribution cost of "on-tray-prepared vegetables", considering that this goods typology represents the 70-80% of the total volume dealt by the companies[3] of the province of Catania. To this end the person we surveyed

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[3] The remaining share concerns the in-bag production above all. We specify that the vegetable mix in bag is identical to that of trays. It still represents the main production item in only one case among the trade companies we surveyed.

was given an *ad hoc* questionnaire to gather average technical data between the years 2003-2005 and economical data for the year 2005. By means of further biweekly surveys in the year 2006 for a total of 7 from the month of February of last year, other useful technical-economical indications were obtained concerning those sections of the questionnaire relative to the stocking of raw material, the manpower and processing, as well as to the quality of the final products and the ways of sale. In particular, we pointed at highlighting the phase of "purchasing and to-warehouse transportation of raw materials", "goods preparation and sale". The average distribution cost pertains to the trade segment that includes the purchase and the loading of stocks on trucks in the field of production and the final destination of the ready-to-sell products. If first phase expenses depend almost exclusively on the logistic system adopted by the company, those pertaining "preparation" are more complex because they involve both the amounts reported on accounts books (explicit costs) and the values calculated according to the opportunity cost (implicit costs), which are distributed according to the way of production they refer to, among the five big expense categories: Materials", "Work wages" (contributions included), "Salaries" (contributions included), "General management expenses" and "Investment shares and interests"[4].

RESULT AND CONCLUSIONS

The survey allowed ascertaining that the universe of "ready vegetables" firms involved in commercialisation in the province of Catania, up to 31st January 2006, is made up of five units prevalently located in the area of Acireale and less in the Etnean Southwest. The processing takes place in recently-built functional facilities even if there are rearranged ones. Equipment is very advanced and 4-5 years old at most. In some cases companies have received public contributions to buy it, but often it has remained underused despite production of *minimally processed products* is throughout the year. In fact, based on the level of specialization of stocks, companies can prepare different vegetable and salad mixes, packaged in different containers as far as the shape, material they are made of, and the weight is concerned. The company surveyed has modern facilities including insulated processing facilities, reefers, offices, etc. and main and secondary equipment, which were bought with public contributions[5], with a processing capacity of 1.4 tons per day, but used up to 1.0 and 1.2 tons. Raw materials come exclusively from other production areas of the South according to a calendar based on the harvest time of the different products, while the processed goods – packaged in proper trays wrapped in neutral plastic film with antifog action– is mainly distributed within the province of Catania and Syracuse.

The average total distribution cost for the year 2005 was equal to 3.40 Euro/Kg[6], 22,1% of which can be assigned to the "purchasing and to-warehouse trans-

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[4] Rough data concerning the company census and the elementary expenses met by the company surveyed, the results of the elaborations carried out by means of a proper software, besides other information about the methodologies, are available at the Department of Economical-Agricultural and Estimate Sciences of the University of Catania.

[5] "Minimally processed product" processing line.

[6] Considering a 200-g salad tray such price is equal to 68 Eurocents.

Table 1 - AVERAGE TOTAL DISTRIBUTION COST (Euro/KG)

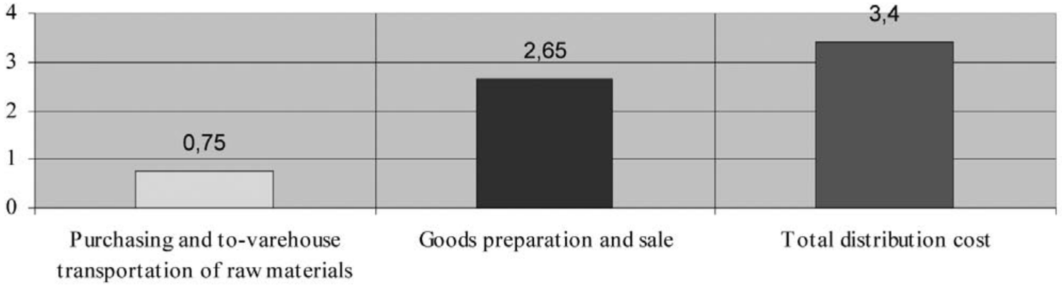
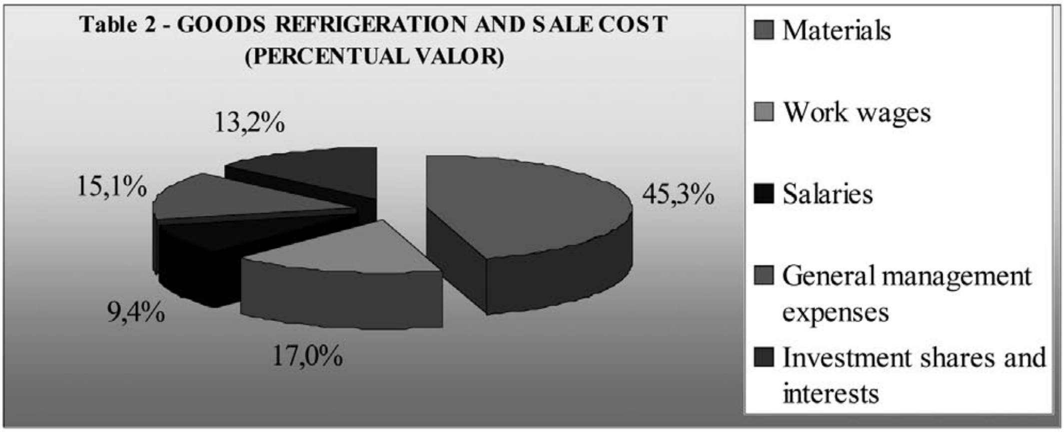


Table 2 - GOODS REFRIGERATION AND SALE COST (PERCENTUAL VALOR)



portation of raw materials" phases (transportation caused the highest expenses, followed by the discarded part and intermediation[7]), while 77.9% derives from the phase of "goods preparation and sale"[8] (table 1). The expenses as far as this latter phase is concerned are equal to 1.20 Euro/Kg for the great category of "Materials" (in particular on the total cost 19.1% for packaging, 16.1% for power, detergents, natural antioxidants and water above all), to 0.45 Euro/Kg for the "Work wages" (processing), to 0.25 Euro/Kg for the "Salaries" (ISO 9001, Haccp and marketing personnel included), to 0.40 Euro/Kg for the "General management expenses" (communication, transportation to retailers, etc.), and to 0.35 Euro/Kg for the "Investment shares and interests" (table 2).

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[7] In particular the discarded part at production fields and warehouses equals 25% of the raw materials collected; while mediation equals 2%.

[8] Actually, we have to say that to the total cost per unit stated should be added the value of the returned goods, that is, the goods unsold by retailers (about 8-10%) within the shelf life and given back to the trade company according to what established by the contract of sale.

This analysis is only a first contribution to what is developing around the ready-to-eat vegetable sector. However, management issues have started coming out and having specific profiles. On costs' side there is a need of reducing average ones by developing ways to boost production and to better quality, such as the shelf life; on earnings' side more and different market niches should be considered and involved by differentiating products and discriminating prices. Finally, the new structure remains to be considered, with the increase in production that the sample company is implementing.

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SHELF LIFE ASSESSMENT AND PREDICTION OF TRADITIONAL FOOD OF CENTRAL ITALY

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ABSTRACT

This study was aimed at assessing the shelf life of some traditional products of Umbria and Marche regions (Central Italy). Each product, packed with or without modified atmosphere or vacuum, was stored at three different temperatures (4°C, 10°C and 25°C) and sampled at different times (0, 0.5, 1 and 1.5 times the labelled shelf life date) to evaluate microbial and chemical profiles. The results allow to evaluate the spoilage mechanisms. The labelled shelf life was confirmed for two of the used products and reduced for the others.

Key words: shelf life, traditional foods, microbial growth, chemical modification

INTRODUCTION

Shelf life is the period of time under defined conditions of storage, after manufacture or packing, for witch the product will remain safe and be fit to use. In other words, during this period, it should retain its desired sensory, chemical, physical, functional or microbiological characteristics and, where appropriate, comply with any label declaration of nutritional information when stored according to the recommended conditions (Man, 2002).

There is no federal standard on stating freshness dates, although the food manufacturers include some form of dating on their labels. Most food spoilage can be explained by one o more of different mechanisms (IFST, 1993). Further, time and temperature are crucial factors affecting the quality of foods and the accelerated shelf life determinations are particularly useful when the pattern of changes are practically identical during normal and accelerated storage so that shelf life under normal storage can be predicted with a high degree of certainty (Labuza and Schmidel, 1985). It should also be noted that different food products retain their freshness at different rates due to the type of ingredients and the methods of preparation, packaging and storage. However, the small and medium traditional food factories that characterise Italy report on their product labels shelf life dates based on empirical and not well known methods.

In order to evaluate the shelf life of foods, this study was aimed at assessing the microbial growth and chemical modification of some traditional products of Umbria and Marche regions (Central Italy), under different storage conditions.

MATERIAL AND METHODS

Five different Italian traditional products were used to fill the purpose: Pizza "Cascione", a meat, cheese and vegetables stuffed pizza, oven backed (220°C, 4 min.) and modified atmosphere packed (N 69.5 %, CO_2 29.85% and O_2 0.5 %) with a 45 days labelled duration time; chicken "Galantina", a meat, cold cuts, cheese, egg, vegetables and preservatives based product, steam cooked (5 hours at 90°C) and vacuum packed; the labelled shelf life is 90 days; "Corallina" salami, packed with and without vacuum with labelled shelf life of 6 months; 3 months labelled duration time, vacuum packed truffle cheese. At first, the manufacturing processes were recorded and controlled. For each of the considered products, different samples with the same manufacturing and packaging date were then stored at three different temperatures (4°C, 10°C and 25°C) and sampled at different times (0, 0.5, 1 and 1.5 times the labelled shelf life date) to evaluate microbial and chemical profiles.

At each sampling time, activity water (Aw), moisture, pH, total volatile nitrogen (TVN), peroxide number, rancidity Kreis test, protein, lipid, sodium chloride, nitrites, nitrates, mesophilic bacteria, *Salmonella* spp., *Listeria* spp., thermophilic *Campylobacter*, *Y. enterocolitica, Coliforms*, *E. coli, S. aureus* and *Staphylococcus enterotoxin*, *Clostridium* spp., *C. botulinum* and botulinum toxin, *Enterococcus* spp., *B. cereus,* Mould and Yeast, *Lactobacillus* spp., *Lactococcus* spp. and *Micrococcus* spp. were determined. The analysis were carried out using ISO or AOAC methods. All the methods are validated and accredited following the UNI CEI EN ISO/IEC 17025.

Statistical analysis were performed using analysis of variance and covariance (STATA, 2005). Predictive models were performed using the obtained results to predict the shelf life.

RESULTS AND CONCLUSIONS

Only the significant mean results are shown in tables 1 to 3.

No highly significant developments were observed for Cascione pizza (table 1). For chemical profile, only the peroxide number value may have a significant expression since it was high already at the earliest packing date but decreased afterwards, maybe due to the vacuum packaging that helps to retard the development of rancidity by excluding oxygen. Regarding bacterial rates, the levels of *Lactobacillus* spp. and *Enterococcus* spp. became very high, and unacceptable for *E. faecalis* (> 10^4/g; Ferrari and Iacono, 1999), since 0.5 times the labelled shelf life at 25°C storage and since 1.5 times the labelled shelf life at all the storage temperatures. The spoilage

Table 1. Mean results of modified atmosphere packed Cascione pizza.

	Storage time (day)				Storage temperature (°C)		
	0	23	46	68	4	10	25
Lactobacillus (Log/g)	1.24^{a}	2.91^{ab}	2.43^{ab}	4.40^{b}	1.52^{A}	2.23^{A}	4.51^{B}
Enterococcus faecalis (Log/g)	ND ^{a}	1.28^{ab}	0.42^{a}	3.11^{b}	0.76	1.22	1.41
Micrococcus (Log/g)	0.77	3.31	3.49	2.72	1.93	2.18	3.89
TVN (mg/100 g)	13	12	13	13	12^{a}	13^{a}	16^{b}
Peroxide N (meqO_2/kg fat).	17	18	10	10	10	14	14

abc: significant differences a $p \leq 0.05$; ABC: significant differences a $p \leq 0.01$; ND: not detected.

Table 2. Mean results of vacuum packed chicken “Galantina”.

	Storage time (day)				Storage temperature (°C)		
	0	30	60	90	4	10	25
Mesophilic bacteria (Log/g)	1.90^{A}	7.94^{B}	8.47^{B}	7.36^{B}	6.69	6.49	6.08
Lactobacillus (Log/g)	1.48^{A}	7.35^{B}	6.84^{B}	5.78^{B}	5.47	5.36	5.25
TVN (mg/100 g)	22^{A}	30^{A}	54^{B}	255^{C}	27	31	100
Peroxide N (meqO_2/kg fat).	4^{ab}	$5^{ab}6^{a}$	0^{b}	3	3.5	4.5	
Moisture (g/100 g)	62.78^{AB}	64.86^{A}	60.63^{B}	60.31^{B}	61.71	62.35	62.38

abc: significant differences a $p \leq 0.05$; ABC: significant differences a $p \leq 0.01$; ND: not detected.

Table 3. Mean results of Corallina salami packed with or without vacuum.

	Storage time (month)			Storage temperature (°C)		
	0	3	6	4	10	25
	Not vacuum packed					
Enterococcus faecalis (Log/g)	ND	5.07	0.78	2.73	2.13	0.98
Lactobacillus (Log/g)	8.20	7.64	6.87	7.69	7.61	7.69
Lactococcus (Log/g)	7.94	7.89	7.87	8.00	7.93	7.72
TVN (mg/100 g)	72	63	190	86	78	162
	Vacuum packed					
Enterococcus faecalis (Log/g)	3.08	4.13	ND	3.39	1.97	1.85
Lactobacillus (Log/g)	8.08	7.51	4.59	7.94	7.28	4.96
Lactococcus (Log/g)	7.51	7.21	4.82	7.47	7.31	4.75
TVN (mg/100 g)	74	88	68	71	76	84

ND: not detected.

association especially of protein-rich foods can be dominated by Gram-positive bacteria, notably lactic acid bacteria which affect vacuum packaged refrigerated processed meats and some dairy products. On the other hand, a causative role in food spoilage has not been established clearly for all those groups, some of which may be opportunistic in their behaviour. The *Lactobacillus* often share similar habitats and show similar physiological behaviour on a number of elective and selective media. (Holzapfel, 1992). *Micrococcus* became too high (> 10^3/g; Ferrari and Iacono, 1999) since 0.5 times the shelf life date, at 10 and 25°C, and 1.5 times the use by date, at 4°C. Indeed, with minimal cooking the surviving microflora (10^3/g) was dominated by species of *Lactobacillus, Brochothrix* and *Micrococcus* (Bell, 1983). Micrococci are not thought to be pathogenic but they are well known spoilage bacteria (Garvie, 1998). The shelf life can be then confirmed at 45 days with 2-4°C of storage condition.

As for chicken Galantina product (table 2), even if the mesophilic bacteria level was found unacceptable (> 5 x 10^6) since less of 30 days of storage, the most critical parameter resulted the TVN for witch a maximum limit was set at 30 mg/100 g. Following Arrhenius equation (Labuza and Riboh, 1982), the activation energy was 29.54 KJ/mol, Q_{10} 1.56 and the shelf life was assessed at 45 days at a refrigerated temperature (figure 1) instead the 60 days assigned by the manufacturer. The evaluated 45 days of shelf is confirmed by the Arrhenius equation of moisture (activation energy -3.69 KJ/mol, Q_{10} 0.95).

Regarding Corallina salami (table 3), non general significant differences were observed. *Lactobacillus* and *Lactococcus* rates increased with the decrease of temperature storage since at higher temperatures we observe an increase of the population and afterwards the plateau and then the decrease, not observed at the lowest temperature. *Lactobacillus*, especially *Lactobacillus acidophilus,* are the most

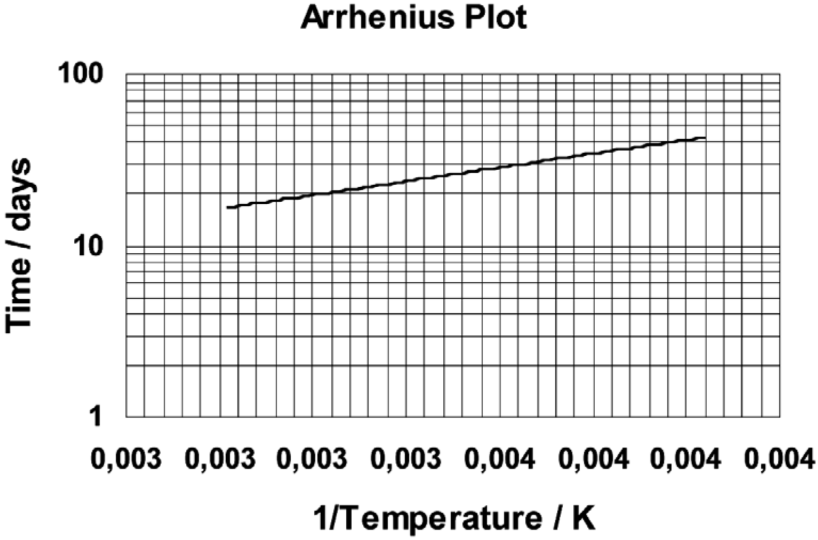

Fig. 1. Chicken Galantina TVN Arrhenius plot.

commonly used probiotics bacteria that protect against the entrance and proliferation of pathogen organisms, through a variety of mechanisms such the breakdown of food that leads to production of lactic acid, hydrogen peroxide, and other by products that make the environment hostile for undesired organisms (Gilliland and Speck, 1977; Brennan *et al.*, 1983). On the other hand the behaviour of *Enterococcus faecalis* must be more extensively investigated since they show, at 0.5 times the labelled duration date corresponding to 3 months, a very higher although not significant increase at lowest temperature and a following lowering overtime or set to zero. Among the chemical parameters, only TVN shows an Arrhenius behaviour (Labuza and Riboh, 1982) that allows to confirm the 6 months shelf life earlier assessed (not published data) for not vacuum packed Corallina salami. For this fermented product, the vacuum packaging does not show a general benefit for shelf life, nutritional and organoleptic quality and has no meaning to be used.

Finally, the shelf life trial of vacuum packed truffle caciotta is still in process. However, based on the first results and the going over, we can assert that the packaging type adopted is absolutely not appropriate since the product shows before 45 days of storage (0.5 times the labelled duration date) strong mould formation at 4°C storage, water overstock in the package at 10°C and 25°C and packing swelling and breakdown at 25°C.

The knowledge of Aw, pH, salt and preservative contents and that of physicochemical changes allow to determine an acceptable shelf life of the product, using appropriate prediction software. More extensive researches to evaluate the shelf life of the traditional foods are needed and are in progress.

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SESSION II

New technologies for extending shelf-life

Chairmen:

Gianna Costa, ISMAC-CNR, Genova - Italy
Francisco Artes, University of Cartagena, Murcia - Spain

NEW TECHNOLOGIES FOR EXTENDING SHELF LIFE

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Some of the most important factors reducing shelf life of many foods are oxygen, light, spoilage bacteria and inadequate cooling. The strategies of Matforsk – Norwegian Food Research Institute to prevent or limit the damage of these factors are to understand the effect of oxygen and light of different wavelengths on different foods to develop protection of the food with adequate light and oxygen barrier packaging and by antioxidative marinades. Application of the right packaging gas for different foods, including the use of an active packaging concept such as a CO_2-emitter, is also crucial for ensuring sensory and microbial shelf life. Fighting spoilage and pathogen bacteria may also be done by means of protective, bacteriocin producing bacterial cultures. Inadequate cooling may be detected by means of intelligent packaging concept such as Time-Temperature Indicators. This paper summarises a number of research tasks recently undertaken at Matforsk to extend the shelf life of a number of different foods, including extruded oats, bologna sausage, cheeses, butter, fresh salmon, smoked salmon, fresh cod, minced meat and fresh and frozen meat cuts.

POLYMERIC CAPSULE PRODUCTION FOR FOOD PACKAGING APPLICATION

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ABSTRACT

Capsules of modified PEEK (PEEKWC) were prepared using a procedure which combines the droplet formation employing membranes with the phase inversion technique. The polymeric droplets were prepared using a mono-pore polyethylene (PE) film with pore diameter ranging from 300 to 800 μm. The capsule morphology, porosity, shell thickness and size were modified changing some process and ingredient parameters such as the polymeric concentration, oil phase and pore film diameter. Active carbon was loaded into the capsules during their production. The interaction of the loaded active carbon and the polymeric capsule matrix was also investigated.

Key words: modified Polyetheretherketone (PEEKWC), polymeric porous PE film, capsule preparation, phase inversion technique

INTRODUCTION

Capsules of different polymeric material are largely employed in industrial applications such as cosmetic, pharmaceutical, chemical and food packaging. Therefore, the preparation of loaded capsules by means of a reliable procedure is an important challenge.

In this work, macro-capsules using the membrane process concept combined with phase inversion technique have been developed. This technique can be considered as an integration between the traditional chemical capsule preparation (coacervation or phase inversion) and the mechanical capsule technique (pressure extrusion).

The polymer employed was the modified polyetheretherekene (PEEWC) which has excellent chemical, thermal and mechanical properties; moreover it has the advantage, compared to traditional PEEK, to be soluble in several common organic solvents which facilitates the membrane preparation [Liu *et al.* (1987), Zhang *et al.*

(1987), Drioli and Zhang (1989)] and capsules. In a previous work, the suitability of PEEKWC as potential food packaging was verified by means of overall migration tests, using different food simulants [Torchia *et al.* (2004)]. Therefore, its excellent thermal and chemical properties combined with its food suitability makes PEEKWC an interesting matrix for preparing capsules in food packaging application. The preparation of PEEKWC membranes by phase inversion has been widely studied and different membrane morphologies have been obtained [Jansen *et al.* (2006), Buonomenna *et al.* (2003)].

The objective of this work was to prepare polyetheretherketone (PEEKWC) capsules of different morphology, porosity, size and shell thickness changing the ingredient and process parameters such as polymer concentration, oil and pore dimension of the PE film.

The morphology and dimension of the prepared capsules are the fundamental parameters to control the release of different active compounds loaded and their adsorbent properties.

The active carbon was loaded into the capsules during their production. The interaction of the loaded active carbon and the polymeric capsule matrix was hence investigated.

Active carbon finds its application in food packaging as ethylene scavengers. Ethylene (C_2H_4) acts as plant hormone which accelerates the respiration rate causing the ripening of many kinds of fruits and vegetables. A reduction of the ethylene level in the packaging extends the ripening stage of the fruit, the storage time of ripe and ready-to-eat fruits. Charcoal, containing PdCl as a metal catalyst, was effective at 20°C in preventing the accumulation of ethylene, in reducing the rate of softening in minimal processed kiwifruits and bananas and in reducing chlorophyll loss in spinach leaves [Abe *et al.*, (1991), Vermeiren *et al.*, (1999)].

Most of the scavengers are supplied as sachets or integrated into films. C_2H_4 scavengers are not yet very successful, probably due of insufficient adsorbing capacity or of the low adsorbent quantity loaded in the film. This could be overcome by encapsulation of the adsorbents.

MATERIALS AND METHODS

PEEKWC was supplied by the Chan Chung Institute of Applied Chemistry, Academia Sinica. N,N-dimethylformamide (DMF) was purchased from Merck and used as polymer solvent without further purifications. The active agent used was the active carbon, purchased by Norit BV with average particle dimension of 5 μm.

The solvents used (dodecane, iso-octane and iso-propanol) were purchased from Sigma-Aldrich and used as oil phase and as polymer non-solvent, respectively. The repeating unit of the polymer is shown in Fig. 1.

Capsule preparation and characterisation

The polymer, PEEKWC (8, 10 and 12 wt.%), with and without active agents was dissolved in DMF at room temperature. The solution was magnetically stirred for at least 1 day to allow a complete dissolution of the polymer.

The polymer solution *(phase 1)* was add to the feed tank of the module used in the capsules preparation (Fig. 2). Then, it was pressed through the mono-pore film of polyethylene (PE) which has a pore diameter ranging from 300 to 800 μm. The

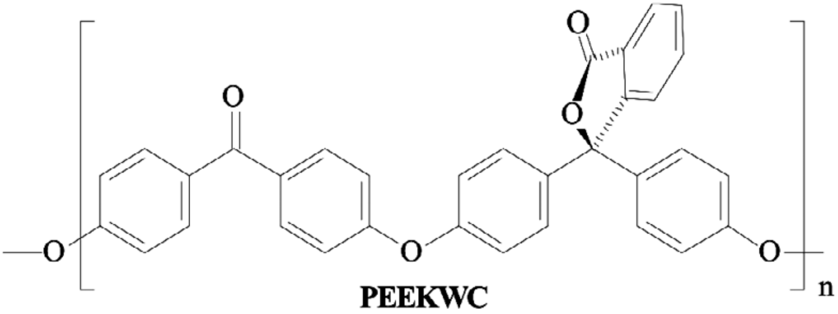

Fig. 1. Chemical structure of PEEKWC.

droplets formed at PE hole border move through the dodecane *(phase 2)*, which should give a spherical shape to the droplets, and immediately polymerised by phase inversion when in contact with the non-solvent phase, water/isopropanol 70/30 *(phase 3)*. The capsules were then recovered using a filter paper. The capsules were left to evaporate over night at room temperature and set in an oven under vacuum for 24h to remove completely the solvent.

In table 1, the summary of the main ingredients and process parameters involved in the capsules preparation is reported.

The morphology and the shell thickness of the dried capsules were determined using a Scanning Electron Microscopy (SEM), Cambridge, Stereoscan 360, at 20kV. The capsules were freeze-fractured in liquid nitrogen for cross-section analysis. All samples were evacuated and then sputter-coated with gold under argon atmosphere before SEM analysis.

The capsules size was measured by a digital micrometer (Carl Mahr D 7300 Esslingen a.N.).

RESULTS AND DISCUSSION

The capsule formation lab set-up is shown in Fig. 3.

The SEM pictures of the capsules, obtained with PEEKWC/DMF 8 wt.%, are shown in Fig. 4. These capsules were made by a pore diameter of about 500 μm, dodecane as oil phase 2 and water:isopropanol as non-solvent (phase 3). The cap-

Table 1. Summary of the ingredients involved in the capsules preparation.

Phase 1	Phase 2	Phase 3
PEEKWC/DMF 8 wt.%	Dodecane	Water:isopropanol (70:30)
PEEKWC/DMF 10 wt.%	Dodecane, Iso-octane	Water:isopropanol (70:30)
PEEKWC/DMF 15 wt.%	Dodecane	Water:isopropanol (70:30)
PEEKWC/DMF 10 wt.%+active carbon 5 wt.%	Dodecane	Water:isopropanol (70:30)

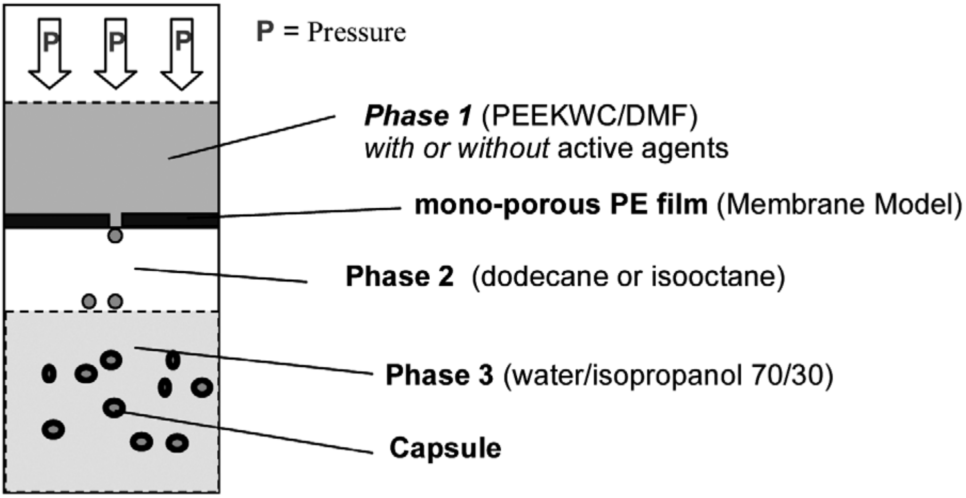

Fig. 2. Scheme of capsule formation unit.

sules were spherical and with a smooth surface (Fig. 4a). Moreover, in Fig. 4b and c, the capsule cross-section shows a central cavity and a asymmetric (finger type) structure with a dense skin layer at the shell side.

Fig. 5 shows the surface of the capsules obtained using the same previous conditions but increasing the polymer concentration from 10 to 15 wt.%.

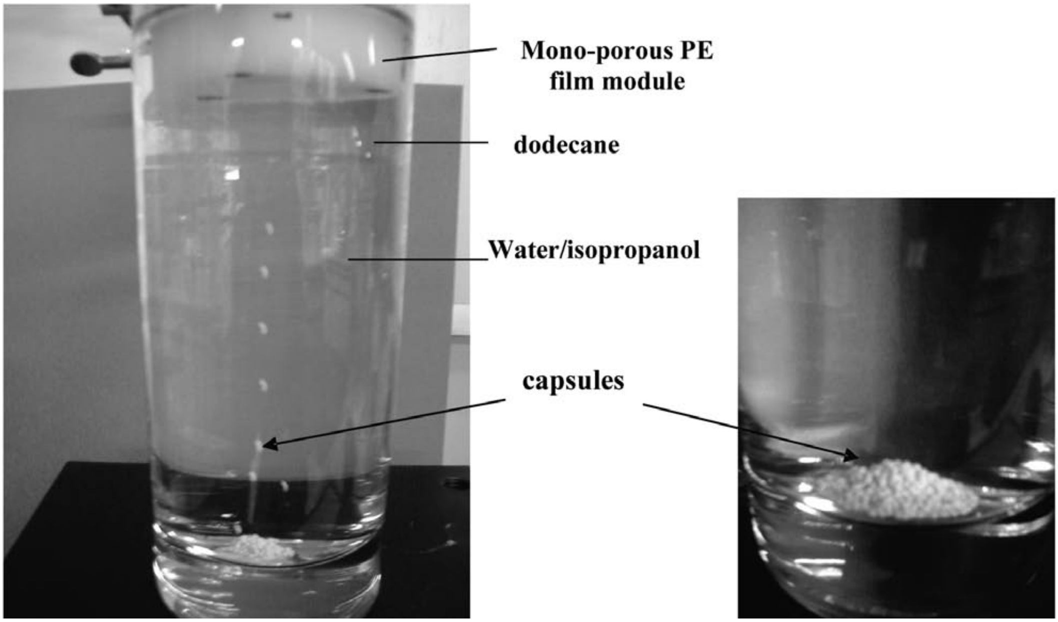

Fig. 3. Picture of the lab device for polymeric capsule preparation.

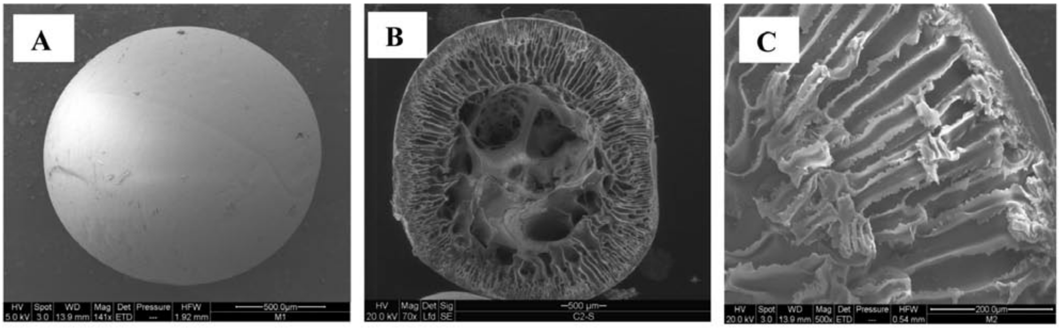

Fig. 4. SEM pictures of the a) Surface and b,c) Cross-section of the capsules prepared using a film with the pore size of about 500 μm and PEEKWC/DMF 8 wt.%, phase 1, dodecane, phase 2, and water: isopropanol, phase 3.

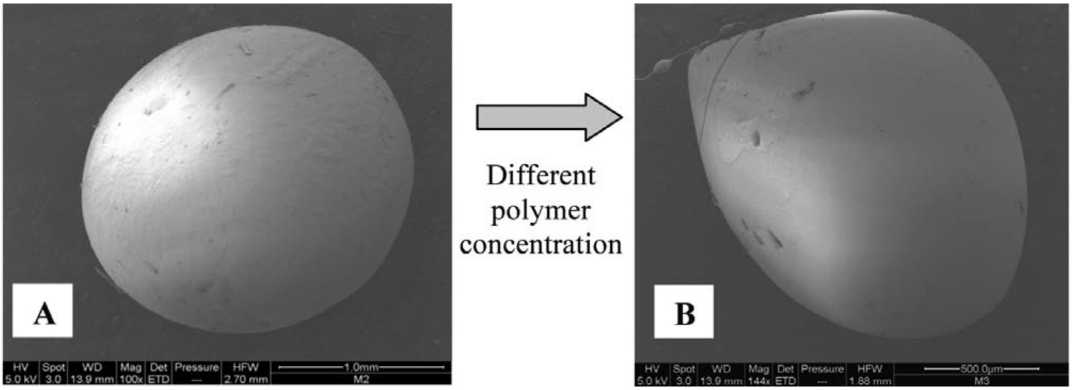

Fig. 5. SEM picture of the capsules surface prepared using a film with the pore size of about 500 μm and as phase 3, water:isopropanol, phase 2, dodecane and phase 1, PEEKWC/DMF: a) 10 wt.% and b) 15 wt.%.

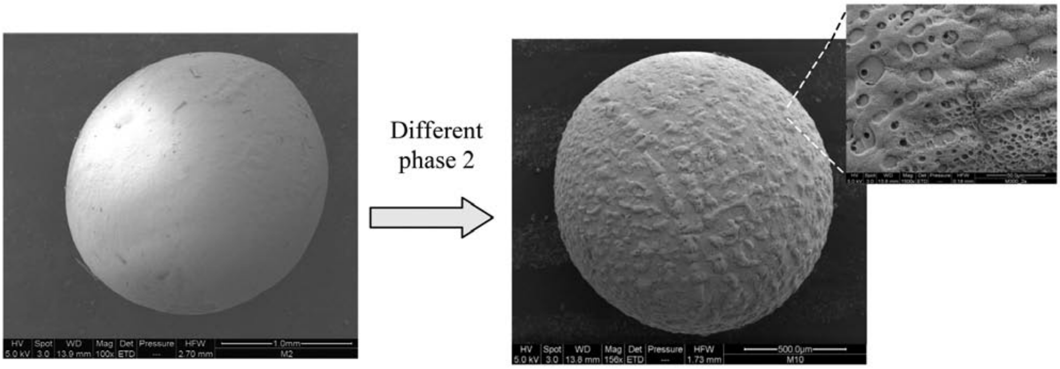

Fig. 6. SEM pictures of different capsules using: pore size (800 μm), PEEKWC/DMF 10 wt.%, dodecane (a) and isooctane (b) as phase 2.

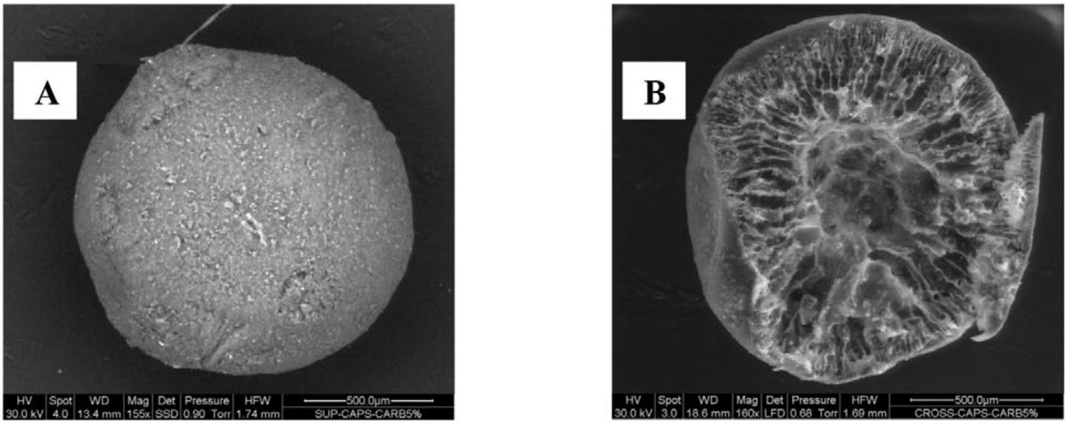

Fig. 7. SEM pictures of the capsule loaded with active carbon using as phase 1, PEEKWC/DMF 10 wt%, phase 2, dodecane and phase 3, water:isopropanol (70:30).

The increase of the polymer concentration determined a deformation of the spherical symmetry, probably, due to a decrease of the droplet interfacial tension.

In Fig. 6, the influence of phase 2, which strongly modify the morphology of the made capsule is reported. In fact, the variation of phase 2 (from dodecane to iso-octane) changed the capsule morphology from a dense to porous surface. The higher affinity of iso-octane caused a starting of the droplet demixing before coagulating at the non-solvent phase.

The diameter of the prepared capsules varied, from 500 to about 2,100 µm, depending on the pore film diameter. However, the change of pore size did not influence the morphology of the capsules.

After the study of the influence of the main variables on the capsule morphology, PEEKWC capsules loaded with active agents were prepared.

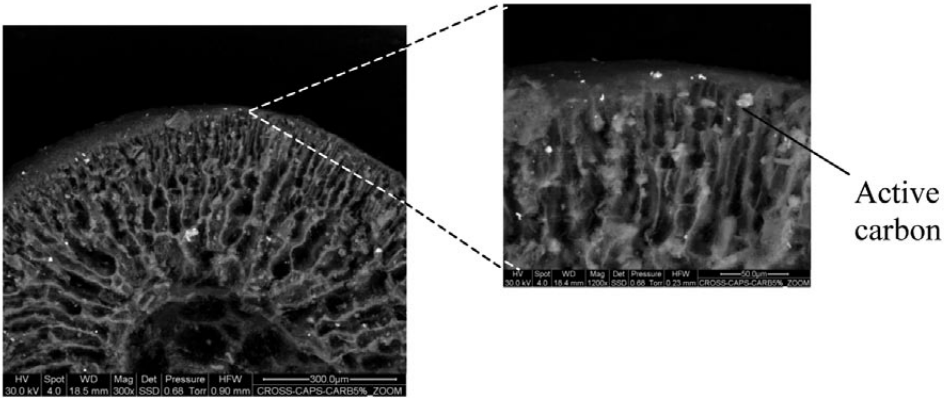

Fig. 8. Cross-section of the capsule loaded with active carbon (at higher magnification, detail of the skin layer).

In Fig. 7, capsules formed using PEEKWC solution containing 5 wt.% of active carbon and pore size of about 500 μm are shown.

The addition of the active carbon to the polymer solution did not affect the final morphology and shape of capsules which resulted to have the same asymmetric morphology and the central cavity of the capsules prepared without adding any active agent. On the other hand, the surface of the loaded capsules showed a pronounced roughness, compared with the capsules prepared with the same solvent, probably due to the presence of the inorganic specie also on the outside of the capsules itself.

The active carbon was randomly distributed in the asymmetric part of the capsule, as shown in the cross-section reported in Fig. 8.

CONCLUSIONS

The formation of mono-dispersed PEEKWC capsules with different morphologies has been carried out. The capsule morphology and dimension could be adjusted changing some process and ingredients parameters, such as the polymer concentration, oil phase and the PE film pore diameter.

These tests showed that the capsules sizes depend on film pore diameter. Furthermore, the PEEKWC capsules were successfully loaded with active carbon for potential applications in food packaging, in which the removal of volatile compounds (e.g. ethylene) or off-flavours is needed.

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PLASMA-ACTIVATED PACKAGING POLYMER FILMS

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ABSTRACT

The present work is focused on the surface modification of commercial plastic materials used in food packaging and deals with the study of barrier coatings deposited on these modified surfaces, improving their “barrier to gas” performance.

The deposited thin coatings are tremendously effective, which makes these materials a valid alternative to coextruded materials, considering both cost and raw materials savings. In fact, the coatings turn out to be equally effective when deposited as a thin layer on common packaging plastics, with the consequent possibility of lightening the pack.

Even not much wettable surfaces have been successfully coated after a preliminary surface treatment: surface modifications have been obtained by cold plasma. The study of these surfaces revealed to be particularly interesting: the combined use of several analytical techniques, such as XPS (X-ray Photoelectron Spectroscopy), DCA (Dynamic Contact Angle), and AFM (Atomic Force Microscopy) allowed to interpret the molecular mechanisms that brings to the improvement of surface properties, for what concerns wettability and adhesion by the deposited coatings.

An accurate study of the thermal treatments and the high barrier obtained allowed to improve the barrier performance of the common plastic materials used for packaging (food and no-food packaging) and to make savings both in raw materials and in pack weight, with the consequent reduction of waste material to be disposed.

Key words: contact angle, packaging, plasma activation, polymer films

INTRODUCTION

To improve the properties of plastics for food packaging water-based lacquers are being extensively used: this brings several advantages to converters, as solvent-recover plants are not necessary and the pack does not show problems of solvent release. Nevertheless, if water is used as the base problems are encountered while handling the substrate to coat. This is the reason why studies are carried on about surface activation systems aimed to make hydrophobic surfaces wettable through the use of plasma. In this context a particular attention is addressed towards the *functionalization* of the surfaces of flexible packages. The strong interest for this kind of innovation comes from the fact that these surface treatments don't generally cause any interference with the intrinsic properties of the modified material, that remain the known ones, but they can rather be regarded as interventions that *give* the materials new properties, giving rise to innovative packaging typologies [1,2,3,4].

In the present work, the plasma treatment is aimed to increase the surface energy, and therefore the wettability of LDPE surfaces, measured in term of reduction of the contact angle, so to form a surface able to actively react with the solution to be deposited.

MATERIALS AND METHODS

To perform the study of the wettability on the plasma- treated LDPE samples preliminary test have been performed to individuate the process variables to control. The most important process parameters for plasma treatment are the power to supply by the frequency generator, the pressure inside the reactor and the time of plasma discharge. The plasma treatments (Mod. Colibrì, Gambetti Vacuum Technology and Related Solutions) were performed on LDPE films by varying only two parameters, i.e. generated power (in the range 10÷56 Watts) and treatment duration (10 or 30 seconds), and maintaining absolute working pressure constant (≈0,1 mbar). Air has been used as the ionization gas, so that one can speak about an "oxidising plasma" treatment.

Several tests have been performed on each sample to control results reproducibility.

Contact angle measured have been performed by using water as the reference liquid.

Possible structural and morphological changes occurred on the LDPE surface due to plasma treatment have been evaluated by means of XPS and SEM/AFM analyses respectively.

Last, to check the surface behaviour after plasma treatment, some test –normally performed for quality control at industrial level- have been carried out: the surface tension of plasma treated LDPE films has been measured by using liquid mixtures with known wettability values (wetting solutions). This is known as the "Test Inks Method" and allows to determine the adhesion level of a liquid on a plastic surface, in order to evaluate the suitability to printing or gluing: it consists of finding out the mixture which is at the edge of drop formation, indicating the solid wettability value expressed in dyne/cm. Solutions based on formamide and cellosolve have been used, containing the dye "Du Pont Victoria Bleu Pure 80". The standard system used includes a set 12 bottles covering the surface tension range 34÷58 dynes/cm. The precision of the test is ± 0,5 dyne/cm.

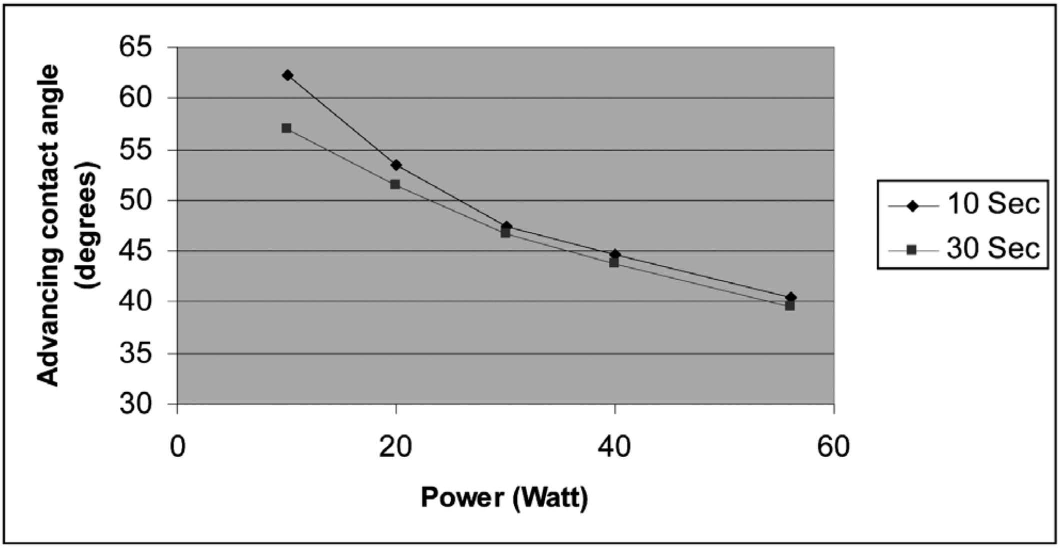

Fig. 1. Variation of advancing contact angle for plasma-treated samples as a function of the generated power.

RESULTS

In all the cases examined it was possible to notice an increase of the wettability after the treatment.

The average value of advancing contact angle for untreated LDPE 150 μm is 95,5° ± 0,1°.

The results (see Fig. 1) show that the cold plasma treatment gives rise to a considerable increase of wettability. The average value of contact angle measured on LDPE samples soon after the treatment drastically decreases with respect to that of untreated samples. From the test performed one can see that the process parameter

Table 1. Contact angle measurements on samples plasma-treated in different conditions.

Power (Watt)	Time (sec)	Advancing angle (degrees) after the treatment			
		Time 0	After 24 h	After 7 days	After 14 days
10	10	62,2 ± 1,1	68,6 ± 0,9	65,2 ± 2,9	65,2 ± 1,6
10	30	56,9 ± 1,4	64,7 ± 1,2	64,6 ± 2,8	63,6 ± 1,6
20	10	53,4 ± 1,9	57,5 ± 1,2	57,9 ± 0,8	58 ± 2,6
20	30	51,4 ± 1,7	55,5 ± 1,8	57,1 ± 1,9	57 ± 0,5
30	10	47,4 ± 0,9	53,7 ± 4,1	54,8 ± 0,7	54,7 ± 2,4
30	30	46,7 ± 1,6	53,6 ± 2,6	54,2 ± 1,6	54 ±1,4
40	10	44,6 ± 1,1	49,6 ± 4,4	51,3 ± 1,8	50,1 ± 2,6
40	30	43,8 ± 0,4	48,4 ± 0,6	50,1 ± 2,6	51,5 ± 1,8
56	10	40,4 ± 1,1	44,1 ± 1,7	46,3 ± 1,2	46,5 ± 1,1
56	30	39,5 ± 1	45,2 ± 1,9	43,4 ± 1,9	45,1 ± 1,9

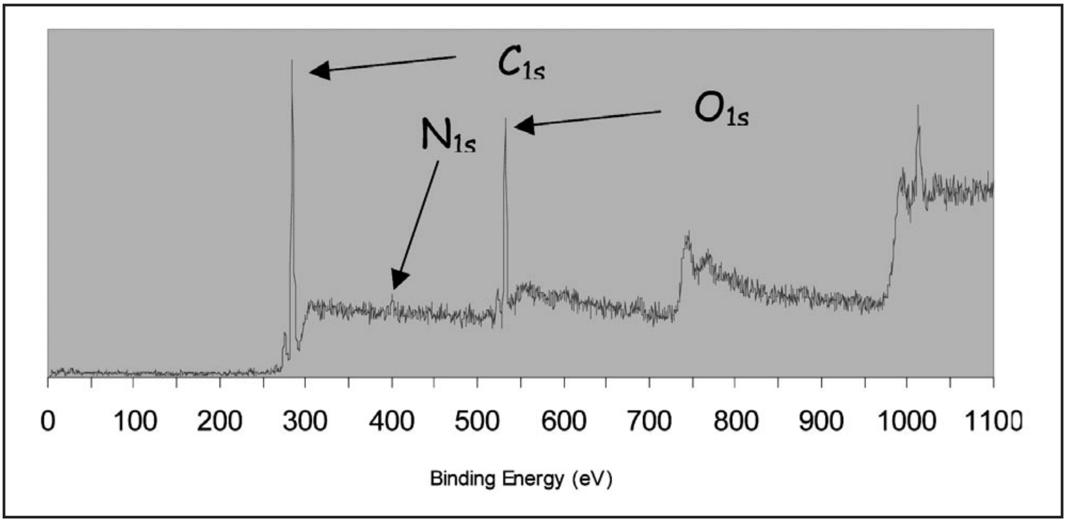

Fig. 2. Example of XPS spectrum of a plasma-treated sample.

most affecting the surface treatment is the generated power, probably because as power increases, the ionization degree of plasma increases as well.

To evaluate the duration of plasma treatment measurements of contact angle have been performed day by day for 14 days: results are summarized in Table 1.

The data obtained show that the values of advancing contact angle soon after the plasma treatment are much lower than those measured afterwards. The difference is probably due to the fact that after 24 hours from the treatment the polymer films loses part of the effect due to plasma, with a consequent decrease in the wettability. Analysing the variation of the values of contact angle in the range of time in which the measurements have been performed, one can notice a small increase of contact angle: therefore, it is possible to say that the activation has a rather long duration, that nevertheless slowly decreases with time.

XPS measurements performed on the untreated LDPE substrate gave a spectrum in which obviously only the signal relative to olefin chains can be noticed. On the contrary, the measurement on plasma treated samples gives rise to as spectrum like the one reported in Fig. 2.

For what concerns curve fitting, in most cases the C1s envelope has been decomposed into three contribution, attributable to the following groups: -CH_2O- at 286,5 eV (alcohols, ethers, esters, peroxides), C=O (aldehydes and ketones) at about 288 eV, and C(O)O at ≈289,5 eV (acids and carboxylic esters).

As one could expect, the increase of wettability can be attributed to the formation, after activation, of hydrophilic function on the polymer surface, as XPS data show. Like previously observed, one can see an increase of the oxidized functions as the power treatment increases and, maintaining the power constant, as treatment duration increases. Fig. 3 schematically shows the results of XPS measurements performed.

The surface activation might also modify the morphology of the substrate: to evaluate this probable effect SEM and AFM measurements have been performed.

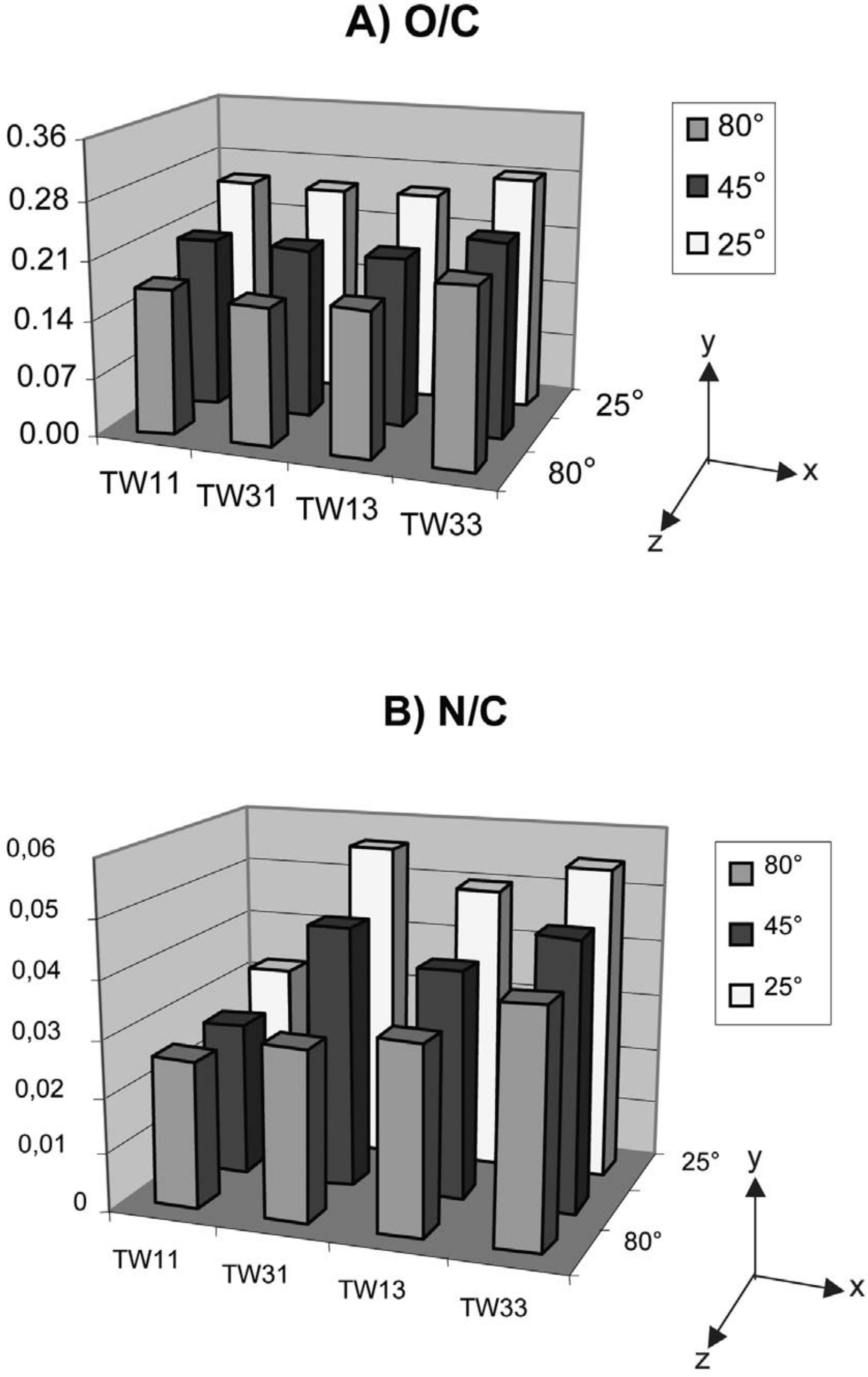

Fig. 3. Results of XPS analysis of plasma-treated sample (x: sample name; y: % of oxidized functions; z: incidence angle of the beam). A) Oxygen to carbon ratio; B) Nitrogen to carbon ratio.

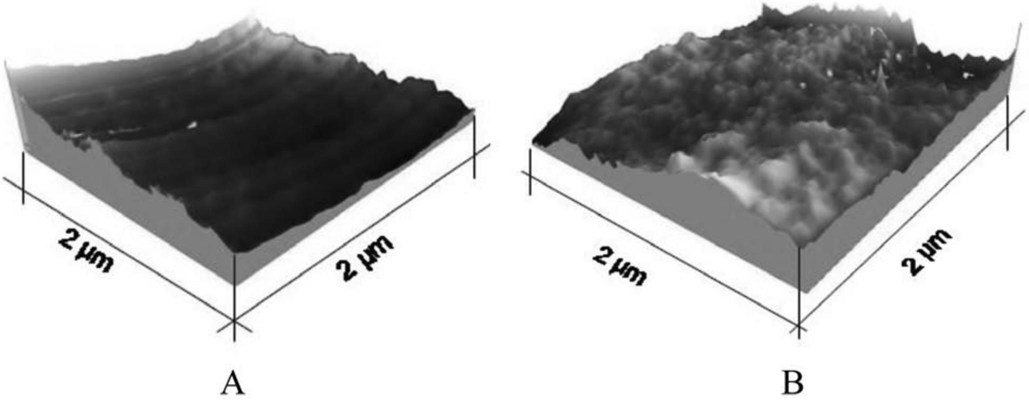

Fig. 4. AFM micrograph of untreated (A) and plasma treated (B) samples.

SEM analysis actually does not allow to distinguish between a treated and an untreated surface.

On the contrary, a difference can be easily seen observing the results of AFM measurements

Fig. 4 (A) shows the AFM image of the surface of untreated LDPE, while in Fig. 4 (B) the image of LDPE plasma-treated at 50 Watt for 30 seconds is reported: it is possible to see that the plasma treatment increases the roughness of the surface. So, the increase of wettability found for the treated surfaces can also be related to irregularities formed by plasma.

The measurements performed showed that the roughness of the samples changes from 2-5 nm for the untreated surfaces to 30-40 nm for plasma-treated samples. The images reported in Fig. 4 are a representative example of those collected also for the other samples treated in different experimental conditions of power and time, in which a remarkable increase of roughness can always be noticed.

Data reported in the literature show that the surface tension of polyethylene

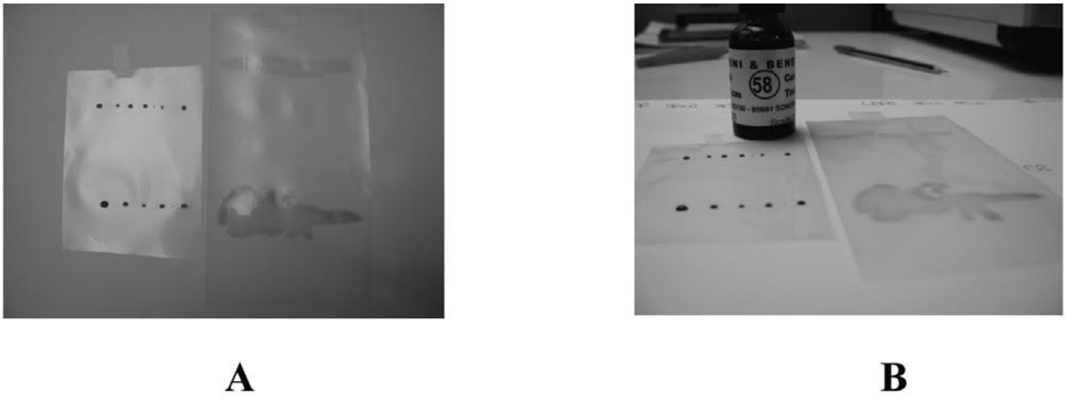

Fig. 5. (A) Test Inks test 34 dyne/cm; (B) Test Inks test 58 dyne/cm.

is about 25 dyne/cm, but for industrial applications a value in the range 48-56 dyne/cm is required.

If LDPE films are subjected to plasma treatment a very low value is obtained for advancing contact angle and so a higher wettability.

Due to plasma treatment (a 30 Watt for 30 seconds) the surface tension of the polyethylene film drastically increases as well.

From Fig. 5 (A) one can see that if a thin layer of a liquid with surface tension of 34 dyne/cm is applied on the surface of untreated polyethylene, one can observe that the liquid non is divided into little drops in less than 2 seconds. So, one can say that the wettability of the sample is lower than the value of the liquid used for the test.

On the other hand, if a thin layer of a liquid with surface tension of 58 dyne/cm is applied on the surface plasma-treated polyethylene, one can see from Fig. 5 (B) that the liquid is not modified after 2 seconds, but it spreads all over the surface completely. drops so, in this case one can conclude that the wettability of the sample is higher than the value of the liquid used for the test.

All the inks tested in this works, i.e. from 34 to 58 dynes/cm, are well spread on activated LDPE, so confirming the effectiveness of the treatment of surface activation.

CONCLUSIONS

The treatment of LDPE with oxidizing plasma produces remarkable improvements in terms of surface wettability, with an increase of this parameter due both to the formation on the surface of polar groups such as hydroxy (-OH), carbonyl (-C(O)-) or carboxylic (-C(O)O-) groups or amino groups, and to the increase of the surface roughness.

There is no clear evidence of a real correlation between time and supplied power, if not that wettability increases as treatment power increases and, keeping the power constant, as treatment duration increases.

The increase of wettability is made even more evident by the high tendency to spread on the treated surface that all tested inks have shown.

ACKNOWLEDGMENTS

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ABSORBENT PADS FOR FOOD TRAYS MADE FROM *SPHAGNUM* MOSS

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ABSTRACT

Absorbent pads were made from chlorite-treated (bleached) *Sphagnum papillosium* and placed on the top and bottom of Atlantic salmon (*Salmo salar*) individual fillet slices. Each fillet slice was packed in a high-density polyethylene tray in a modified atmosphere of 60:40 N_2/CO_2 to a gas:fillet slice volume $\approx$ 5:1 and stored at +4°C. These trays, and controls (fillet slices with a standard paper absorptive pad or no pad) packed in the same way, were independently removed from cold storage and sampled (n=3) at selected time intervals over a 30 day period. Tray gas composition, water content, water holding capacity, pH, colour, texture, smell, viable bacterial count, amount of soluble protein, amount of acid soluble peptides and free amino acid were assessed for each fillet slice. By the end of the experiment and compared to controls, the smell of the fillet slices stored with *Sphagnum* pads was deemed to be acceptable to a consumer. Between storage days 7-12 and 19-30 days in the stationary bacterial growth phases these same fillet slices had roughly half the viable bacterial counts per gram slice. No significant differences, between all fillet slices at each sampling interval, were found for any of the other parameters which were assessed, except for free amino acid content. These results show that absorbent pads made from *Sphagnum* moss may, with further development, have a commercial potential in that they can help extend the shelf-life and the quality of packaged fresh foods such as fish.

Key words: fish, food packaging, shelf-life

INTRODUCTION

Previous experiments have shown that *Sphagnum* moss bleached with chlorite displays microbiocidal properties (Børsheim *et al.*, 2001). When used as a packing material it had the ability to seemingly preserve and slow-down the on-set of putrefaction of whole Zebra fish for a number of days at room temperature and in the presence of oxygen (Børsheim *et al.*, 2001). Historical evidence for the preservative properties of *Sphagnum* moss is also abundant. Sterile *Sphagnum* moss bandages show excellent wound healing properties and their use dates back to the Bronze Age (Varley & Barnett, 1987). In particular they were extensively used in World War I (Riegler, 1989). *Sphagnum* moss is also used to preserve post-harvest fruit and vegetables, and even freshly caught fish (Riddervold, Pers. comm.). These functional properties of *Sphagnum* have been attributed to its remarkable ability to absorb liquid and to as yet an unidentified microbiocidal entity.

The aim of our current study is therefore to evaluate the use of chlorite-treated *Sphagnum* as a packaging material for fresh food stuffs. Fillet slices of salmon were selected for study because at present only 1 in 3 temperate marine fish is sold to the consumer (mostly export) as high-quality, high-profit fresh fish. If the shelf-life of fresh fish can be extended by only 20-30 % (2-3 days) without sacrificing sensory or nutritional quality (Declerck, 1976) then the economic and health benefits are potentially substantial. The salmon fillet slices were packaged in trays under modified atmosphere to mimic the present commercial standard. The only difference was that chlorite-treated *Sphagnum* was applied to the top and bottom of the fillet slice in the form of a pad made according to the ISO standard traditionally applied in the production of paper sheets from cellulose fibres and pulp. These trays, and controls (fillet slices with a standard paper absorptive pad or no pad) packed in the same way, were independently removed from storage at +4°C and sampled (n=3) at selected time intervals over a 30 day period. Tray gas composition, water content, water holding capacity, pH, colour, texture, smell, viable bacterial count, amount of soluble protein, amount of acid soluble peptides and free amino acid were assessed for each fillet slice.

MATERIALS AND METHODS

Pad production

66 *Sphagnum* sheets (12 x 24 x 0.1 cm) were produced to ISO standard 5269-1 using a custom-made sheet former, and a press (PTI, model 40140). Each sheet comprised 4 g of ground chlorite-treated whole *S. papillosum* plants (Ballance *et al.* In Press). Finally the sheets were cut down to 9.5 x 11 x 0.1 cm to form the pad.

Salmon packaging, storage and sampling

Fifteen salmon (*Salmo salar*) of 13-15 kg were obtained from a commercial fish farm. These were live-chilled, bled and gutted, and immediately iced prior to transport. All fish were manually filleted 3 days after slaughter to obtain fillet slices of 8 x 9 x 3 cm· One day later (defined as day 0) the fillet slices were individually packed in high-density 750 ml polyethylene trays and sealed with a polyethylene film in a modified atmosphere of 60:40 N_2/CO_2 to a gas:fillet slice volume ≈ 5:1. Three different sample series (treatments) were prepared: fillet slices packed without a pad, slices packed on their underside with a standard absorbent paper pad currently used in food packaging, and fillet slices packaged with *Sphagnum* pads on the top and bottom side. In total 65 fillet slices were packed; 21 in each pad treatment and 23 without a pad. These were immediately stored at +4 °C. After 2, 6, 9, 13, 16, 20, 27 and 30 days three replicate fillet slices from each treatment were removed from cold storage for analysis. At day 0 only 2 fillet slices packed with no pad were sampled.

Analytical analysis (n=3) of sampled fillet slices

The O_2, CO_2 and N_2 content of the tray were quantified by a gas analyser. Muscle pH was measured in the fillet slices. Water content was determined by drying 2 g samples (n=3) of each fillet slice at 105 °C for 24 h. Water holding capacity was determined on ground muscle as described by Eide *et al.* (1982). Fillet slice colour was determined with a salmon graduate colour card. Extractable protein, acid soluble peptides and free amino acids were determined as previously described (Hultmann & Rustad, 2002; Hultmann & Rustad, 2004).

Microbiological analysis

A 50 g piece of fillet slice (n=1) was aseptically cut and homogenized at 200 rpm for 30 second in filter bags with 4 parts sterile peptone saline, pH 7.2, using a stomacher. These homogenates were further diluted (1:5) with peptone saline. Appropriate dilutions (10^{-2} – 10^{-6}) were spread on Long and Hammer agar plates (n=3) with 1% (w/v) added NaCl (van Spreekens, 1974) and incubated at 15 °C for 7 days, prior to counting the number of colony forming units (CFU) and expressing them per gram wet weight of fish (N.B. The water content of each fillet slice was relatively constant). Typical isolated colonies were identified by 16S rDNA sequence analysis (NCIMB Ltd, Aberdeen, Scotland).

Textural properties and smell analysis

Textural properties were measured with a TA.XT2 Texture Analyser as previously described (Hultmann & Rustad, 2002). Smell was determined by sensory analysis by a panel of 5-7 scientists each smelling the fillet slice immediately and 10 min after opening the package. Smell was ranked on a integer scale from 1 to 6 (1- fresh seaweed-like smell, acceptable, 2- odourless, acceptable, 3- slight fishy odour, acceptable, 4- significant fishy odour, borderline acceptable, 5- strong fishy odour, not acceptable, 6- totally off, i.e. putrid smell, not acceptable).

RESULTS AND DISCUSSION

The proportion of CO_2 in the atmosphere of all the packages (no significant differences between treatments) decreased from 60% at day 0 to a near constant value of around 44-45% after day 6. This is possibly because of diffusion of CO_2 into the fish muscle (Killeller, 1930). On the other hand the concentration of O_2 in the trays increased slightly from not detectable at the start of the experiment to a maximum of 0.18% by day 6. This is probably because of a slow diffusion of air into the tray. By day 9 no O_2 was detected in any tray because all is consumed, primarily by bacterial respiration. The pH and colour of all fillet slices remained constant at around 6 and salmon red respectively throughout the experiment. It is likely that the CO_2 content inside the tray had a buffering effect on fillet slice pH and a stabilising effect on the fillet slices pigments (Killeller, 1930).

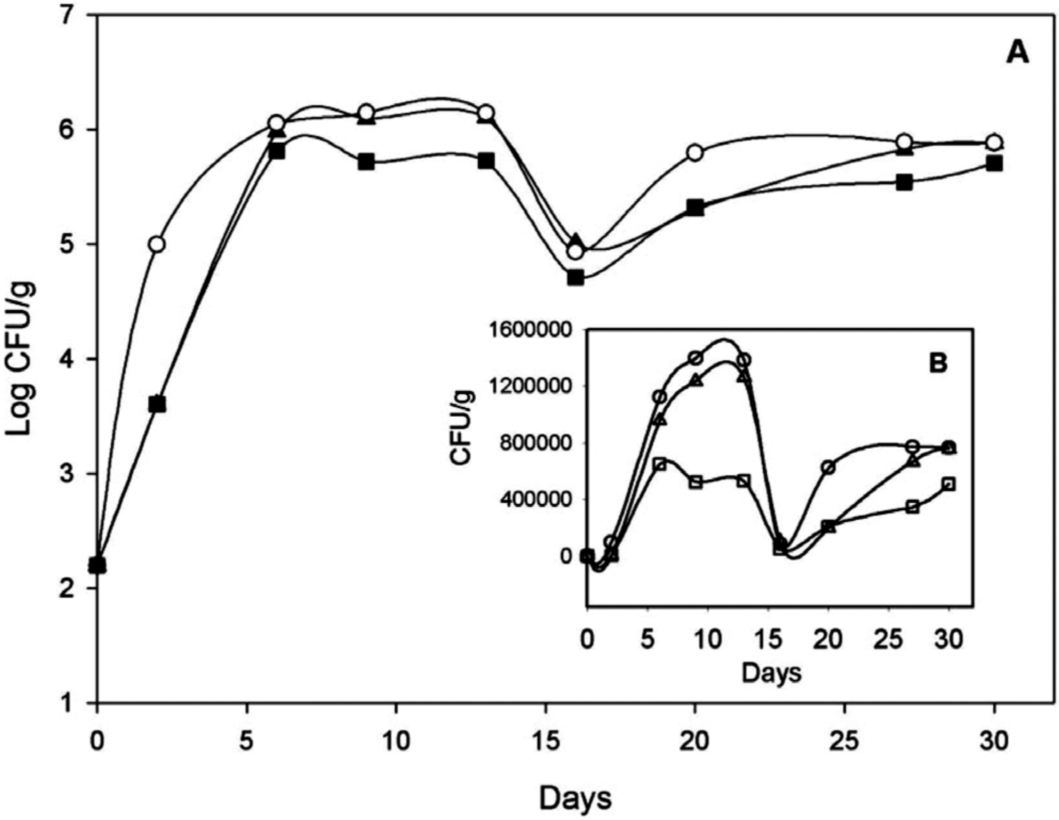

Fig. 1. Log (**A**) or number (**B**) of colony forming bacterial units (CFU) (mean, n=3 ±S.D.) per gram wet weight fillet slice packed without a pad (triangles), packed on the underside of the fillet slice with a standard absorbent paper pad (circles), and packed with *Sphagnum* pads (squares) on the top and bottom side. Fillet slices (n=1) were sampled on the time internals depicted.

Not surprisingly the modified atmosphere packaging (MAP) also had a large influence on the microbiology of the fillet slices over the course of the experiment. At the start of the experiment the observed viable bacterial count was about 10^2 CFU/g which increased at an exponential rate in the log-growth phase to 10^6 CFU/g after 6 days (Figure 1a). Such levels of viable bacteria are typical for MAP packed fish fillet slices (Reddy, 1997, Cann, 1984, Pastoriza, 1996, Stier, 1981). Although there is only one sampling point it is interesting to note that after 3 days storage in the log growth phase, fillet slices in contact with a standard pad used in current food packaging (Figure 1), had about a one and a half magnitude greater viable bacterial count (10^5) than fillet slices in the other treatments. The reason for this is currently unclear.

Between 6 and 12 days of storage the stationary growth phase became established were the viable bacterial count remained relatively constant (Figure 1a). The dominant bacterium in this growth phase was identified as *Photobacterium phosphoreum*; typical of MAP packaged fish (Dalgaard *et al.*, 1996). Between day 12 and 16 there is a death phase followed by a secondary log and stationary phase

(Figure 1a). *P. phosphoreum* remains the dominant bacteria in this growth phase, but in addition colonies of *Lactobacillus maltaromaticus* start to appear. In food microbiology it is usually standard practice to consider significant differences in bacterial growth in terms of orders of magnitude. In this case no marked difference is seen in the stationary growth phases between fillet slices in each of the three treatments. However, examination of Figure 1b reveals that the viable count of bacteria in the fillet slices treated with the *Sphagnum* pad, in both the first and second stationary growth phases, is consistently about 50% less than the other control treatments. It therefore seems clear that the presence of such a pad has some influence in terms of decreasing the overall maximum viable bacterial count of the fillet slices. A number of explanations are possible: 1. the presence of the extra *Sphagnum* pad on the top of the fillet slice, 2. interaction of the pad with the fillet slice or with the bacteria (see Painter, 2003). 3. the *Sphagnum* pad has some anti-microbial properties in which the division of absorbed bacteria is impeded.

In addition to these properties we also found that the smell of the fillet slices stored with the *Sphagnum* pads were deemed acceptable to a consumer, in contrast to control fillet slices, right up to the end of the experiment. All fillet slices that contained pads had no free liquid floating around in the trays because as expected all was absorbed by the pad. Nevertheless, pad treatment had no significant effect on fillet slice texture, water content, water holding capacity, extractable protein or acid extractable peptides. The content, however, of extractable free amino acids was significantly different.

In conclusion it is clear to see that the application of *Sphagnum* pads to foods or food packaging may have a commercial potential. Further research, however, is required in designing a more durable pad and investigating the true extent of it apparent antimicrobial properties and how these may be optimised.

ACKNOWLEDGEMENTS

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DETERMINATION OF THE SHELF LIFE OF A NEW ANTIOXIDANT ACTIVE PACKAGING

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ABSTRACT

A new antioxidant active packaging containing rosemary extract or oregan as active agents is produced and evaluated versus time using fresh meat with and without modified atmosphere. The analytical method to extract and analyze the carnosic acid content, the main antioxidant in the rosemary extract, along the time in the packaging material has been developed. Such a method consists of an extraction with methanol followed by a clean-up step using an anionic exchange cartridge and then analyzing the carnosic concentration by UPLC-MS-MS. In the case of active packaging with oregan carvacrol concentration is monitored with time and the shelf life of the active packaging is established. TBARS test was applied to the meat packaged with these active materials. The results obtained are shown and discussed.

Key words: rosemary, carnosic acid, shelf-life, food storage

INTRODUCTION

The development of active packaging materials and specially those having antioxidant or antimicrobial properties is a challenging area in which several technologies are now under study. In this frame, the use of natural extracts from plants is becoming more and more interesting, as the synthetic chemicals are very limited when being in contact with food. This is particularly true when the natural extracts are accepted as food additives and this is the case of rosemary and oregan extracts. Our research group in collaboration with a Company has studied and produced new active antioxidant materials for food packaging using essential oils or their extracts as active agents (Pezo *et al.*, 2006). However, the technical evaluation, that means the behavior of the new material in food applications, as well as the knowledge about the mechanism of food protection is required. Previous work (Tovar *et al.,* 2005, Nerín *et al.,* 2006a, 2006 b) showed that no direct contact between the food and the active material is necessary. It is important to emphasize that no an universal solution can be proposed in terms of antioxidant or antimicrobial active packaging, as each food application need a different concentration of active components and then each material should be optimized *ad-hoc*.

On the other hand, the optimization of the concentration involves the analysis of the active agents in the new material versus time, in order to determine the shelf life of the material and the prediction of the shelf life of the packaged food. As the active agents are complex mixtures of organic compounds and only som of them are been recognized as antioxidants, the first problem is the identification of the individual compounds with antioxidant properties in each case and then, the quantitative analysis in both the material and the food or food simulant. This is the main focus of this research. That means, i) the optimization of the analytical procedure for carnosic acid, the main antioxidant in Rosemary extract and thus in the antioxidant packaging-rosemary –based; ii) the optimization of the carvacrol analysis in the atmosphere generated from the oregan-based active material; iii) the technical evaluation of the antioxidant activity in fresh meat packaged with the oregan-based material; iv) the study of the shelf life of the active material itself versus the storage time. The results obtained are shown and discussed.

MATERIAL AND METHODS

Rosemary Active Packaging

Determination of carnosic acid in the active film is performed as following. The active film is extracted with methanol in an ultrasonic bath for 15 minutes. An interference, which can't be injected in the liquid chromatograph, is also extracted from the active packaging. An anionic exchange cartridge is used to recover the carnosic acid in a formic acid in methanol solution, but not the interference. Afterwards, the recovering solution is injected in a UPLC-MS-MS and therefore carnosic acid is quantified. This analytical method is explained in depth in previous work (Bentayeb *et al.,* 2006).

Meat was selected as the test food since the potential migration of some flavours from rosemary doesn't reduce its consumer acceptance. Meat was packaged combining modified atmosphere with rosemary active packaging and stored at -1°C in dark for about a month.

A simulation of the worst storage conditions was used to estimate the film shelf-life. Several samples of film were stored at 40°C for 11 days. Carnosic acid measures were taken after a half, 1, 2, 6, 8 and 11 days.

Oregano Active Packaging

Carvacrol is sampled from the atmosphere by single drop micro-extraction. A drop of two and a half microliter is suspended on the tip of a micro-syringe as the extraction media and exposed to the packaging headspace for 5 minutes at room temperature. In this way, some of the compounds from the generated atmosphere come into the extraction agent. Then, the drop is recovered and injected in a GC-MS device and carvacrol is subsequently quantified. This analytical method is also explained in depth in previous work (Romero *et al.,* 2005).

In this case meat was packaged without modified atmosphere in trays covered either by oregano active film or common polyethylene film and was stored at 4°C for 11 days. Meat degradation was screened by thiobarbituric acid reactive substances assay (TBARS) and was expressed as malondialdehyde (MDA) equivalents. At the same time, other trays without meat were stored in order to control the generated atmosphere.

RESULTS AND DISCUSSION

Rosemary Active Packaging

Two complementary experiments were carried out to understand the relevance of carnosic acid in the antioxidant capacity of the active packaging. Carnosic acid was screened both in usual meat-on-sale conditions and in industrial film storage conditions.

Shelf-life of packaged meat was slightly increased in those trays covered with active film, in comparison with the use of modified atmosphere alone. At the same time, the carnosic acid concentration in the active film decreases during meat degradation (figure 1). It means that carnosic acid is also being degraded. Furthermore, its initial concentration is only a tenth of the nominal amount of samples, so that the active film was previously degraded before its use. It was prepared 2 days before.

Active film shelf life at industrial storage conditions is presented in figure 2. Carnosic acid concentration fell down to 17% after the first day and, after two days, only 4% of the initial concentration remains and it goes on decreasing slowly. However, residual concentration is detected even after eleven days.

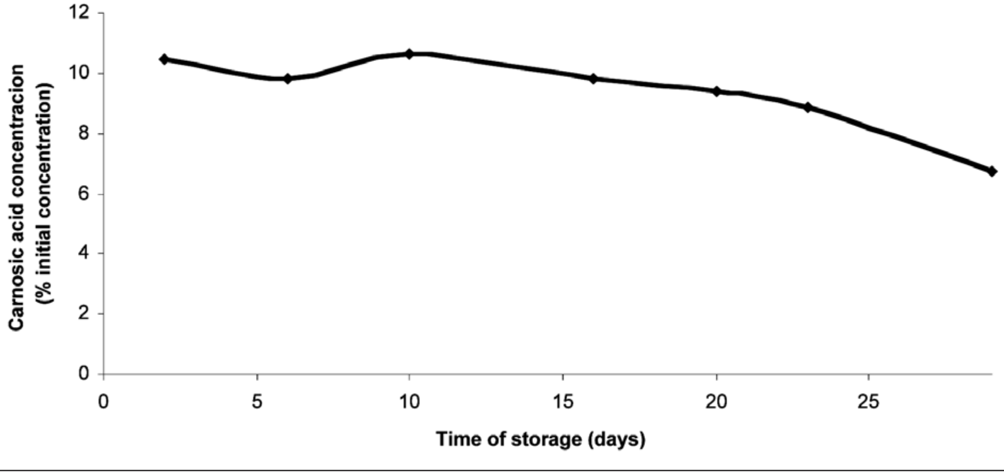

Fig. 1. Carnosic acid concentration (% of the initial concentration) versus time. Storage conditions: -1°C in dark.

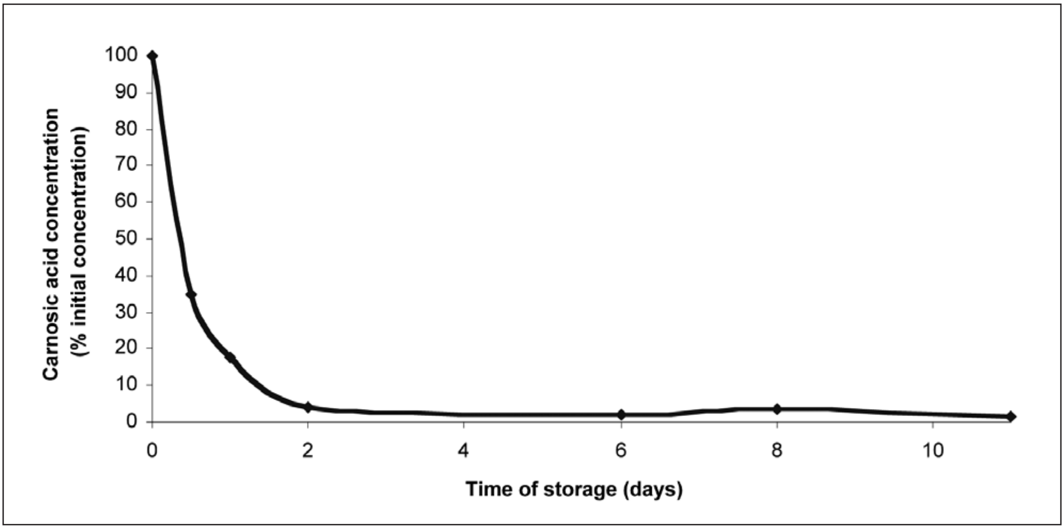

Fig. 2. Carnosic acid concentration (% of the initial concentration) versus time. Storage conditions: 40°C in dark.

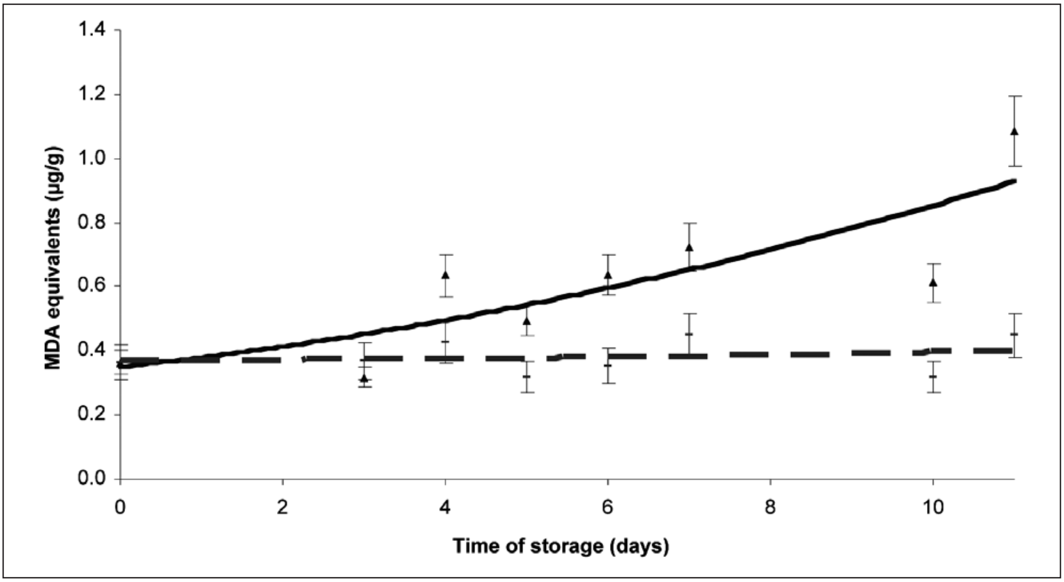

Fig. 3. Meat degradation (expressed as MDA equivalents) in trays covered either with a common polyethylene film (continuous line) or oregano active packaging (discontinuous line). Relative standard deviation expressed as vertical bars (n =3).

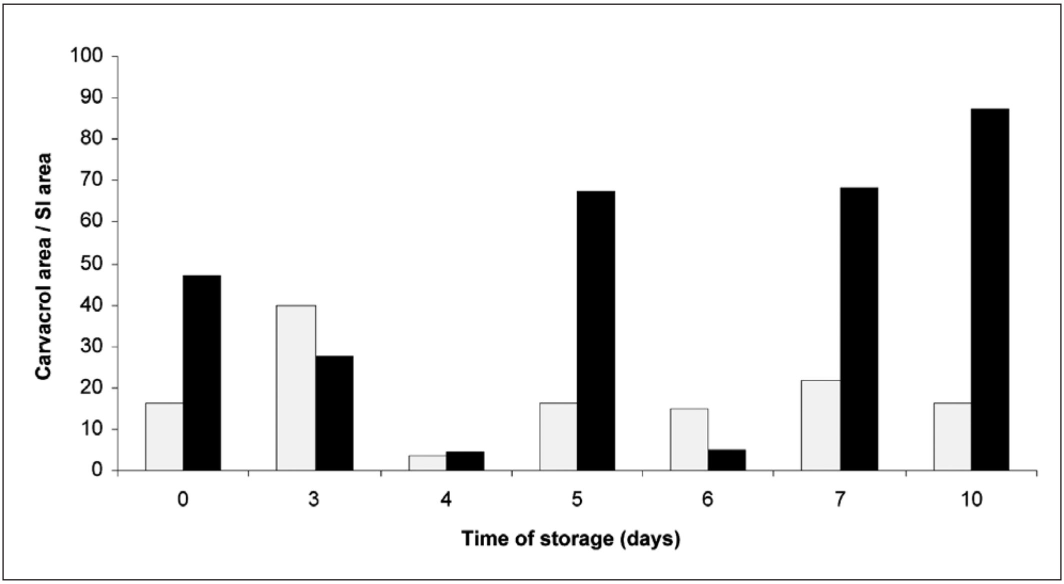

Fig. 4. Carvacrol concentration in the generated atmosphere with packaged meat (light) or without packaged meat (dark).

These results agree with the previous described ones. Despite of the antioxidant activity shown, most of the initial carnosic acid disappear quickly from the active film after its preparation. It means that other antioxidants are produced at the same time as carnosic acid degradation. Such antioxidants are related to carnosic acid through the called antioxidant cascade, which provides additional antioxidant activity to the active packaging thus making longer the shelf life of this active packaging. On the other hand, not to be neglected is the fact that such little amount of carnosic acid could be able to give antioxidant properties to the active package.

Oregano Active Packaging

As for rosemary active packaging, carvacrol was screened in the generated atmosphere in order to understand its relationship with the antioxidant activity of the packaging. Evolution of meat degradation is presented in figure 3. According to TBARS results, much more degradation occurs in trays covered with active film supporting the antioxidant capacity of the film developed.

On the other hand, carvacrol concentration was higher at the second half of the experiment time in those trays where no meat was packaged (figure 4). It means that carvacrol could be absorbed on the meat surface avoiding its degradation.

CONCLUSIONS

Despite of the fact that the rosemary active packaging enhances meat shelf-life, its main antioxidant component, carnosic acid, has not a representative behaviour of the evolution of the film antioxidant capacity. Carnosic acid concentration fells down quickly in a few days after active packaging preparation. Rosemary active film with less quantity of carnosic acid has to be studied to find out this point. Besides, other antioxidant compounds have to be screened in the film.

Oregano active packaging antioxidant capacity seems to be provided by carvacrol in the atmosphere. Furthermore, this antioxidant compound could be absorbed on meat avoiding its degradation. Quantification of carvacrol on the meat surface is a good study to clarify this point.

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VIRGIN OLIVE OIL PRODUCTION FROM DE-STONED PASTES: A NEW TECHNOLOGY TO IMPROVE THE SHELF LIFE OF THE PRODUCT

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ABSTRACT

The aim of increasing the quality standards for virgin olive oil is continuously stimulating the search for new technologies. In this sense, a new technological procedure is being developed that includes stone removal before the olive oil extraction process. Virgin olive oil contains a great number of volatile and non-volatile compounds mainly phenolic compounds responsible for its fragrant and peculiar flavour. These substances also contribute to the stability of the oil. Since the occurrence of phenols in virgin olive oil is strictly related to the activities of various endogenous enzymes of olive fruit, their concentration in the oil is strongly affected by the extraction conditions. In the olive seeds there is highly concentrated the peroxidase, an enzyme that catalyze phenol oxidation. The de-stoning process, excluding the olive seed before malaxation, partially remove the peroxidase activity in the pastes and consequently reduce the enzymatic degradation of the phenols in the oils improving their concentration and oil oxidative stability. Our results shown that the use of a de-stoner during the extraction process of olive oil increased total phenol content of the oil. A relationship between total phenol content and Induction time was found.

Key words: virgin olive oil, de-stoner, total phenol content, olive oil stability

INTRODUCTION

The quality of virgin olive oil is strictly correlated with the quality of fruits, with the harvesting systems and mostly with the techniques utilized to extract the oil, especially the machines for crushing the olives, for the kneading of the olive paste and separation of the oil phase. All operations required in the oil extraction process take aim at to obtaining the highest quality of oil from fruits. In such context the phase of preparation of the olive paste is one of the most important stage of the process where oil is mechanically extracted from the fruits. A large increase in demand for high-quality virgin olive oil during the past few years can be attributed not only to its potential health benefits, but also to its particular organoleptic properties. The aim of increasing the quality standards for virgin olive oil is continuously stimulating the search for new technologies. In this sense, a new technological procedure is being developed that includes stone removal before the olive oil extraction process (Amirante, Catalano, Amirante, Montel, Dugo, Lo Turco, Baccioni, Fazio, Mattei & Marotta, 2001). Virgin olive oil contains s a great number of volatile and non-volatile compounds mainly phenolic compounds responsible for its fragrant and peculiar flavour, (Caruso, Berra, Giovanini, Cortesi, Fedeli & Galli, 1999; Ragazzi & Veronesse, 1973). These substances also contribute to the stability of the oil and prolonging the shelf-life of the product (Caruso *et al.*, 1999; Di Giovacchino, Solinas & Miccoli 1994; Montedoro, Servili, Baldioli, Selvaggini, Miniati, & Macchioni, 1993, Papadopoulos & Boskou, 1991) and moreover protect consumers against cancer and atherosclerosis by impeding the oxidative modification of LDL and its adherence to the arterial wall (Armstrong, Paganga, Brunev, Miller, Nanchahal, Shipley, Rice-Evans, Marmot & Diplock, 1997; Nicolaïev, Lemort, Adorni, Berra, Montorfano, Rapelli, Cortesi & Jacotot, 1998). The different kinds of crushers used to produce olive pastes (Angerosa & Solinas, 1990; Angerosa & Di Giacinto, 1995) modify phenolic compound contents. In this work, we tested an innovative continuous processing line made up a de-stoner (instead of the usual metal crusher) and a new generation three phase decanter (without adding water), witch was compared with a stone mill-decanter processing line.

MATERIALS AND METHODS

Sample preparation: Olive fruits (*Olea europaea* L.) of the Coratina variety were harvested in olive groves of the same area near Foggia (Apulia–Italy) during the crop season 2004/2005. Olives were randomly picked at industrial optimum ripening stage, according to their skin colour. Harvesting was done by hand, using rakes. Ten oil samples were extracted, using a stone mill triple-phase decanter line (Alfa Laval Olive Oil, San Buca Val di Pesa, Florence, Italy) and ten with a de-stoner triple-phase decanter line (Alfa Laval Olive Oil, San Buca Val di Pesa, Florence, Italy). In each test olive paste was kneaded for 30 minutes at 27°C. The centrifugal decanter employed is a new generation three phase decanter Alfa Laval X32 model, therefore no water was added to olive paste prior to centrifuge. The decanter process parameters were fixed in the same way for all trials. The flux diagram of the processing procedures are shown in figure 1. All samples were filtered with anhydrous Na_2SO_4 and stored at 20°C in darkness using amber glass bottles without head space until analysis.

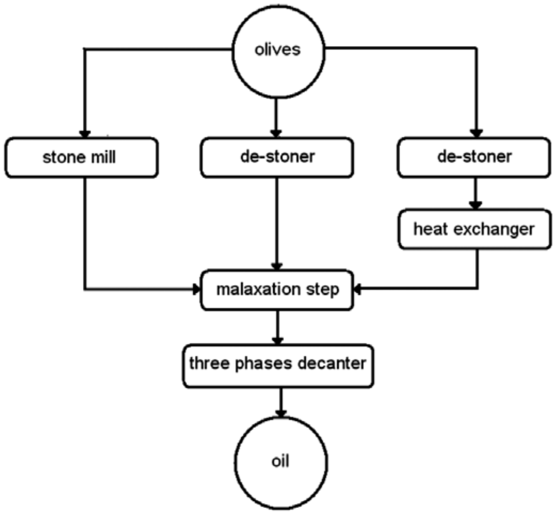

Fig. 1. Flux diagram of the processing procedures.

Acidity value, peroxide index and ultra-violet light absorption: Acidity value, peroxide index and ultra-violet light absorption (K_{232}, $K_{270)}$ were determined following the analytical methods described in the Regulation EEC/2568/91 of the Commission Regulation, 1991.

Oxidative stability: Oxidation induction time was evaluated by the Rancimat method. Stability was expressed as the oxidation induction time (h), measured with the Rancimat apparatus (Metrohm AG, Herison, Switzerland) using an oil sample of 2,5 g, warmed to 120°C and a purified air flow rate of 20 l/h. In the Rancimat method, the volatile degradation products were trapped in distilled water and determined conductometrically. The induction time was defined as the time necessary to reach the inflection point of the conductivity curve (Halbault, Barbè, Aroztegui, & De La Torre, 1997).

Total phenol content: Phenolic compounds were isolated from a solution of oil in hexane by triple-extraction with water–methanol (60:40 v/v). Total phenols, expressed as gallic acid equivalents (ppm), were determined with a UV visible spectrophotometer (Beckman) at 765 nm using the Folin-Ciocalteau reagent (Swain & Hillis, 1969).

Statistical Analysis. Experiments to determine the different olive oil parameters were performed in five times, the result are expressed as means ± standard deviations. One-way analysis of variance (Anova) were used. Differences between groups were considered statistically significant at $p<0,05$.

RESULTS AND DISCUSSION

Acidity value, peroxide index and ultra-violet light absorption: The oils examined were defined as belonging to the commercial class of extra virgin olive oil. No substantial difference was noticed which was related to the olive paste preparation technique.

Oxidative stability and total phenol content: Since the occurrence of hydrophilic phenols in virgin olive oil is strictly related to the activities of various endogenous enzymes of olive fruit, their concentration in the oil is strongly affected by the extraction conditions. Crushing and malaxation are the most important critical points of the oil mechanical extraction process (Servili, Selvaggini, Esposto, Taticchi, Montedoro & Morozzi, 2004). In these tests, when the stone mill was used to prepare the paste, the oils extracted contained much lower amounts of polyphenols (322±56 mg/kg) than the oils obtained using the de-stoner, (443±18 mg/kg). These variations were statistically different ($p\leq0.05$). The amount of polyphenols obtained using the de-stoner was higher in comparison of than oils obtained from the whole paste with an average difference of about of 30%.

The results obtained employing the de-stoner machine confirms previous researches (Servili, Baldioli, Begliuomini, Selvaggini, Montedoro, Lanzotti & Taglialatela-Scafati, 2000; Amirante *et al.*, 2001; 2002). In fact, oil mechanical extraction from de-stoned pastes can improve the oil phenolic concentration and it seems to confirm the relationships between the control of oxidative reactions during processing and the phenolic concentration in the oil because of the peroxidase is highly concentrated in the olive seed. The de-stoning process, excluding the olive seed before malaxation, partially remove the peroxidase activity in the pastes and consequently can reduce the enzymatic degradation of the hydrophilic phenols in the oils during the process thus improving their concentration and oil oxidative stability (Servili, Baldioli, Mariotti, Montedoro, Metzidakis & Voyiatzis, 1999).

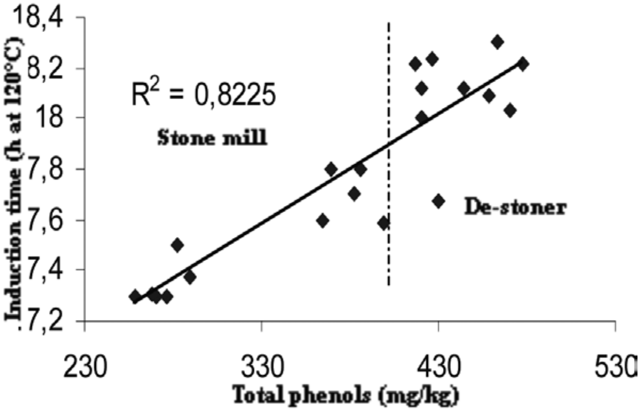

Fig. 2. Correlation between total phenols and induction time.

The antioxidant activity of hydrophilic phenols of virgin olive oil has been well studied (Servili *et al.*, 2004). In fact, as reported by different authors, the concentration of phenolic compounds, evaluated colorimetrically and expressed as total phenols, was highly correlated to the shelf life of virgin olive oil, tested using accelerated methods such as Rancimat (Gutierrez Gonzales-Quijano, Janer del Valle, Janer del Valle, Gutierrez Rosales & Vazquez Roncero, 1977). The mean value of Rancimat induction times (h at 120°C) of the samples obtained employing the de stoner (18.10±0.17 h at 120°C) was about 10% higher than stone mill oils (17.10±0.20 h at 120°C) ($p<0.05$). Fig. 2 relates the total phenols data to the corresponding induction times for the oils of the two extraction systems. The overall values show that a direct correlation exists between the two parameters. The regression line computed yielded $r^2=0.82$ ($p<0.05$).

CONCLUSIONS

The oils obtained from de-stoned pastes had a higher polyphenol content in comparison with the oils obtained from whole paste. The increment of the amount of phenolic compounds determined an increase of induction time to the oxidation. A significant correlation has been found between the content in total phenols and the induction time. The de-stoner is a soft technique of olive paste preparation that guarantees the highest amount of phenols, then a best shelf life of the oils.

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STUDY ON THE QUALITY OF 'SALAMI' WITH EXTRA-VIRGIN OLIVE OIL

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ABSTRACT

The effects on chemical, microbial and sensory quality of the partial substitution of pork backfat with extra-virgin olive oil were studied in 'salami' products. Five 'salami' formulations with total fat between 10 and 35%, were prepared. Two of these, were made up only with pork backfat, while other three formulations at 10% of total fat, in which 5% of pork backfat was substituited with extra-virgin olive oil, were prepared with or without added humectant (dry milk powder and maltodextrins) and treated with forced drying (ventilation). For all formula, three samples were analysed at each step of following process: making into sausages, beginning and end of ripening and after 15 and 30 days of storage at 4°C. The addition of extra-virgin olive oil, did not decrease the shelf-life in terms of hygienic and organoleptic quality. The results showed that this type of product, similar to the traditional one (at 35% of pork backfat), but with healthier features, could be produced with safety and a suitable shelf life.

Key words: biogenic amine; fat substitution; fermented sausages; microbiological analysis; olive oil; sensory evaluation

INTRODUCTION

Dry fermented sausages are meat products with a high fat content, as visible in the sliced product. Dry sausages made with a normal recipe have fat content around 32% directly after manufacture, but during the first week these value rises to about 40%, as a result of drying, and after 4 weeks to about 40-50% (Wirth, 1988). Pork backfat used in fermented sausages is rich in saturated fatty acids (about 40%) and cholesterol is the most important sterol present. It has been shown that high saturated and cholosterol consumption was linked to the development of cardiovascular diseases (Mattson & Grundy, 1985). Therefore, the world health organizations have promoted the reduction of fat in total dietary, particulary saturated fatty acids and cholesterol, as a means of preventing cardiovascular diseases (AHA, 1986; NCEP, 1988; Department of Health, 1994). For this reason, nutritionists, generally, recommend a change in composition of the fat associated with meat products by substitution of saturated fatty acids with monounsaturated fatty acids (Zanardi *et al.,* 2000). A possibility to reduce the negative effects derived from the high fat amount of 'salami' products, is the partially substitution with other ingredients; the use of vegetable oil containing unsaturated fatty acid instead of animal fat is one of these strategies. Olive oil is the most monounsatured vegetable oil (56.3-86.5%) with a high biological value attributed to the high ratio of vitamin E and polyunsatured fatty acids contents (Viola, 1970). It is also rich in tocopherols and phenolic substances which act as antioxidants. Therefore, the positive effects on the consumer health could be further improved by producing fermented sausages with the simultaneous reduction of fat level and partial replacement of pork backfat with olive oil. Pork backfat plays a significant role in the processing and quality characteristics of 'salami' products, because it affects drying process, flavour, texture and colour. For these reasons dry fermented sausages involve many technological drawbacks because of fat reduction (Wirth, 1988). The purpose of the present research was to improve the quality of 'salami' with extra-virgin olive oil using some usual humectants (dry milk powder and maltodextrins) and applying a step of forced drying, to reduce the water activity and to obtain a structure similar to conventional products. The effects on chemical, microbial and sensory quality of these products were studied during ripening and preservation.

MATERIALS AND METHODS

Five formulations (table 1), differing in pork backfat and extra-virgin olive oil content were studied. 'Salami' samples were hand-produced in the laboratory of foods preserves technology (Agricultural Faculty of Foggia University). Both fresh lean pork meat and backfat (previously minced using a draw of 4.5 mm holes diameter) were supplied by a local market, as also the extra virgin olive oil and other ingredients. The samples with extra-virgin olive oil differed in applied technology (forced drying process before ripening or traditional process) and added of humectants (dry milk powder from Digei s.r.l., Foggia, Italy and maltodextrins from Nutrivis s.r.l., Milan, Italy (table 1). All other ingredients were added in the same concentrations to each formulation. The prepared sausage mixture, were then stuffed using vacuum stuffer into 40 mm diameter collagen casings to produce a 'salami' 200-220 g in weight. Drying and ripening steps were carried out in the fermentation room (BINDER KBF240) at 24°C - 85% RH and 20°C-80% RH rispectively. Forced drying was carried out using a pilot plant (tunnel with punched tape) SANDVIK Process System (Schiedam, Netherlands) in the following conditions: air mouvement 3 m/s, air temperature 19-22°C. The length of each step was following: 1 day for equilibration and mixing, 1 day for manufacture, 2 days for drying and 19 days for ripening. For all formulations, three samples were analysed at each step of the process (equilibration/mixing, making into sausages, beginning and end of ripening and after15 and 30 days of storage at 4°C). Measurements of pH were carried out with a digital pH-meter Crison 2001 (Barcelona, Spain) on 10 g of sample mixed with 20 ml of water and homogenized in a blender (Stomacher 400, PBI International, London, England). Water activity (a_w) was measured with a point of dew hygrometer

Table 1. Ingredients, formulations and technology applied for 'salami' production (% on total weight of each formula).

Ingredients	F1	F2	F 3	F4	F5
Lean pork meat	90%	90%	90%	90%	65%
Pork backfat	5%	5%	5%	10%	35%
Oil	5%	5%	5%	-	-
NaCl	2.5%	2.5%	2.5%	2.5%	2.5%
D-glucose	0.5%	0.5%	0.5%	0.5%	0.5%
Finnel seeds	0.1%	0.1%	0.1%	0.1%	0.1%
White wine	0.2ml/100g	0.2ml/100g	0.2ml/100g	0.2ml/100g	0.2ml/100g
Garlic	0.01%	0.01%	0.01%	0.01%	0.01%
Pepper	0.1%	0.1%	0.1%	0.1%	0.1%
Potassium nitrate	187ppm	187ppm	187ppm	187ppm	187ppm
Sodium nitrite	75ppm	75ppm	75ppm	75ppm	75ppm
Milk powder	-	-	3%	-	-
Maltodxtrine	-	-	1.5%	-	-
Technology	Ventilated	Traditional	Ventilated	Traditional	Traditional

Aqualab CX-2 (Decagon Devices Inc., Pullman, WA). Calibration was performed using saturated solutions of known a_w. The microbiological analysis were carried out to determine the presence of lactic acid bacteria (growth on MRS agar - Oxoid, Milano - + 0.17g/l of cicloeximmide - Sigma Aldrich, Milano - at 30°C for 48-72 h) and moulds (growth on MEA - Malt Extract Agar, Oxoid - substratum not acidified at 25°C for 4 gg). The content of biogenic amines, were determinated according to the method of Palermo et al, 2004. Sensory evaluation was performed at the end of ripening by 33 untrained panel members using a triangular test. The panellists were to identify the odd sample between three slices of product (two equal ones and only one different) and they were to indicate the perceived difference. The basic formulation (without extra-virgin olive oil) was used as a discriminant sample. The difference between samples is considered to be significant, if the number of correct answers is 17 (α=0.05), 18 (α=0.01) and 21 (α=0.001) (Porretta, 2000). Results were expressed as frequency percentages of the panelist responses.

RESULTS AND DISCUSSION

The figure 1 shows the evolution of pH and the cell load of lactic acid bacteria and moulds in 1 and 3 formulations. The cell load of lactic acid bacteria of formula 1 increased within the storage; due to their metabolism pH decreased from 5.72 to 5.07 at the beginning of ripening. The growth of the moulds after 3 days caused an increase of pH up to 6.57. Their presence allowed to obtain an uniform ripening and a regular dehydratation. The cell load and the pH values showed the same trends for the formulation 2, 4 and 5 (data not shown). Significant differences were observed for the formulation 3; in fact, the pH values decreased from 5.72 to 4.9 at the beginning of ripening, then it remained constant within the whole phase of ripening and storage. The absence of deacidification phase depended on the low

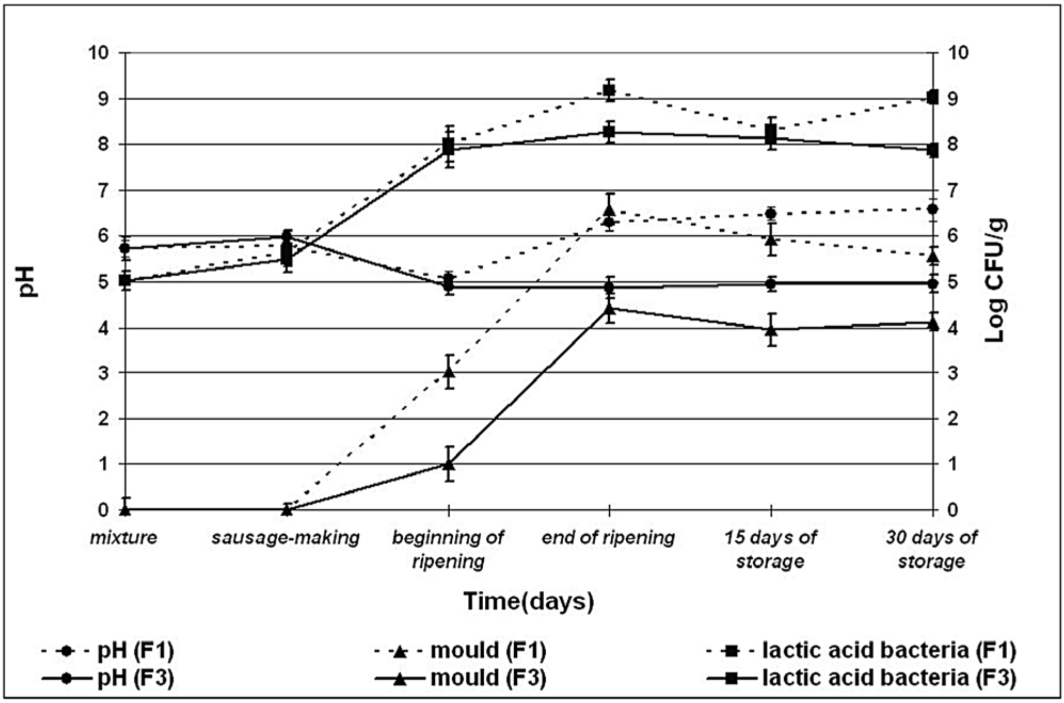

Fig. 1. Evolution of pH and the cell load of lactic acid sacteria and moulds in the formulations 1 and 3 (F1: 5% oil + forced drying; F3: 5% oil + forced drying + humectants).

cell load of moulds at the beginning of ripening (1 Log CFU/g); but they increased up to 4.42 Log CFU/g at the end of the storage. The figure 2 reports the evolution of 'salami' water activity of formulation 1, 3 and 5. The samples of formulation 1 showed a decrease of water activity from 0.993 to 0.879 at the end of storage. Water activity showed the same trends for the formulation 2 and 4 (data not shown). A different trend was pounted out for the formulation 3; in fact a greater decrease of this parameter was observed (0.833) at the end of storage. The excessive and rapid dehydratation of bowels during the ripening, because of the combined effect of the humectants and the forced drying, could explain the moderate cell load of the moulds. The 'salami' water activity of formulation 5 atteined at 0.842 at the end of the storage; however a higher cell load of moulds was observed. The formulation with the highest fat content (F5) showed a greater dehydratation than those added with olive oil without humectants (F1, F2). Olive oil covered probably the pieces of meat in the mixture and prevented the release of moisture (Bloukas *et al.*, 1997). A similar decrease of this parameter, instead, was not observed for the salami with the least fat content (F4). The content of biogenic amine in the five formulations after ripening and refrigerated storage is shown in table 2. Spermine, spermidine, putrescine and cadaverine were found in the samples, but its amounts resulted very lower than results reported in literature (table 3). With regard to organoleptic characteristics (taste, texture and colour) the difference (table 4) between the sample with 5% oil and the basic formulation was to be significant (α=0.01).These differences were evident (45.84%) because the oil substitution was considered to be

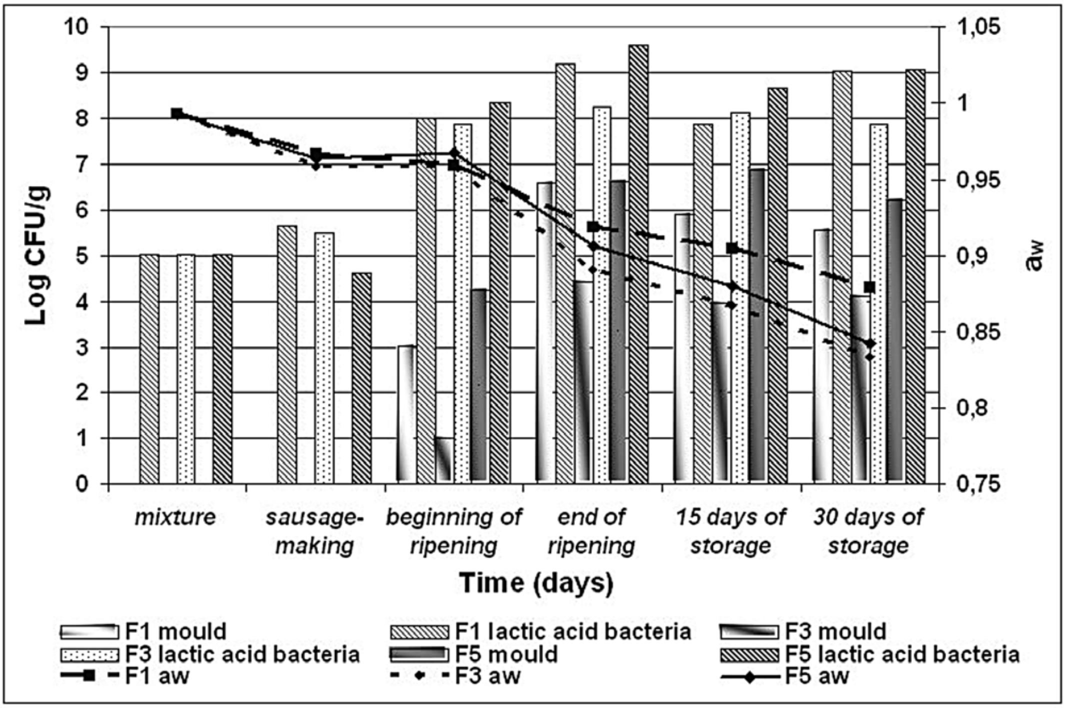

Fig. 2. Evolution of water activity and the cell load of lactic acid sacteria and moulds in the formulations 1, 3 and 5. (F1: 5% oil + forced drying; F3: 5% oil + forced drying + humectants; F5: 35% pork backfat).

Table 2. Biogenic amine contents (mg/Kg) of 'salami' after ripening and refrigerated storage (T2: end of ripening; T3: 15 days of refrigerated storage; T4: 30 days of refrigerated storage).

Formulations	Putrescine			Cadaverine			Spermine			Spermidine		
	T2	T3	T4	T2	T3	T4	T2	T3	T4	T2	T3	T4
F1	9,42	21,28	30,24	1,00	6,59	11,66	2,27	1,00	1,00	1,00	2,09	4,82
F2	10,16	14,89	13,69	8,12	11,17	9,25	3,18	2,93	1,00	0,57	2,70	4,54
F3	15,19	15,97	17,91	5,99	6,88	6,78	3,31	3,67	1,00	0,63	1,64	2,88
F4	7,57	18,03	16,17	11,19	40,20	40,41	2,33	1,00	1,00	0,13	1,85	1,97
F5	5,24	6,41	5,82	2,42	2,72	2,21	4,61	1,00	1,00	0,27	1,27	1,47

Table 3. Biogenic amine contents (mg/Kg) of different types of dry-fermented sausages after ripening (Suzzi and Gardini, 2003).

	Putrescine	Cadaverine	Spermine	Spermidine
Industrial dry-fermented sausages	279	103	91	5.1
Traditional dry fermented sausages	223	71.3	83.7	4.3

Table 4. Triangular test between F2 (5% oil + 5% porkbackfat) and F4 (control with 10% porkbackfat).

Answers %		Perceived difference (degree %)			Perceived difference (type %)		Perceived difference (sensorial characteristics %)			
Right	Wrong	Little evident	Evident	Very evident	Positive	Negative	Texture	Taste	Colour	Odour
72.73	27.27	29.17	45.83	20.83	45.84	54.16	33.33	29.17	4.17	16.67

Table 5. Triangular test between F1 (5% oil + 5% porkbackfat ventilated) and F2 (5% oil + 5% porkbackfat not ventilated.

Answers %		Perceived difference (degree %)			Perceived difference (type %)		Perceived difference (sensorial characteristics %)			
Right	Wrong	Little evident	Evident	Very evident	Positive	Negative	Texture	Taste	Colour	Odour
57.58	42.42	26.32	63.14	10.54	52.63	42.10	21.05	47.37	5.26	5.26

negative by the panelists (54.16%) in terms of texture (33.33%) and taste (29.17%). The forced drying application has improved this characteristics (table 5). The panel carried out on samples with 5% oil submitted and not to the forced drying was significatively different (P=0.001) and evident for 63.13% of panelists. The differences perceived were positive for 52.63% of correct answers in terms of taste, texture (more compact), colour (red intense) and odour. Most panelists appreciated 'salami' made with oil more than basic formulation, because of its pleasant aromatic note.

CONCLUSION

The partial substitution of pork backfat with extra-virgin olive oil did not substantially affect the microbial, chemical and sensory characteristics of the products, with of exception of texture. The addition of extra-virgin olive oil did not reduce the shelf-life in terms of microbial growth and biogenic amine contents. The forced drying application has improved the sensorial characteristics of 'salami'. The sample with 5% oil, submitted to forced ventilation was judged the best of all.

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SHELF-LIFE OF VEGETABLE OILS BOTTLED IN PLASTIC (PET) CONTAINERS WITH OXYGEN-SCAVENGERS

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ABSTRACT

The present work reports a study on the shelf-life of extra virgin olive oil (EVOO) and of sunflower oil (SO) bottled in containers with different oxygen barriers and stored at room temperature under diffuse lighting conditions for 6 months. Four materials were tested: glass, polyethyleneterephthalate (PET), polyethyleneterephthalate including 1% of oxygen scavengers (PET 1%), polyethyleneterephthalate including 5% of oxygen scavengers (PET 5%). Free acidity, peroxide value (PV), spectrophotometric indices (K_{232}, K_{270}, ΔK), antioxidant (biophenols and tocopherols) content, pigments (chlorophylls and carotenoids) evolution and the amount of oxygen dissolved in the oil were monitored during storage. A significant influence of the packing material on the quality decay was not found. The difference in the shelf-life observed between oils bottled in PET and oils bottled in glass are attributable to a difference in the initial content of dissolved oxygen in the oils. The study showed the usefulness of monitoring changes in dissolved oxygen level, antioxidants (phenols and tocopherols) and pigments (chlorophyll and carotenoids) profiles during oil storage. The evolution of these compounds could in fact supply important information on oxidation processes which occur in bottled oils and on effectiveness of the material employed in oil packaging.

Key words: vegetable oils, shelf-life, oxygen scavengers, PET, oxidation

INTRODUCTION

The main cause of qualitative deterioration during the storage of vegetable oils is represented by the oxidation of polyunsaturated fatty acids, which gives rise to the formation of hydroperoxides (primary oxidation products). These compounds undergo a subsequent decomposition which originates a set of volatile compounds responsible for the onset of rancid off-flavours, with consequent decline in the nutritional and sensory quality of the product (Frankel, 1998; Guillén and Ruiz, 2005). Beyond meeting the practical needs of consumers, food packaging is intended to slow down the qualitative decay of the product protecting it from biological contaminations and undesirable chemical reactions. In vegetable oils packaging, one of the most important characteristics of a container is represented by its barrier properties against natural pro-oxidant agents such light and oxygen. Nowadays, plastic polymers, and in particular PET (polyethyleneterephthalate), cover a wide range of the packaging structures used in food industry due to their "thermoplastic" nature, that guarantees the recyclability and consequent low environmental impact. Moreover, PET satisfies many important requirements: good aesthetic aspect (brilliance and transparency); suitability for colouring; good mechanical, thermal and chemical resistance; low production cost; suitability for prolonged storage and low weight with respect to glass bottles (Tsimis and Karakasides, 2002). While glass containers are able to completely prevent oxygen exchange, plastic materials are only able to slow down this exchange, because of their oxygen permeability (Del Nobile *et al.,* 2003a). However, in order to reduce the diffusion of oxygen into the bottled oil, various solutions have been tried, the most valid of which is probably represented by the use of "oxygen-scavengers", which are used in order to prolong the *shelf-life* of foods whose kinetic of degradation depends on the partial pressure of oxygen inside the container (Bove *et al.,* 2002, Vermeiren *et al.,* 1999; Kaya *et al.,* 1993). The fundamental characteristic of these molecules is the ability to remove the oxygen dissolved into the oil and to provide a barrier to oxygen diffusion from atmosphere into the bottled liquid; moreover, scavengers can be easy incorporated into the packing material without altering its properties (Maloba *et al.,* 1996). Previously studies regarding the stability of vegetable oils showed an effect of type of packaging materials on the oil quality (Gambacorta *et al.,* 2004; Kaya *et al.,* 1993; Del Nobile *et al.,* 2003b). The aims of this research were: i) to study the oxidation process of extra virgin olive oil and sunflower oil stored in glass, PET and PET loaded with oxygen-scavengers, ii) to evaluate the influence of industrial packaging on the shelf-life of oils during 6 months of storage at room temperature under diffuse lighting conditions, iii) to correlate the antioxidants content decline to the oxidation parameters and to the evolution of dissolved oxygen in the oil.

MATERIALS AND METHODS

Sampling

Italian extra virgin olive oil (EVOO) and sunflower oil (SO) were packed in 1 l glass bottles (glass), in 1 l PET bottles (PET) and in 1 l PET bottles loaded 1% *oxygen scavengers* (OS) (OXISTOP^{TM,}, SIPA, Italy) (PET 1%) and 5% (PET 5%). PET bottles were characterized by a thickness of 0,35 mm.

Glass and PET bottles were rinsed by two automatic packing equipment (IOBM, Industria Olearia Biagio Mataluni, Montesarchio (BN) Italy). Bottles were sealed with standard polypropylene threaded caps and were stored at controlled room temperature (25°C ± 4°C). During night-time, bottles were exposed to a constant light intensity of 300 LUX originated from 8 artificial neon lights, while during daytime light intensity reached 400 LUX because of the effect of daylight. Three bottles per treatment (combination oil/packing material) were analyzed monthly up to 6 months.

Oil quality parameters. Determinations of free acidity, peroxide values (PV), spectrophotometric indices (K_{232}, K_{270}, ΔK) were carried out according to the European Official Methods of Analysis (European Economic Community Regulation 2568/91).

Tocopherol content. Tocopherols content was determined by reversed phase HPLC analysis on a LC-10AD Shimadzu (Milan, Italy) liquid chromatograph equipped with a SPD M10A VP diode array detector (Shimadzu). The chromatographic separation was achieved on a 5 µm ODS-3 Prodigy (250 mm, 4.6 mm i.d.) reversed-phase column (Phenomenex, Macclesfield, UK). The elutographic conditions were those reported in previous works (Tonolo and Marzo, 1989; Napolitano *et al.*, 2004).

Biophenol content. Extraction of EVOO phenolic compounds and their quantification by reversed-phase HPLC according to the method previously reported (Sacchi et al. 2002; Fogliano et al. 1999).

Pigments. Chlorophylls and carotenoids evaluation was carried out by measuring the absorbance in the visible region of an oil solution in hexane (1:1 v/v). The wavelengths for chlorophylls and carotenoids were 670 and 450 nm respectively (Minguez *et al.*, 1991).

Panel Test. EVOO organoleptic assessment was carried out according to European Official Method of Analysis (EEC Regulation 796/2002). Analogous evaluation was carried out for rancid perception in SO.

Dissolved oxygen in the oil. Dissolved oxygen level in the oil was measured by an oxygen analyzer equipped with an electrochemical oxygen sensor (model OX 22; Aqualytic, Langen, Germany). A 12- mm diameter membrane was used. The probe was immersed in the oil in the middle portion of the bottle and the electrical signal was registered 3 minutes after the immersion.

RESULTS

The evolutions of chemical-physical quality parameters (free acidity, peroxide value, spectrophotometric indices) during storage of EVOO and SO bottled in different containers are reported in Figure 1 and Figure 2 respectively. The level of free acidity did not change significantly during 6 months storage in all oils ranging from 0.54 to 0.61 and the official limits for this parameter (0,8% for EVOO – EU Reg. 1989/03; 0,5% for SO – NGD, 1994) have not been exceeded. Peroxide values (PV) increased during the first 35 days, then decreased; anyway, their values were fully within the European Official limits (20 meq O_2/kg) for EVOO and the Italian limits (10 meq O_2/kg) for SO. EVOO showed an increase during storage in

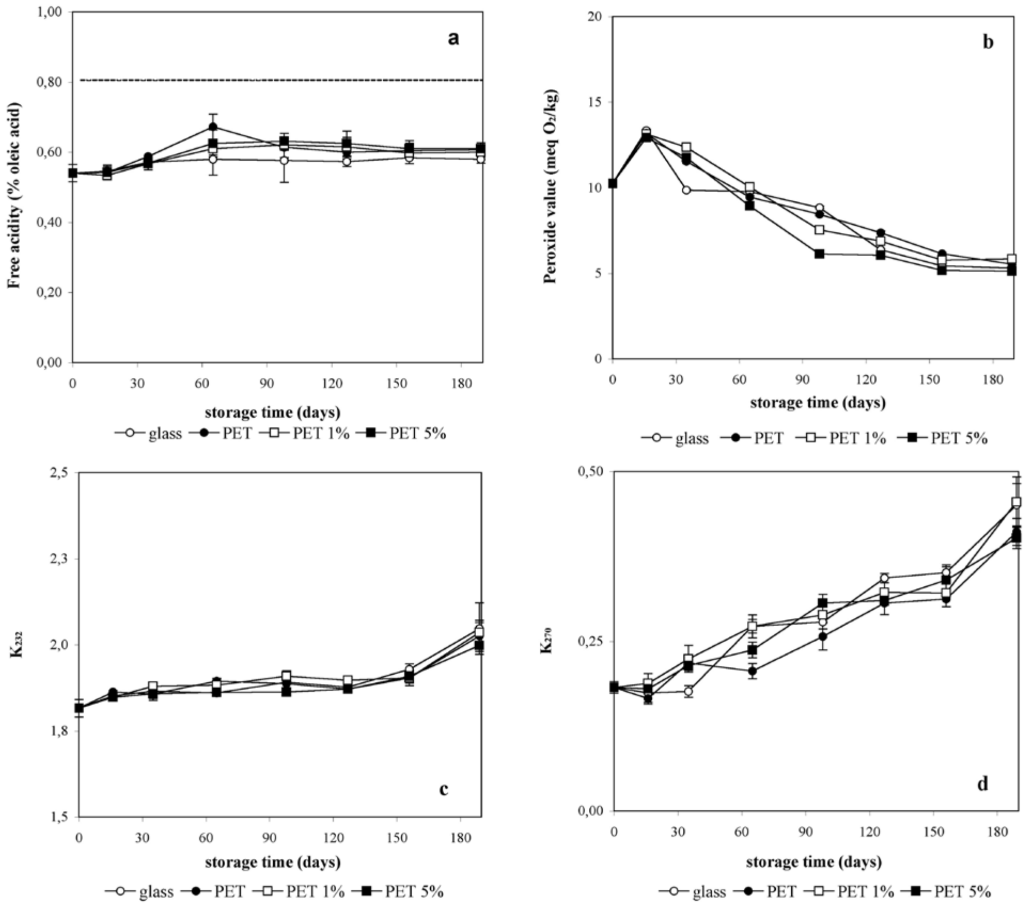

Fig. 1. Evolution of quality parameters (Free acidity, a; Peroxide value, b, K_{232}, c; K_{270}, d) measured (mean ± st. dev., n=3) in EVOO bottled in different containers during storage.

the values of the extinction coefficient at 232 nm (K_{232}), which is expression of the presence of conjugated dienes. This evidence, associated with the decrease of PV, can be related to the formation of aldehydes (2-alkenals) arising from peroxide decomposition and absorb at the same wavelength as conjugated dienes (Frankel, 1998). On the whole, however, the influence of the different packaging materials tested on the evolution of the primary oxidation indices (PV and K_{232}) was found to be negligible.

Dissolved oxygen evolution in the oils bottled in different containers is shown in Figure 3. During the first 3 months of storage, a decrease in dissolved oxygen was observed. During this period, the effect of scavengers included in the packaging material on dissolved oxygen content of oils was evident: oils bottled in PET 5% showed a dissolved oxygen content always lower than oils bottled in PET 1% and PET. Between 3 and 6 months of storage, the level of dissolved oxygen in the oils remained almost constant in all packages, indicating that the oxygen consumed

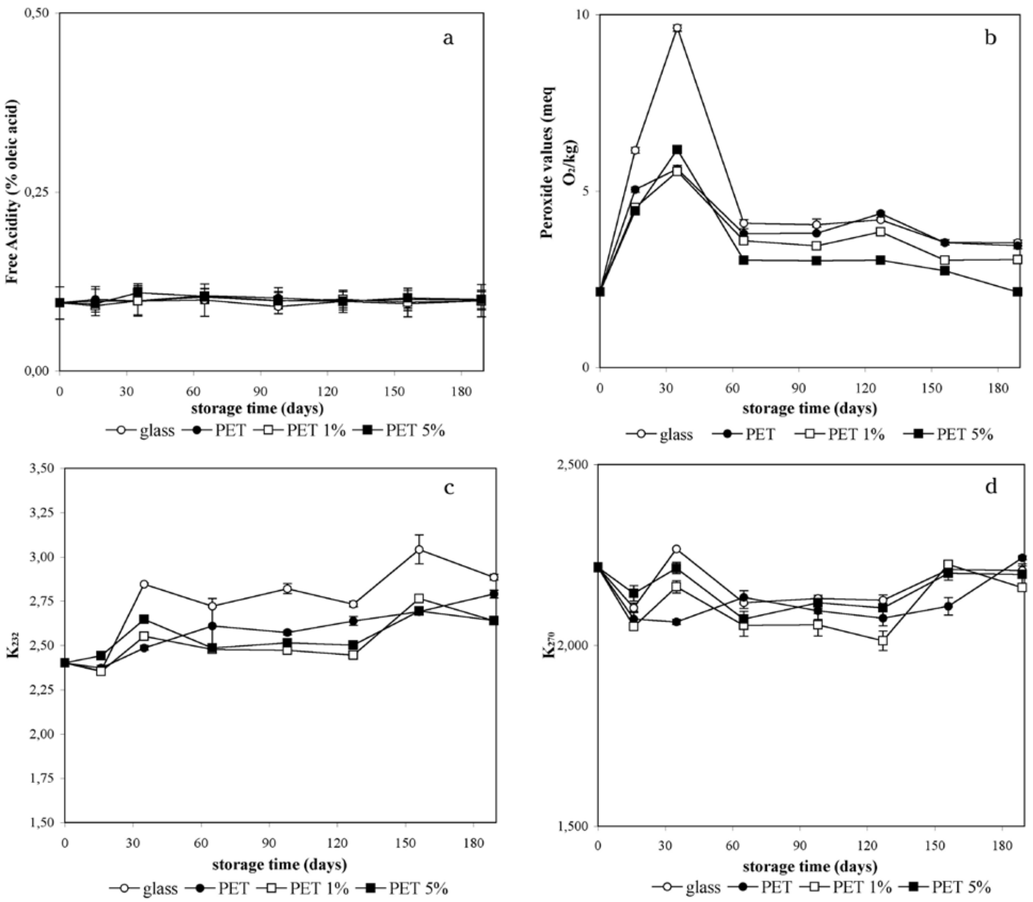

Fig. 2. Evolution of quality parameters (Free acidity, a; Peroxide value, b, K_{232}, c; K_{270}, d) measured (mean ± st. dev., n=3) in SO bottled in different containers during storage.

during storage was nearly limited to the initial content in the oil. This finding could lead to hypothesize a high oxygen barrier in all the plastic packaging materials tested (PET, PET 1% and PET 5%). Differences in initial values of dissolved oxygen (higher in glass than in the other containers tested) could be related to the different bottling lines used for glass and PET.

The evolution of α-tocopherol content during 6 months of storage is reported in Figure 4. α-tocopherol decreased with time, with higher intensity in oils bottled in glass than in oils stored in PET containers, at least in the first months of storage. Furthermore, from a comparison with the trends showed in Figure 5, a correlation between oxygen consumption and decrease of α-tocopherol is evident, suggesting that, in these experimental conditions (very low partial pressure of oxygen and diffuse light), the contribute of lipophilic antioxidants such us tocopherols is determinant in controlling and limiting the oxidation processes. This confirms what reported on α-tocopherol oxygen quenching activity, which is more intense

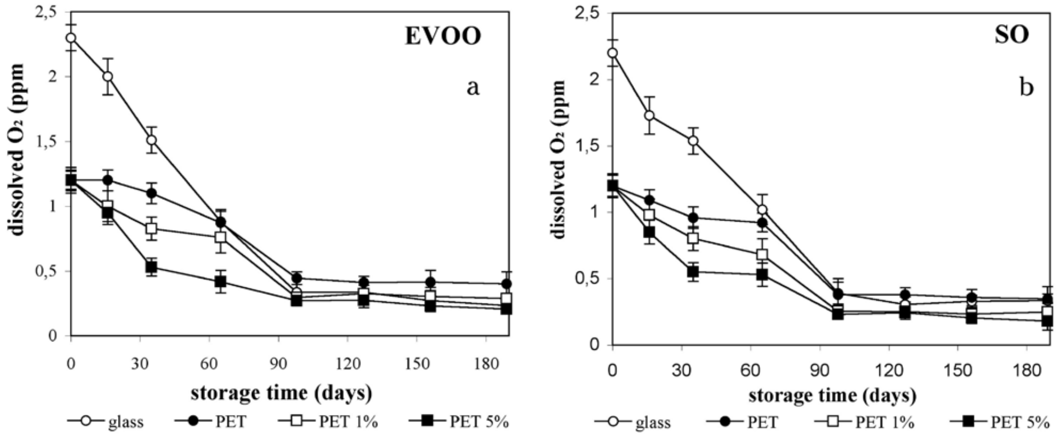

Figure 3. Evolution of dissolved oxygen (ppm O_2) in EVOO (a) and SO (b) bottled in different containers (glass, PET, PET 1%, PET 5%) during the shelf-life test (6 months, 25 ± 4°C; 400 LUX).

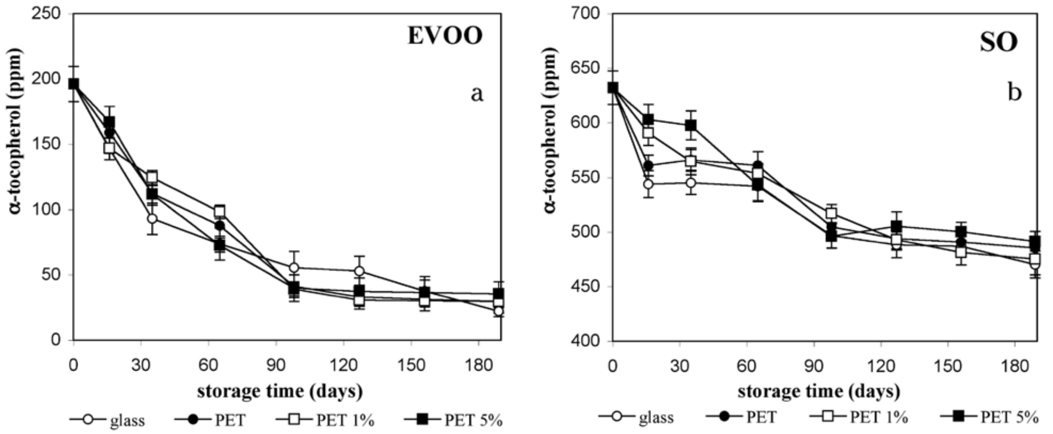

Fig. 4. Evolution of α-tocopherol (ppm) in EVOO (a) and SO (b) bottled in different containers (glass, PET, PET 1%, PET 5%) during the shelf-life test (6 months, 25 ± 4°C; 400 LUX).

in the photo-oxidation phenomenon (Psomiadou and Tsimidou, 2002; Frankel, 1998; Kamal-Eldin and Appelqvist, 1996; Gutierrez and Fernandez, 2002). Figures 5a and 5b show the evolution of chlorophylls and carotenoids in EVOO during the shelf-life trials. Pigments showed a decreasing trend in all oils independently from the packaging materials. Chlorophylls exhibited a strong decrease during the first two months of storage and was almost totally consumed after 3 months; carotenoids also decreased during the first period of storage, though slowlier than chlorophylls: after six months an appreciable amount of these pigments (around 40% of the initial content) was still observed. Such behaviour could be associated with the protective action exerted by α-tocopherol against carotenoids oxidation (Frankel, 1998).

The evolution of biophenols measured in EVOO during the six months of storage is

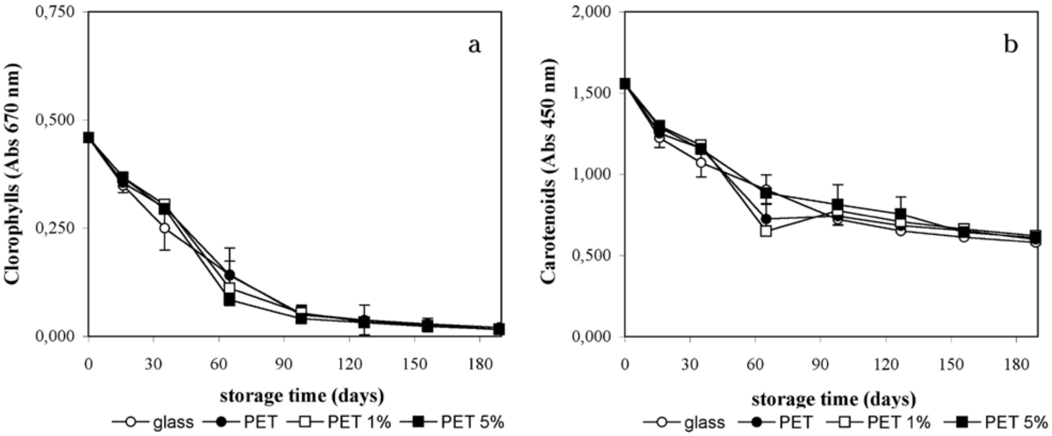

Fig. 5. Evolution of clorophylls (a; Abs 670 nm) and carotenoids (b; Abs 450 nm) in EVOO bottled in different containers (glass, PET, PET 1%, PET 5%) during the shelf-life test (6 months, 25 ± 4°C; 400 LUX).

reported in Table 1. The total content of phenolic antioxidants didn't show a significant decrease; only a slight increase of simple phenols (hydroxytyrosol and tyrosol) was observed, probably due to hydrolysis of complex phenolic compounds.

The organoleptic characteristics of EVOO didn't show significant differences during the storage period (data not shown), while in SO the onset of rancid perception (Figure 6) was evident already from the first months of storage. Increase of rancidity resulted more intense in oil bottled in glass than in oils in PET confirming the observations made for peroxide values (Figure 2b) and for dissolved O_2 (Figure 3b).

CONCLUSIONS

During storage at room temperature (25°C ± 4°C) under diffuse lighting conditions (about 400 LUX), significant effects attributable to the action of oxygen scavengers on the kinetic of oil oxidation were not observed. During the first months, in fact, the scavengers reduced the amount of dissolved oxygen in the oils but this decrease did not give rise to significant differences in quality parameters during the successive months. It was notable that the oxygen consumption was limited to the initial content in the oil and that there was no permeation through the package; nevertheless, these results are limited to a period of storage of only six months: scavengers might prove more efficient during a longer storage period. The differences in shelf-life found between oils bottled in

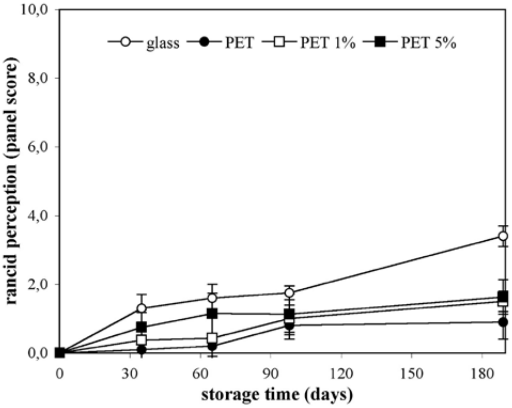

Fig. 6. Evolution of rancid perception (panel score) in SO bottled in different containers (glass, PET, PET 1%, PET 5%) during the shelf-life test (6 months, 25 ± 4°C; 400 LUX).

Table 1. Evolution of biophenols content measured in EVOO bottled in different containers during storage.

Biophenols* (ppm tyrosol)	Containers [1]	initial	1 month	2 month	3 month	4 month	5 month	6 month
OHTy	glass	12,0	16,2	14,5	13,7	14,4	17,0	19,0
	PET	12,0	13,5	14,7	14,8	14,0	19,0	16,0
	PET 1%	12,0	14,5	15,8	15,3	14,2	17,0	17,0
	PET 5%	12,0	13,3	13,7	15,3	14,1	17,0	20,0
Ty	glass	10,0	14,2	13,0	13,5	13,1	15,0	17,0
	PET	10,0	11,9	12,5	13,5	12,9	16,0	16,0
	PET 1%	10,0	11,8	13,6	15,1	13,1	15,0	15,0
	PET 5%	10,0	11,1	11,7	14,6	12,9	14,0	17,0
OHTy-DEDA	glass	20,0	25,1	22,2	15,8	12,4	19,0	23,0
	PET	20,0	15,2	22,0	19,5	17,9	20,0	18,0
	PET 1%	20,0	21,4	23,5	20,8	17,9	19,0	20,0
	PET 5%	20,0	20,4	21,0	20,6	18,1	19,0	24,0
Ty-EDA	glass	36,0	36,6	33,1	26,7	22,1	32,0	38,0
	PET	36,0	28,1	32,6	32,1	29,6	34,0	31,0
	PET 1%	36,0	31,3	34,4	34,4	29,5	31,0	32,0
	PET 5%	36,0	29,7	31,0	33,3	30,1	32,0	39,0
PR	glass	2,0	4,0	3,0	2,0	2,0	2,0	2,0
	PET	2,0	3,0	2,0	2,0	1,0	3,0	2,0
	PET 1%	2,0	3,0	3,0	1,0	1,0	2,0	1,0
	PET 5%	2,0	3,0	3,0	2,0	1,0	3,0	2,0
OHTy-EA	glass	53,0	61,8	55,1	47,8	48,3	51,0	59,0
	PET	53,0	54,2	53,7	52,0	46,4	54,0	58,0
	PET 1%	53,0	52,7	56,4	54,6	47,2	50,0	50,0
	PET 5%	53,0	50,5	50,6	53,6	47,4	51,0	58,0
Ty-EA	glass	21,0	26,1	23,6	22,1	23,1	22,0	27,0
	PET	21,0	23,2	22,9	23,5	20,3	23,0	27,0
	PET 1%	21,0	22,2	24,3	24,9	21,0	21,0	22,0
	PET 5%	21,0	21,2	21,2	23,8	21,0	22,0	26,0
Total phenols**	glass	154,0	184,1	164,6	141,6	135,4	158,0	185,0
	PET	154,0	149,2	160,4	157,4	142,1	169,0	168,0
	PET 1%	154,0	156,9	170,9	166,1	143,9	155,0	157,0
	PET 5%	154,0	149,1	152,3	163,1	144,6	158,0	186,0

* Compounds identification: OHTy = hydroxytyrosol, Ty = tyrosol, OHTy-DEDA = dialdehydic form of decarboxymethyl oleuropein aglycon, Ty-DEDA = dialdehydic form of decarboxymethyl ligstroside aglycon, PR = pinoresinol, OHTy-EA = aldehydic form of oleuropein aglycon, Ty-EA = aldehydic form of ligstroside aglycon.
** Total phenols = sum of HPLC identified peaks.
[1] PET = polyethyleneterephalate; PET 1% = polyethyleneterephalate + 1% oxygen scavenger; PET 5% = polyethyleneterephalate + 5% oxygen scavenger.

PET and in glass containers (faster oxygen consumption and slightly higher oxidation indices shown by glass-bottled oils) are attributed to a different initial content of dissolved oxygen in the oils. This evidence, due to the more or less intense stirring undergone by oils on the two (PET and glass) bottling lines, suggests that a correct management of the bottling process is extremely important in order to improve oil shelf-life. So, not only the type of packing material, but also the route covered by the oil masses are to be carefully analysed (reduction of turbulent motions, use of nitrogen, reduction of oil movements, etc.) and the critical points which influence the amount of dissolved oxygen are to be identified. The present study showed the usefulness of monitoring not only the analytical oxidation indices during oil storage, but also dissolved oxygen level, antioxidants (phenols and tocopherols) and pigments (chlorophyll and carotenoids) contents. The evolution of these compounds could in fact supply important information on oxidation processes which occur in bottled oils and on the effectiveness of the material employed in oil packaging.

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MICROBIAL SPOILAGE IN REFRIGERATED BEEF DURING STORAGE IN DIFFERENT PROTECTIVE ATMOSPHERES

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Key words: protective atmosphere, refrigerated meat, spoilage bacteria, PCR-DGGE

The effect of package gas composition on the development of spoilage microbial flora was investigated by packaging freshly cut beef with gas mixtures containing: A) air, B) 60:40:0 and C) 20:40:40/O_2:CO_2:N_2 with 3:1 headspace - meat volume ratio. Sample A was used as control. Packed samples were stored at 7°C and the microbial spoilage populations were monitored at time zero and after 2, 4, 7 and 14 days of storage. The microbial colonies from the plate counts of each microbial group were identified by PCR-DGGE of the variable V6-8 region of the 16S rDNA. At each sampling time, the head space gas composition (O_2%-CO_2%). the weight loss and the surface meat colour (Hunter L*, a*, b values) of the beef were also determined. The head space gas analysis was a good index of microbiological spoilage. In fact during storage time, a decrease of the oxygen and an increase of the carbon dioxide concentration were observed only in the control samples. Due to the barrier properties of the package. the atmosphere composition changed from 21 to 0% of O_2 and from 0 to 25% of CO_2 after 14 days of storage. This modification may be caused by a major aerobic microbial contamination in the control samples compared to sample B and C for which no changes in gas composition were observed up to 14 and 7 days. respectively. This result was confirmed by the microbiological analysis. In particular. the effect of package gas composition was significant on *Brochotrix*, *Pseudomonas*, *Enterobacteriaceae*, mesophilic and psychrotrophic microbial counts, although no effect was registered on LAB growth. The atmosphere B proved the most protective- the viable counts were always lower than the control during the storage. Colour results were in agreement with microbiological results showing a protective effect of the atmosphere B on the browning of the beef. During storage in air, the colour of the beef changed from red to brown in 7 days and to a cherry red colour after 14 days due to increase of anaerobic conditions inside the package. On the other hand, the beef packed with the atmosphere B showed a constant red colour up to 7 days of storage. The influence of storage conditions on the occurrence of microbial species was evaluated by PCR-DGGE Overall, *Carnobacterium divergens, Lactobacillus graminis, Weissella hellenica* and several *Leuconostoc* species were identified among the LAB while *Enterobacter, Serratia, Hafnia, Pantoea* and *Ranhella* among the *Enterobacteriaceae.* Besides, *Brochotrix thermosphacta* and *Pseudomonas* spp. occurred in all the samples during storage, especially in the last phases of the trial.

The knowledge of the influence of packaging conditions on the development of microbial spoilage is essential for the improvement of quality and preservation of fresh meat.

ACKNOWLEDGEMENTS

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EFFECT OF OXYGEN SCAVENGER FILLERS ON MECHANICAL AND BARRIER PROPERTIES OF A PET MONOLAYER FILM

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ABSTRACT

In the recent years, consumer and industry preferences are leading towards new systems for extending the shelf life of food or medical substances. In particular, the so-called “active packaging” technologies have been developed as a response to the needs of mildly treated foodstuffs in order to preserve freshness and flavours. These are often compromised by gases, in particular oxygen, which can be present in the package or can permeate from the surrounding ambient. The oxygen is, in fact, the responsible of the main degradative phenomena (oxidation reactions, growth of aerobes micro and macro organisms, alteration of colour and flavour, loss of nutritional properties, etc.), which affect products quality. One of the most promising approach for the realization of an active packaging consists in the use of scavengers for the undesired gas. The gas scavenger function is to extend the shelf life of the packaged product, by actively removing the gas present in the packaging. Currently, different kind of scavengers are available on the market but, actually, their applications are still few due to the difficulty of incorporate them in the packaging.

The aim of this work was to perform preliminary tests in order to evaluate mechanical and barrier properties of monolayer polyester films containing an active oxygen scavenger. These active films were produced with different percentages of the scavenger by extrusion process, using a laboratory cast film extrusion line. The produced films were analysed in order to verify the activity of the scavenger. In particular, permeability was assessed as time function until saturation of the scavenger was achieved. Moreover, mechanical measurements have been carried out for evaluating the effect of the scavenger blend on tensile properties.

Key words: active packaging, barrier properties, mechanical properties, oxygen scavenger

INTRODUCTION

During the last decades, the food and pharmaceutical industries have seen several changes in packaging technology and application, due to the new consumer demands and market trends. These requests can be resumed in an higher freshness and quality of the products; a longer shelf life; an handle and resistant packaging made with lighter, cheaper and recyclable materials. The use of polymers in food packaging has continuously increased due to their advantages respect to other traditional materials, such as glass or metal. Nevertheless, the polymers are permeable to gases and low molecular weight molecules. The use of plastics for packaging gases sensitive products requires an improvement of their barrier performances, through innovative technological processes (Lagaròn *et al.,* 2004). The current tendencies include the development of packaging materials that interact with the environment and with the food, playing an active role in quality preservation. The so-called "active packaging" are able to modify the gas environment by removing the undesired gases from the package or by regulating the package headspace through the addition of useful gases. In particular the presence of oxygen in the package can be the cause of different detrimental phenomena as microbiological, chemical, macrobiologic or physiological, that affects products quality.

One of the most promising approach for the realization of an active packaging consists in the introduction of scavengers into a polymeric matrix. Currently, different kind of oxygen scavengers (OS) are available on the market (Schroeder *et al.,* 2001; Vermeiren *et al.,* 1999) but their applications are still limited due to the difficulty of realizing a functional and efficient active film, having high mechanical and optical properties.

The present work was focused on the production and characterization of polyethylene terephthalate (PET) monolayer films containing different concentrations of a polymeric oxygen scavenger. The samples were produced using a laboratory cast film extrusion line. The thermal properties and the permeability were analysed in order to verify the activity of the scavenger; while the tensile characteristics were determined to investigate the performances of the films. This preliminary study can be useful to investigate the behaviour of a scavenger into a polymeric matrix in order to optimize the process and to evaluate the most performing design of the active system.

MATERIAL AND METHODS

Materials. The polymer used as matrix was a PET Cleartuf P60 amorphous, supplied by MG Polimeri Italia S.p.A. The chemical-physical properties of the matrix are reported in Table 1. The oxygen scavenger added to the polymeric matrix was the Amosorb DFC 4020 (AMS) supplied by ColorMatrix Europe ltd. Amosorb 4020 is a transparent copolyester-based polymer, designed for PET containers. The scavenger element consists of transition metal, as cobalt salt, catalyzed unsatured hydrocarbon dienes such as polybutadiene linked to polyester (Cahill *et al.,* 2000).

Conditioning. The PET P60 was previously crystallized at 130°C for 6 h under vac-

Table 1. Chemical-physical properties of the PET used for the analyses.

Properties	Values
I.V. (dl/g)	0.58
DEG (% w/w)	1.4
COOH (meq/kg)	49
Acetaldehyde (mg/kg)	38
Water (% w/w)	0.1
Colour b*	-2
Melting point (°C)	250
Weight 100 chips (g)	1.8
Contaminants	none

uum and then dried under vacuum at 120°C for 16 h before processing. The AMS was stored at environmental temperature into aluminium bags sealed under vacuum.

Processing. Dry blends at different composition of 100/0 PET/AMS, 99/1 PET/AMS, 97/3 PET/AMS and 95/5 PET/AMS were extruded in order to obtain films with a laboratory cast film extrusion equipment. The laboratory line system consisted in a single screw extruder, a flat die and a take up system. The extruder was equipped with a screw diameter of 12 mm and an L/D ratio of 24. The extrusion die was a coat hanger type with a lip width of 200 mm and thickness of 0.25 mm. The take-up system consists in a couple of chill rolls thermally controlled by conditioned water circulation at 10°C. The extruder temperature profile was 280°C and the die temperature was 270°C. The chill roll speed was 2.5 m/min, thus allowing film stretching to its final dimension, with a thickness of about 50 μm. The produced films have been tested after production and during the time, to see the impact of aging on the mechanical properties and to investigate their barrier behaviour during the time. The aging lasted three months, by storing the samples at ambient condition.

Methods. The thermal behaviour of the polymers was analysed using a Differential Scanning Calorimeter (model DSC 822, Mettler Toledo). Experiments were carried out on samples (10-13 mg) of pure PET and pure AMS pellets and on the produced films, under nitrogen gas flow (50 ml/min). Samples were first heated at a rate of 10°C/min, from 25 to 300°C, held for 5 minutes at 300°C to allow the complete melting of the crystallites; then, they were cooled at a rate of 10°C/min until 25°C and reheated at the same conditions set for the first heating.

The oxygen permeability of the produced films were performed by means of a permeabilimeter (GDP-C, Brugger), connected to a thermo-controlled bath (ThermoHaake). The test temperature has been set at 23°C and 60% R.H, the flow rate of oxygen was 80 ml/min (ASTM D 1434-82).

Tensile properties were determined at room temperature with an Instron Universal Testing Machine (model 4301), operating at a rate of 5 mm/min for the determination of the Young's modulus and a crosshead velocity of 50 mm/min for the measurement of the stress and deformation at break (ASTM D 638–99).

RESULTS AND CONCLUSIONS

The activity of an oxygen scavenger decreases during the time, due to the continuous reaction with the oxygen. This phenomenon influences the shelf life of the packaged product, but could also have an effect on the performances of the package itself, in particular on the gas permeability and the mechanical characteristics. At this regard, the films permeability has been measured after the production, when the scavenger is active, and after three months of storage at ambient condition, when the AMS is supposed to be partially oxidised. The results are reported in Fig. 1. The standard PET film shows a constant permeability, while the values relative to scavenger films increase during the time. In fact, the permeability value of PET + 5% AMS after the production is significantly lower than pure PET film. This difference is reduced after three months, according to the activity of the OS.

Since the permeability is strongly effected by matrix crystallinity, thermal analyses have been performed in order to assess the crystallinity of the active system. In table 2 the thermal behaviour of samples analysed by DSC is reported. The values are relative to the second heating for T_g and T_m, while the % of crystallinity has been evaluated from the first heating (Fig. 2), normalizing the ΔH_{cc} and ΔH_f respect to the weight of the matrix. The calorimetrical analysis does not show evident differences in T_g and T_m values between the films containing the scavenger and the pure PET film. The T_c, instead, is higher for active films, as the thermogram reported in Fig. 3 shows. It is also possible to observe an increasing of %

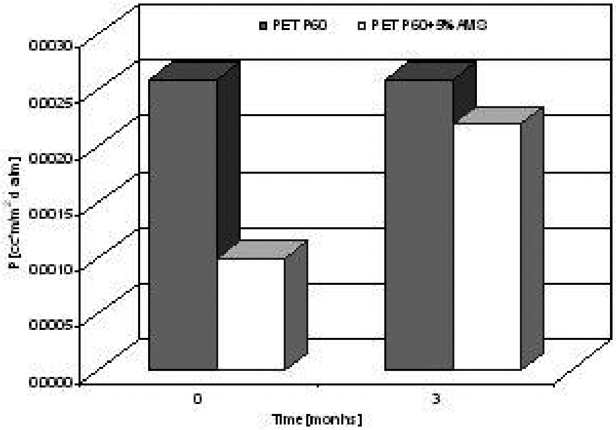

Fig. 1. Permeability of pure PET and active (+5% AMS) films tested after production (0 month) and after three months.

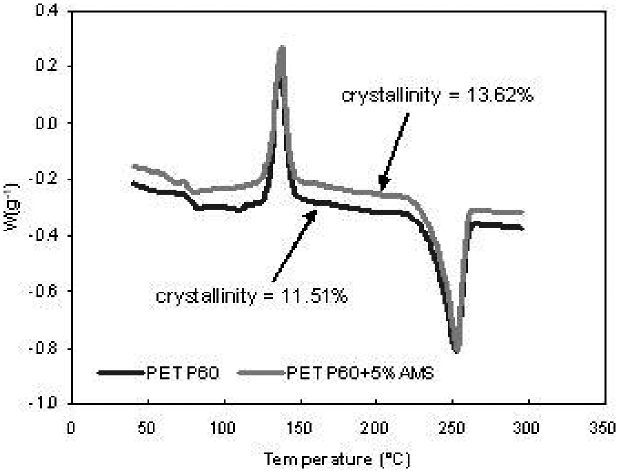

Fig. 2. Second heating DSC thermograms of PET P60 and PET P60+5%AMS films.

Table 2. Thermal behaviour of samples analyzed with DSC technique.

Samples	T_g [°C]	T_{cc} [°C]	T_m [°C]	T_c [°C]	ΔH_c [J/g]	ΔH_{cc} [J/g]	ΔH_f [J/g]	% Crystallinity[1]
AMS[2]	n.d.	n.d.	250.12	189.97	41.42	n.d.	- 41.98	n.d.
PET P60[2]	78.94	128.67	250.33	178.00	39.16	30.27	- 43.45	11.26
PET P60[3]	78.98	137.28	250.20	186.20	39.77	30.23	- 43.70	11.51
PET P60+1%AMS[3]	78.75	137.78	248.47	194.22	39.33	31.80	- 43.45	10.06
PET P60+3%AMS[3]	78.97	140.67	252.50	190.50	42.81	30.13	- 44.65	12.79
PET P60+5%AMS[3]	78.62	137.47	251.19	193.74	42.62	28.97	- 44.11	13.62

[1] The value of ΔH_0 for PET has been assumed 117J/g and the % of crystallinity has been evaluated on the % of PET content.
[2] Pure AMS and PET in pellets.
[3] Film samples.

Table 3. Tensile properties of films tested after production and after three months.

Film Production	Samples	E [MPa]	δ_b [MPa]	ϵ_b [%]
Tested after production	PET P60	1360.30±427.30	37.45±4.44	211.70±219.97
	PET P60+5%AMS	1884.70±182.92	41.97±8.44	395.57±145.27
Tested after three months	PET P60	1442.00±132.23	50.17±10.10	3.95±0.77
	PET P60+5%AMS	1594.75±160.30	36.12±4.39	3.11±0.27

crystallinity raising the amount of AMS fed. In fact, the % of crystallinity ranges from 10.06 for the PET containing 1% of AMS, to 13.62 for the samples with the maximum content of scavenger. These results suggest that the AMS can behave as a nucleating agent on the PET crystallization process. Moreover, the presence of 1% AMS in the matrix does not cause any thermal differences respect to the pure PET film. The effect of the higher crystallinity for samples containing AMS on barrier properties, cannot to be determined during the scavenger reaction because it is hided by its activity. Nevertheless, in order to investigate the transport properties of the active films is necessary to deeply study the activity of the scavenger during the time, by determining its kinetic reactions. For this purpose a new oxygen measurement system, based on luminescence lifetime detection, have been realized and experimental tests are in progress.

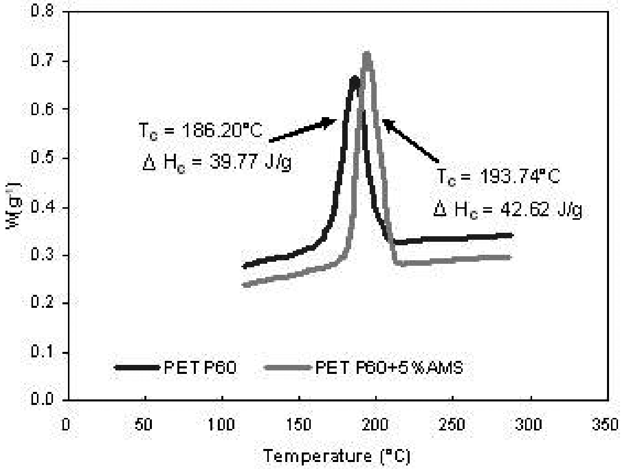

Fig. 3. Cooling DSC thermograms of PET P60 and PET P60+5%AMS films.

The addition of a filler into a polymeric matrix can influence the crystallinity and permeability, as above observed, but can also have effect on tensile properties. Therefore the mechanical properties of produced films have been investigated and the results are reported in Table 3. The aging effect results to be quite severe since the films became extremely fragile after three months of storage. Nevertheless, samples tested after production, when the scavenger is still active, show a very interesting behaviour: the modulus and the elongation at break of the active films are higher than the pure PET ones (tested in machine direction). These mechanical behaviours require a deeper investigation on the active films structure, through Scanning Electron Microscopy (SEM), X-Rays and Dynamic Mechanical Analyses (DMA).

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THE USE OF NATURAL ESSENTIAL OILS AS ANTIMICROBIAL SOLUTIONS IN PAPER PACKAGING

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ABSTRACT

The main aim of this contribution is to develop a new alternative for active paper packaging, based on the antimicrobial effects of different essential oils. A potential advantage of this alternative over other existing methodologies is the use of natural products, validated for food-usage, thus raising no health concerns. The essential oils will be evaluated as a function of their activity in vapour phase, meaning that no direct contact with packaged food is needed, thus minimising the chances for food alteration due to these components.

Key words: paraffin wax, active coating, antimicrobial packaging, food protection, essential oils

INTRODUCTION

Food technology is changing according to the consumer habits. Nowadays the consumer demands more natural products, without chemical additives, and simultaneously safer products in which the chemical and microbiological pollution are under control. The new technology of active packaging can let us have packaged food with longer shelf life, by introducing antimicrobial compounds into the packaged instead of adding them to the food. In this frame, several active packaging systems have been developed. One of the most advanced system deals with the introduction in the packaging material of a little amount of some extracts of essential oils that have been recognized as antioxidant and antimicrobial agents (1-3). However, most of the new active materials are based on plastics and only some references deal with paper and board (4).

Paper and board are porous materials only appropriate for dry foodstuffs. However, their application field can be broaden by coating with paraffin waxes, plastic layers or aluminium foil. Using a specific formulation of paraffin wax, a new active material can be obtained in which the antimicrobial or antioxidant properties are considerably improved. This contribution summarises the experimental work carried out to produce a new active material paper-base as well as its performance versus a large series of bacteria, yeast and moulds and a real application with strawberries. The results obtained are shown and discussed.

MATERIALS AND METHODS

A wax formulation containing a little amount of a commercially available extract of essential oil is prepared. The preparation as well as the final formulation are patent protected and cannot be described here.

Using this active formulation, sheets of common paper are coated and used in microbiological tests with the specific culture of the bacteria, yeast and moulds. Microbiological analyses were carried out using Petri Dishes each one with the specific culture required for each microorganism and covered with the active paper, without direct contact between the culture and the active paper layer. They were also evaluated for selected food.

The following Gram negative bacteria were tested: *E. coli, P. aeruginosa, Y. enterocolitica, S. choleraesuis.* As Gram positive bacteria, *E. faecalis, S. aureus, L. monocytogenes and B. cereus* were tested. *C. albicans* was used as yeast and *A. flavus, P. roqueforti, P. nalgiovense* and *E. repens* were tested as moulds. All the results are based on 3 replicates of each active paper.

Strawberries were selected for testing the performance of the active packaging with real food. Then some paper trays were constructed with the active material and the strawberries were directly placed on them and covered by a sheet of active material. The texture, external appearance and the presence of moulds were monitored.

RESULTS AND DISCUSSION

It is well known that the system food-packaging-environment presents different types of interactions, mainly permeation, adsorption and migration. Migration, which is the key of this contribution can have a negative impact in food (when migrating substances are monomers, undesirable chemical additives or contaminants) or positive, as for instance in the case of antimicrobial compounds. This concept has been used to incorporate some substances with antimicrobial activity into the headspace inside the package to prevent microbial proliferation which could result in food spoilage.

So, what is the idea? The idea is to develop a paper (or board or even wood) containing natural extracts which could be released to the headspace thus protecting food from microorganism’s infestation. Thus paper is treated with the active coating based on paraffin wax including the active compounds as well as some additives which are included in the final formulation.

Antimicrobial activity tests were performed in order to evaluate the antimicrobial performance of the concept. A Petri dish with the appropriate culture medium was inoculated with 100 μL of a 10^6 CFU/mL of the microorganism. Activity was evaluated as a count of the viable cells remaining after the test. Blank analyses, meaning that no paper is included were carried out for each test.

Three different essential oils were considered as active components: clove, cinnamon and oregano. Cinnamon was used in two different formulations. Essential oil selection was performed according to bibliographic data on their successful antimicrobial activity. Different concentrations of the oils were tested as well.

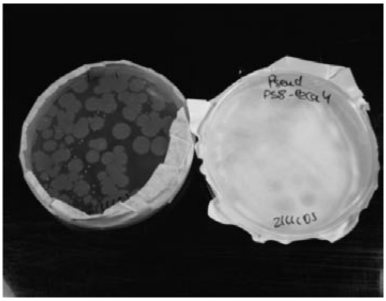

Fig. 1. *P. aeruginosa* in the presence of a non active paper (left) and the active paper with cinnamon II (right).

The array of food-borne pathogens selected was presented previously. As can be seen, a wide range of microorganisms including Gram positive and negative bacteria as well as moulds and a yeast were selected. They are among the most prevalent microorganisms found in foodstuffs.

Encouraging results were obtained for Gram negative bacteria. With the only exception of oregano, inhibition was obtained for all the tested oils for *E. coli.* Better results were obtained for cinnamon II, an essential oil enriched in cinnamaldehyde, which is proven to be the most active compound in terms of inhibition. Going into more detail, *Yersinia* is inhibited to an 80% ratio and a 50% reduction was obtained for *Yersinia* and *Pseudomonas.* Even at lower concentrations differences in size and colour of the colonies were observed, demonstrating the positive effect of the active paper. Explanation should be related by the differences between the cellular walls between both kinds of bacteria.

Illustration of the results is depicted in Figure 1. As can be seen (blank on the left) clear inhibition is obtained. This is a quite interesting finding, since *Pseudomonas aeruginosa* is known to be one of the more resistant food-borne microorganisms.

For fungi, different results were obtained for mould and yeast. As can be seen in Figure 2, *Candida* was inhibited (20%) for both oregano and clove, whereas *Aspergillus* was only inhibited for cinnamon. Total inhibition was obtained for both microorganisms when working with cinnamon II, which is a quite relevant result since fungi proliferation is of paramount relevance in a number of food industries such as bakery. These results were not totally unexpected since fungi have been consistently reported in the scientific literature to be more sensitive against antimicrobial products than bacteria are.

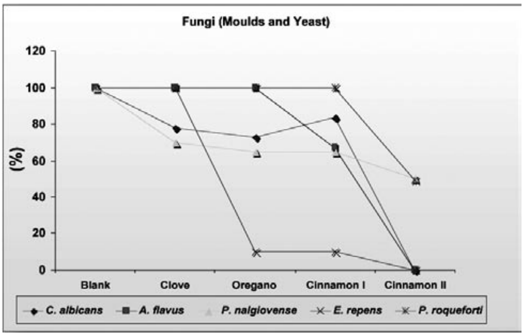

Fig. 2. Results obtained using different essential oils as active agents in paraffin wax coated paper.

Inhibition is demonstrated in Figures 3 and 4. No growth at all was obtained for any of the fungi under test.

Of high relevance, is the shelf life of the proposed alternative, that means

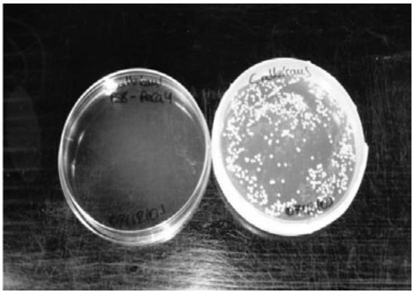

Fig. 3. Yeast: *C. albicans* with active paper (left) and non active paper (right).

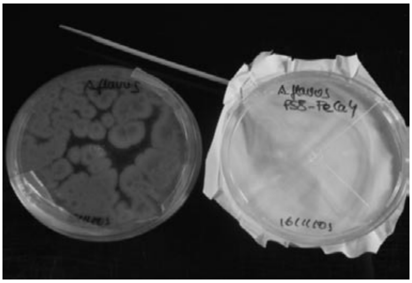

Fig. 4. *A. flavus* in the presence (right) and absence (left) of the active paper.

for how long the active paper retains its capability to inhibit the microorganisms after been manufactured. The results were calculated for *Aspergillus* and *Candida*, which showed the best behaviour in the preliminary tests. After been manufactured, active paper was stored at room temperature in the light, which were the worst conditions. The results showed that *Aspergillus* was completely inhibited even after 71 days, whereas inhibition for *Candida* was dependent on the initial concentration of the yeast inoculated in the tests. Nevertheless, total inhibition was also achieved for the total tested time of 71 days.

Then, testing with real food is the next logical step to be taken. Differences have been reported when an antimicrobial solution is tested with real food and compared with the results obtained in culture conditions. Strawberries were selected as the test food since they are usually wrapped in paper or board and they are prone to fungi infestation. Two varieties of strawberries were also selected for testing: pale red in colour and dark-red colour. Packaging was done as illustrated in Figure 5, no-hermetic. Cinnamon II (4%) was selected as the antimicrobial agent and after packaging; strawberries were stored in a fridge at 4 °C.

Figure 5 shows the results obtained after 5 days of storage for the pale red variety. The blank test (on the right) was significantly more spoiled than these packed with the active compounds. Clear presence of fungi (probably *Botrytis cynerea*) nests is to be reported as well as significant amount of juice exudation which is known to be a preliminary step in fungi infestation. These are quite encouraging results with a direct relevance for this particular food industry.

CONCLUSIONS

The active paper packaging system developed with the use of natural essential oils as antimicrobial active agents has demonstrated potential usefulness. Cinnamon II (the enriched in cinnamaldehyde) demonstrated the best inhibitory performance against Gram negative bacteria and fungi, with a shelf-life for the later of 71 days. Moreover, application to real food has been demonstrated. Future development will be focused in organoleptical testing, specific migration as well as industrial testing.

Fig. 5. Strawberries in the presence (left) and in absence (right) of the active paper.

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EFFECT OF DIFFERENT PACKAGING ON ENZYMATIC AND CHEMICAL DEGRADATION OF CHILLED CUT ORANGES DURING SHELF-LIFE

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ABSTRACT

Demand for fresh, convenient, minimally processed vegetables has led to an increase in the quantity and variety of products available to the consumer. The aim of this work was: to examine the effect of different packaging on chemical and enzymatic degradation of chilled cut oranges during shelf-life. Particularly were studied Pectinesterase (PE) as index of firmness, Ascorbate oxidase (AAO) as index of acid ascorbic's degradation and poliphenoloxidase (PPO) as Browning's index. . In the slices packaged in passive modified atmosphere, PE enzymatic activity and AAO enzymatic activity increased to the increase of permeability of packaging film. In the slices packaged in active modified atmosphere, PE and AAO enzymatic activity decreased to the increase of CO_2 content in the gaseous mixture. A correlation has been individualized among pectinesterase activity and loss of texture, and among AAO activity and ascorbic acid degradation of slices during shelf-life. Regarding the PPO, it was noticed that enzymatic activity was quite probably because of the natural chemical compositions.

Key words: orange slices, AAO, PE, ascorbic acid, ready to eat fruits

INTRODUCTION

Consumers are increasingly demanding convenient, ready-to-use and ready-to-eat fruits and vegetables with a fresh-like quality, and containing only natural ingredients. Because minimally processed fruit and vegetable are not heat treated, regardless of the use of additives or packaging, they must be handled and stored at refrigeration temperatures. Ingallinera *et al.* (2006) have confirmed that the oranges, thanks to their biochemical composition, are adapt to the production of ready to eat fruits. As a result of peeling and cutting, oranges will change from a relatively stable product with a shelf life of several weeks to a perishable one that has only a very short shelf life. Minimally processed oranges, as other fruits, deteriorates because of physiological ageing, biochemical changes and microbial spoilage, which may result in degradation of the colour, texture and nutritional value. The Modified Atmosphere Protective packaging (MAP) could minimize this alterations as showed in different fruits and vegetables. The basic in MAP is that a modified atmosphere can be created either passively by using properly permeable packaging materials, or actively by using a specified gas mixture together with permeable packaging materials. The aim of both principles is to create an optimal gas balance inside the package, where the respiration activity of a product is as low as possible, but the levels of oxygen and carbon dioxide are not detrimental to the product. The aims of this work were: to examine the effect of different packaging on chemical (polyphenol, anthocianyns, ascorbic acid, colours, firmness) and enzymatic degradation of chilled cut oranges, particularly are studied Pectinesterase as index of firmness, Polyphenoloxidase as index of browning, Ascorbate oxidase as index of acid ascorbic's degradation.

MATERIALS AND METHODS

Samples preparation

The samples of pigmented oranges: *Moro nucellare* clone and *Sanguinello nucellare* clone came from Palazzelli experimental farm of the Experimental Institute for Agrumiculture (Acireale, Italy) in Lentini countryside (Siracusa, Italy). The oranges samples were processed to fresh cut fruits trough a pilot system at Experimental Institute for Agrumiculture (Acireale, Italy).

Every clone was packed under normal atmospheric conditions by two high barrier films with increasing O_2 permeability and under modified atmosphere (MAP) with three mixtures at different and increasing concentrations of O_2 and CO_2. Totally impermeable film was made by OPA, Adesive e PET 30 MELINEX 850 (named film A); Totally permeable film was made by PET, adesivo, PP.(named film B); The mixture of gas used in modified atmosphere were generally those literature recommend for fresh cut vegetables (Cantwell *et al.,* 1995): Mixture 1 (named A_1): 2,5% O_2, 8% CO_2, 89,5% N_2; Mixture 2 (named A_2): 5% O_2, 8% CO_2, 87,5% N_2; Mixture 3 (named A_3): 2,5% O_2, 15% CO_2, 70% N_2.

For each clone, for each packaging film, and for each kind of modified atmosphere, we have considered six wrappings, each containing six orange slices from six different orange fruits.

Samples were stored at 4 ± 0.5°C with 85% R.H. and tested every two-three days until 15 days of chilled storage.

Physical - Chemical Analysis

Firmness test: firmness test was made by weighing orange juice released by fresh cut slices.

Total Polyphenols: total polyphenols analysis was made according to Waterman and Mole.

Anthocyanins: the anthocyanins in orange juice were quantitatively analysed by spectrophotometer, through the use of the balance between the coloured cationic form in acid, and the colourless form at about pH 4.5, according to Rapisarda et al. (2000).

Ascorbic acid: to 5 ml of depigmented orange juice was added an extracting solution of methaphosphoric acid-acetic and the solution was titrated with 0.02% 2,6 dichlorophenolindophenols, according to Rapisarda and Intelisano (1996).

Colour: the colour parameters (CIE L *, a * and b *) in slices were determined with Adobe Photoshop in six replicates. We determined the chromatic variations of the juice extracted by the slices measuring, in three replicates, the transmittance in the range between 389 and 780 nm.

Enzymatic Analysis

Pectinesterase assay: Pectinesterase assay was made according to Ingallinera *et al.,* 2005.

Ascorbate Oxidase assay: Ascorbate oxidase assay was made according to Saari *et al.,* 1999.

Polyphenoloxidase assay: Polyphenoloxidase was made according to Espin.

RESULTS AND DISCUSSIONS

In this study, red colour, expressed by a* parameter, was decreasing in all samples during chilled-storage because of oxidative phenomena against orange's pigments. Samples of *Moro nucellare* and *Sanguinello nucellare* packaged with film B and A_3 mixture had the greatest a* decreasing in both clones, probably related to their higher oxygen content. Lightness, expressed by L* parameter, was increasing during chilled-storage in every samples without regularity, for this reasons it

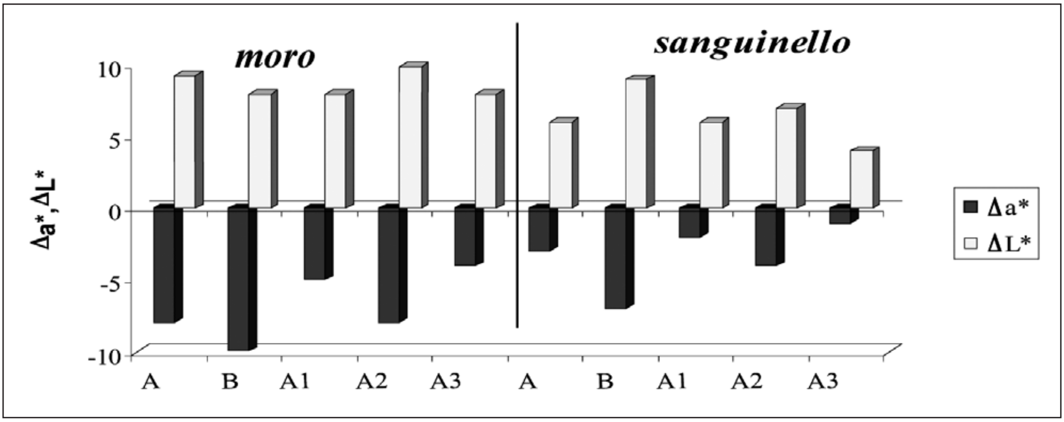

Fig. 1. Chromatic parameters ΔL* (□), Δa*(■) of orange slices on *Moro nucellare* A, B, A_1, A_2, A_3 and *Sanguinello nucellare* A, B, A_1, A_2, A_3 during chilled storage.

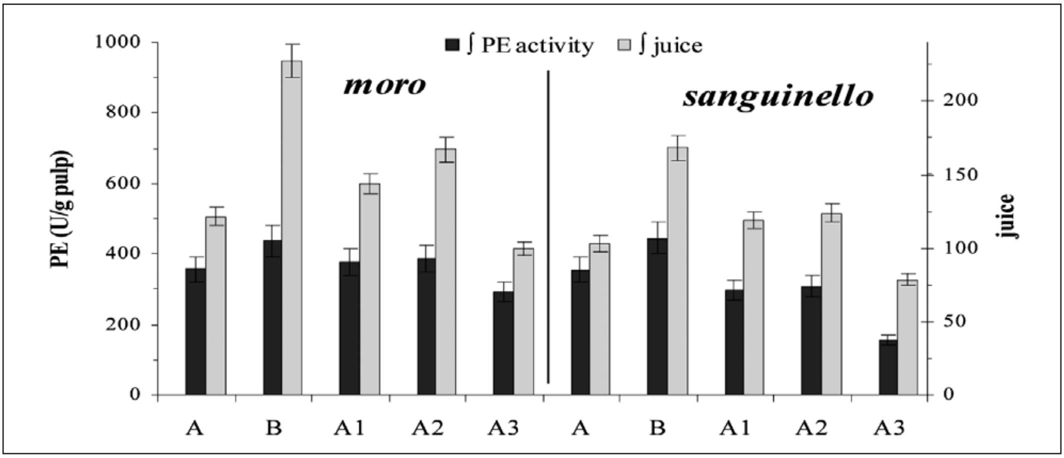

Fig. 2. Relationship between ∫ PE activity (■),and ∫ juice loss (□) in orange slices on *Moro nucellare* A, B, A_1, A_2, A_3, and *Sanguinello nucellare* A, B, A_1, A_2, A_3 during chilled storage.

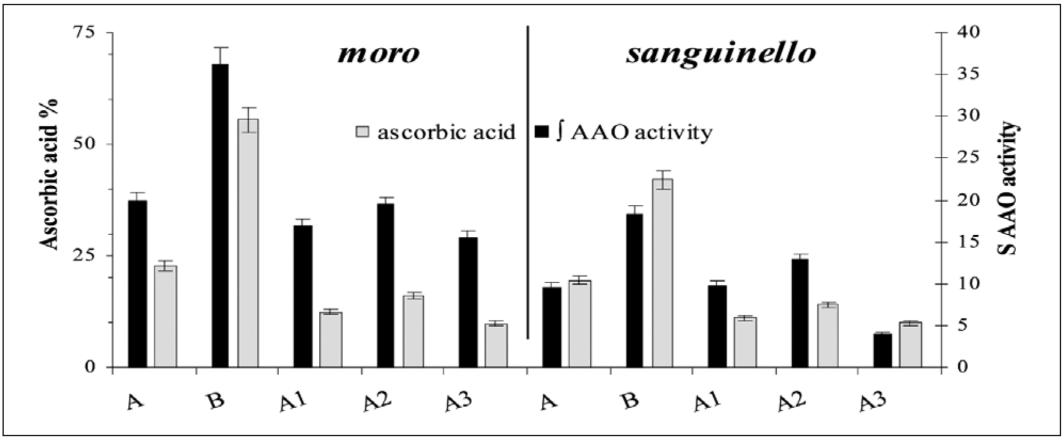

Fig. 3. Relationship between ∫ AAO activity (■), residual ascorbic acid % (□) in orange slices on *Moro nucellare* A, B, A_1, A_2, A_3 and *Sanguinello nucellare* A, B, A_1, A_2, A_3 during chilled storage.

was very difficult understand the effect of packaging on L* variations. We did not reported significative variations related to the parameter b * to the blood oranges. All these results are showed in figure 1.

Average total polyphenol content in both clones was 20 ±0.1 mg/g. Total polyphenol content didn't show hard variations in both clones and in every packaging solutions during chilled-storage.

Average anthocyanins content in both clones was 72.50±0 mg/L. This content didn't show hard variations in both clones and in every packaging solutions during chilled-storage.

PE activity was higher in *Moro nucellare* clone than in *Sanguinello nucellare* clone. Generally the samples of *Moro nucellare* and *Sanguinello nucellare* packed under ordinary atmosphere showed a different PE activity in different packaging solutions, in fact enzymatic activity increased when film permeability increased (Fig. 2). The samples of *Moro nucellare* and *Sanguinello nucellare* packed under modified atmosphere showed a different PE activity in different packaging solutions, in fact enzymatic activity decreased when CO_2 content in mixture increased (Fig. 2). Probably CO_2 had a protective effect against softening loss. Firmness' loss of slices showed the same variations, so it was possible to find a good correlation between PE activity and firmness loss (Fig. 2).

Regarding PPO, it was noticed that enzymatic activity was very low and quite similar in every samples, probably because of low pH and high concentration of citric and ascorbic acid, which are natural inhibitors of this enzyme. This low activity could explain the lack of browning process and the low variation of anthocyanis in the slices.

AAO activity was higher in *Moro nucellare* clone than in *Sanguinello nucellare* clone. Generally the samples of *Moro nucellare* and *Sanguinello nucellare* packed under ordinary atmosphere showed a different AAO activity in different packaging solutions, in fact enzymatic activity increased when film permeability increased (Fig. 3). The samples of *Moro nucellare* and *Sanguinello nucellare* packed under modified atmosphere showed a different AAO activity in different packaging solutions, in fact enzymatic activity decreased when O_2 content in mixture decreased (Fig. 3). Ascorbic acid degradation showed the same variations, so it was possible to find a good correlation between AAO activity and ascorbic acid degradation (Fig. 3).

CONCLUSIONS

Some of quality markers of fresh cut oranges were influenced by different packaging solutions. In this study, red colour, expressed by a* parameter, was decreasing in all samples during chilled-storage because of oxidative phenomena against orange's pigments Lightness, expressed by L* parameter, was increasing during chilled-storage in every samples without regularity. Total polyphenol content and anthocyanins content didn't show hard variations in both clones and in every packaging solutions during chilled-storage.

The samples of *Moro nucellare* and *Sanguinello nucellare* packed under ordinary and modified atmosphere showed a different acid ascorbic's degradation in different packaging solutions, in fact acid ascorbic's degradation increased when film permeability and O_2 content increased. The samples of *Moro nucellare* and *Sanguinello nucellare* packed under ordinary and modified atmosphere showed a different PE and AAO activity in different packaging solutions, in fact enzymatic activity increased when film permeabily and CO_2 content in mixture, increased.

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ENHANCEMENT AND INDICATION OF FOOD QUALITY BY COMBINATIONS OF OXYGEN SCAVENGER AND INDICATOR SYSTEMS

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ABSTRACT

Within the ACOSIC project supported by the European Commission novel oxygen scavenger and indicator systems were developed to remove the residual oxygen from inside the packaging and simultaneously to display the functionality of the oxygen scavenger or the integrity of the food packaging. The oxygen scavenger/indicator systems can be applied by conventional film conversion and printing techniques and can be adapted to different substrate materials. The oxygen scavenger and indicator systems are incorporated into packaging film materials and can be utilized in flexible and rigid packaging applications for sensitive foods or pharmaceuticals, in which a low oxygen environment is beneficial. Data obtained on selected foods packed in a structure that incorporates the novel developed oxygen scavenger/indicator systems illustrates the advantage of the new concepts. Developments are planned for further improving the scavenging capacity and kinetics and the determination of the threshold value for oxygen indication of the demonstrator films to adapt the scavenger/indicator system for selected applications.

Key words: active packaging, oxygen scavenger, oxygen indicator, intelligent packaging

Table 1. Maximum allowable oxygen uptake of different products

Product	Maximum Uptake of Oxygen [mg O_2 / kg of food] Reference [1]	Maximum Uptake of Oxygen [mg O_2 / kg of food] Reference [2]
Beer	1 - 4	
Fruit juices	10	50 - 200
Sterilised milk	1 - 8	1 - 5
Meat, fish	1 - 5	1 - 5
Nuts, snack products	5 - 15	10 - 40
Baby food		1 - 5
Vegetable oil	50 - 100	50 - 200
Ketchup	70 - 100	
Instant coffee		5 - 15

INTRODUCTION

The changing lifestyle leads to the demand for a high diversity of convenience food products and for minimally processed and naturally preserved foods which should be nevertheless healthy, fresh and nutritious. These novel foods are highly perishable products. The influence of oxygen, light, moisture and aerobic microbes can reduce their shelf life and nutritional and sensory quality. Even low concentration of oxygen, i.e. in the range of 1 to 200 mg/kg (see table 1), can adversely affect the flavour and colour of a wide range of foods.

The residual levels of oxygen found in most packaging systems are much higher than this. The consumers want to be informed of the origin, treatment and quality/safety of the food. All these factors put high demand on the shelf life extending capacity of food packaging systems. These demands can be met by the use of active and intelligent packaging concepts. The application of combined systems consisting of oxygen scavenger and indicator integrated into the packaging material (not as a separate element) is a novel and innovative approach. These combined oxygen scavenger /indicator systems will allow to extend the shelf life, to improve the quality and sensory properties and will provide an extended type of information -including options for the tamper evidence- and will reduce risks in hygiene and food safety.

Therefore, in 2003, eight project partners (research institutes, industrial companies and consumer alliance) started the European project "ACOSIC" [3] to develop oxygen indicator/ –scavenger systems for removal the residual oxygen from inside the packaging. These novel developed oxygen scavenger and indicator systems are integrated into the packaging film materials to monitor the functionality of the oxygen absorber and the integrity of the packaging and, simultaneously, to enhance the product quality and shelf life. This paper discusses the first developed prototypes of these novel active packaging and examines some of the factors that affect the performance of the systems incorporated into multilayer film structures.

MATERIALS AND METHODS

Gallic acid – based oxygen scavenger

Matrix system

The matrix consist of component 1 (basic component), component 2 (acetone) and component 3 (accelerator). The component 1 and 3 is a commercial available 2-component solvent based lamination adhesive.

A solution of 25 weight-% gallic acid (relating to the component 1) and component 2 is stirred for 30 min at room temperature. This mixture is solved – under stirring for further 30 min – in the component 1 with a mixing ratio of 50:50 weight-%. To this mixture 1 weight-% of the component 3 is added. The generated composition can be used after a further stirring time of approximately 10 min.

Application

The manufactured composite is applied via a lamination process onto a PE-film filled with an alkaline substance. The thickness of the applied adhesive layer is 5 μm.

Activation

The oxygen scavenging system incorporated into a multi-layer structure is activated by a relative humidity > 92 %. This scavenger system is suitable for the application in packaging of wet food.

Cyclo-olefin-silane based oxygen scavenger [4]

Synthesis – matrix system

A solution of 44 mmol 2-cyclohexenylethyltriethoxysilane and 44 mmol n-octyltriethoxysilane in 2-butoxyethanol was placed in a three-necked flask and cooled in an ice bath. After hydrolysis with 50 % of the stoichiometric amount of water (in the form of 1n hydrochloric acid) with respect to the hydrolysable alkoxy groups the mixture was stirred at room tempera-ture for 2 hours. To this mixture a solution prepared by mixing of zirconium-n-propoxide (12 mmol) and ethyl acetoacetate (molar ratio metal alkoxide/complexing agent: 1/2) was added. The completion of the hydrolysis reaction was controlled by Raman Spectroscopy (solids content: 33%). To the obtained matrix, cobalt(II)-oleat (≤ 2 % by weight), 1 % by weight of the photoinitiator Lucirin TPO and vitamin E as antioxidant (1 % by weight) were added. The mixture was stirred to give a clear solution. Curing conditions: 1 h at 130°C.

Application

The sols were applied with a spiral applicator, the coating thickness was 4 μm.

Activation of the scavenger layers

The activation of oxygen scavenger systems were carried out on a Beltron 22/III with a UV-performance of 8,2 J/cm^2 (2 UV-lamps, each 1200 W, throughput speed of 0.4 m/min. and exposure time of 30 sec).

Gallic-acid-derivates indicator

Matrix system

The matrix of the O_2-indicator system is a commercial available lacquer which consists of component 1 (basic component) and component 2 (methyl-ethyl-ketone). To generate a printable solution of the indicator 6 weight-% gallic acid (relating to the component 1+2) is solved into the component 2 by stirring for 30 min at room temperature. This mixture is solved in component 1 – under stirring for further 30 min. The ratio of the component 1 to 2 is 60:40 weight-%. This solution shows a slow-down time of approximately 15 s with the DIN-beaker (DIN 53211). Therefore the solution could be applied via a printing process.

Application

The produced printable indicator solution is partially applied via a printing process onto a PE film which is extended and filled with an alkaline substance. The thickness of the applied indicator dot is 4 μm.

Activation

The oxygen indicator system incorporated into a multi-layer structure is activated by a relative humidity ≤ 84 %. This oxygen indicator system is suitable for the application in packaging for wet food.

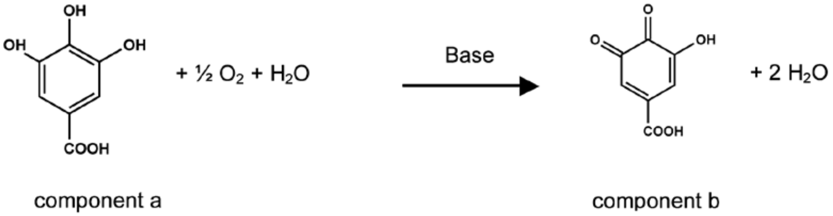
RESULTS AND CONCLUSION

The scavenger and indicator systems developed within the project ACOSIC are based on the following functional principles:

- gallic-acid solved into a specific adhesive in contact with an alkaline environment acting as oxygen scavenger,
- cyclo-olefin bonded chemically to a silicate backbone acting as oxygen scavenger,
- gallic-acid solved into a specific lacquer in contact with an alkaline environment acting as oxygen indicator.

Gallic-acid based oxygen scavenger

The functional principle of the developed oxygen scavenger is based on a redox reaction. The oxygen is reduced by the gallic acid and chemically bonded as H_2O. The gallic acid is oxidized to the ortho-chinoide state. This reaction needs an alkaline milieu which is generated by an alkaline substance in presence of water. The reaction mechanism is expressed by the following equation:



Eq. 1. Mechanism of the reduction of oxygen by gallic acid (component a) in presence of an alkaline milieu; component b: 5-hydroxy-3,4-dioxo-1,5-cyclohexandien-1-carboxylic acid.

This concept permits the activation of this O_2-scavenging functionality by humidity. For that reason the O_2-scavenger can be stored and processed under normal atmospheric conditions.

The manufactured O_2-scavenging packaging material shows after the activation of the O_2-scavenger functionality an excellent oxygen scavenging capacity and kinetic (see Fig. 1).

Cyclo-olefin-silane based oxygen scavenger

The functional principle of the newly developed oxygen scavenger system is based on a photo-initiated, metal catalyzed oxidation of a cyclo-olefin bonded chemically to a silicate backbone. This concept permits the activation of the scavenging process by UV light and prevents the formation of low-molecular oxidation products which may decrease the quality of the packaged goods or may be toxic. This silica-

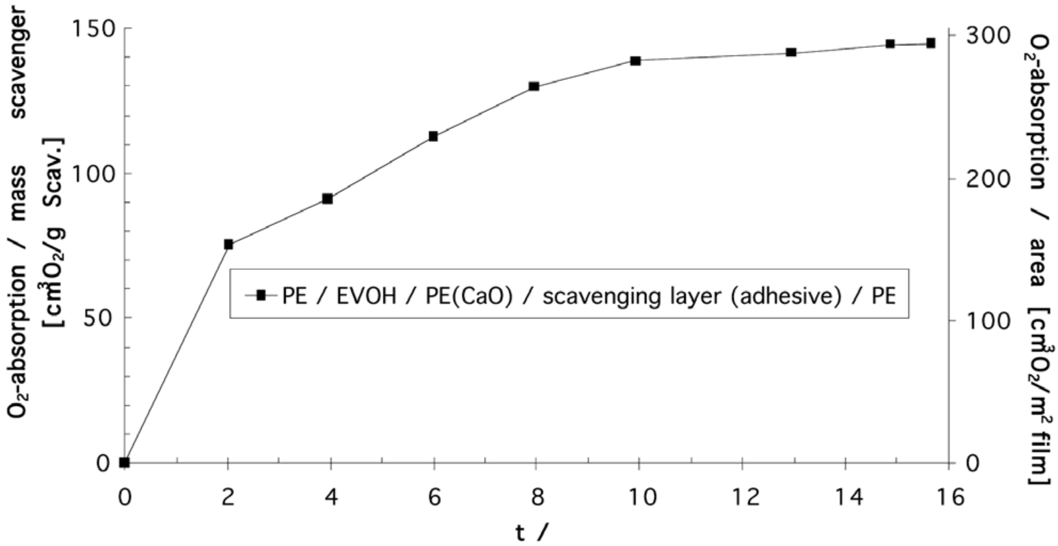
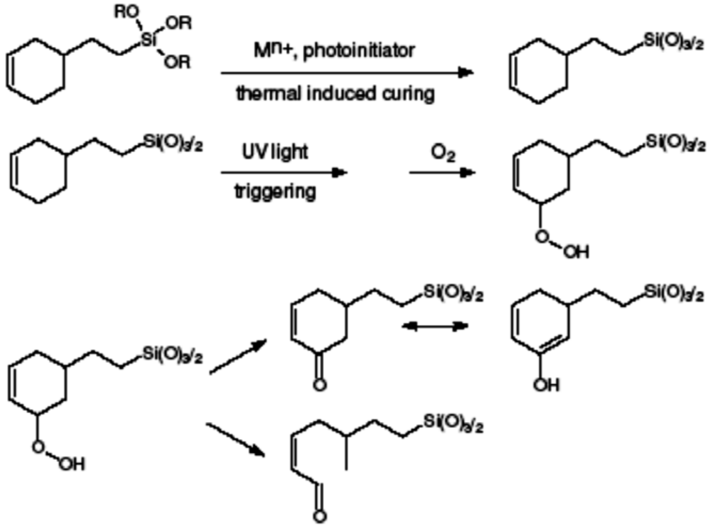


Fig. 1. Oxygen scavenging characteristic of packaging film containing the gallic acid based scavenger.

based system is formed in a one-step synthesis by hydrolysis of the corresponding alkoxysilanes. In addition, the resulting sol can be applied to any suitable film substrates by common coating techniques.

The transition metal catalyzed oxidation of the silylated cyclic olefin compound proceeds in the following way (Eq.2):



Eq. 2. Proposed mechanism of the transition metal catalyzed oxidation of the silylated cyclic olefin compound.

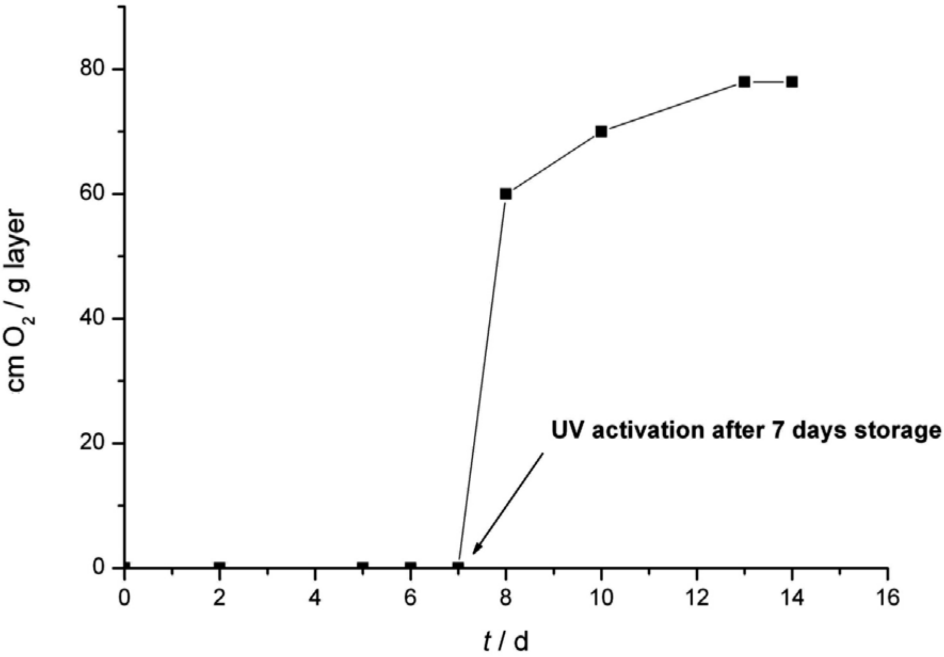


Fig. 2. Oxygen scavenging activity of cyclo-olefin-silane based scavenger system after 7 days of storage under air and subsequent UV activation.

For practical applications it is very important that the film coated with this oxygen scavenger system can be stored under air exposure for some time. A scavenging composition containing an antioxidant (vitamin E, content ≤ 1 % by weight) could be found. The scavenger system was applied onto a PET film by spiral applicator method and subsequently cured thermally. The coated films were UV activated and then evaluated with regard to their oxygen scavenging capacity and kinetics.

The oxygen scavenging system shows a good shelf life with regard to storage under air and subsequent UV activation combined with an excellent oxygen scavenging capacity and kinetics (see Fig. 2). Rapid oxidation consumption by the scavenger is a matter of high importance especially in food and pharmaceuticals packaging where remaining oxygen in the headspace may be detrimental to product quality within a short period of time.

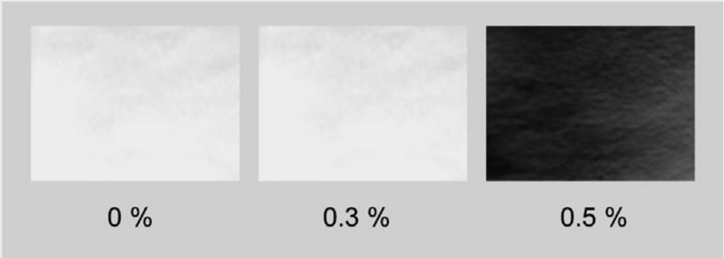


Fig. 3. Colour change of the gallic-acid-derivate based oxygen indicator at different levels of oxygen concentration: 0 %, 0.3 % and 0.5 % of oxygen at 100 % r. h. and 23 °C.

Gallic-acid based oxygen indicator

To provide the gallic

acid with an O_2-indication property a specific formulation is necessary as described in chapter 4.3.1 in combination with an alkaline milieu. At present the O_2-indicator system is adjusted in order to indicate the exceeding of an O_2-threshold value of 0.3 % (see figure 3) by changing the colour from white to dark purple. The indicator function is a irreversible one. The parameters to describe the functional characteristic of the developed O_2-indicator system are presented in table 2.

Table 2. Functional characteristic of the gallic-acid-derivate based O_2-indicator.

Functionality	value
Colour change	White => dark-purple
O_2-threshold value	0,3 %-O_2
O_2-induction time	33 h by 0,3 %-O_2
Trigger	Relative humidity ≤ 85 %

The O_2-induction time is the time which the system needs to be able to indicate the oxygen. This time depends on different parameters which are determined by the headspace conditions and the structure of the packaging material. The main points are the relative humidity and the O_2-concentration in the headspace of the package, the oxygen and water vapour permeation of the packaging material and the storage temperature of the package.

Testing of filled packaging

To determine the scavenging efficiency of film structures containing the novel developed O_2-scavenger tests were performed for packing selected foods under modified atmosphere. A known quantity of the active film structure was placed in a lid which was sealed onto a tray packed with sliced cheese. The oxygen start concentration and the storage temperature were chosen with respect to the target

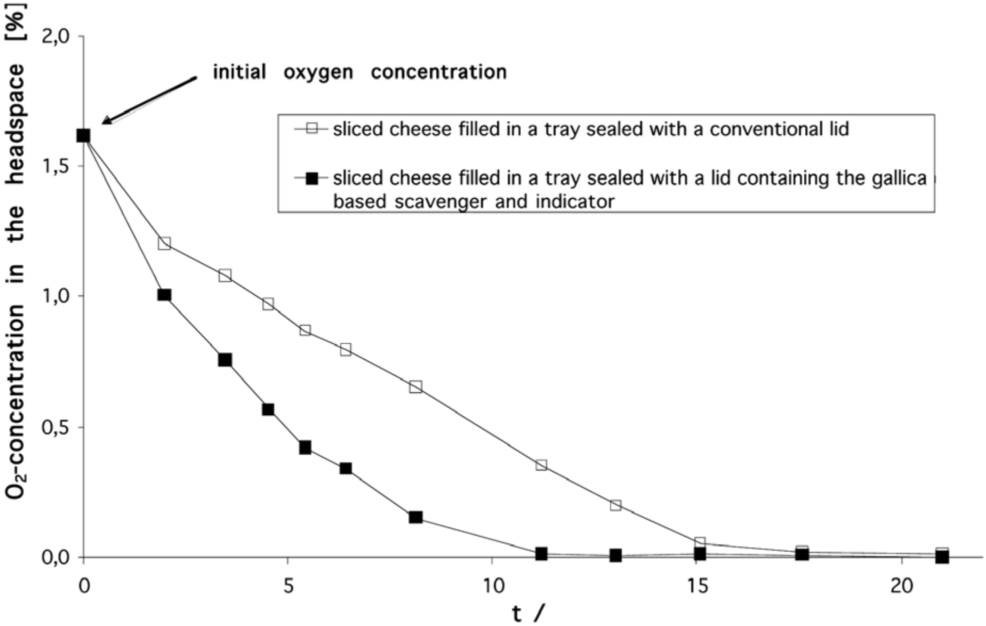


Fig. 4. Oxygen concentration in the headspace of a filled conventional packaging in comparison to the packaging containing the novel developed scavenger incorporated in the lid (packed food: sliced cheese; packing under modified atmosphere; oxygen start concentration: 1,6 % ; storage temperature 5 °C).

application. A sealed standard barrier tray with the same structure but without an incorporated O_2-scavenger was used as reference system. Figure 4 shows the measured characteristic of the O_2-concentration in the headspace of these two packaging types in relation to the storage time.

The O_2-concentration is reduced approximately to a zero level in both packages. The oxygen concentration in the headspace of the packaging containing the O_2-scavenger is lower than in the reference system. Compared with the standard barrier tray the “zero” level is reached in the O_2-scavenger package 7 days faster. The incorporated scavenger system serves as a scavenger of the residual oxygen in the headspace but the packed food is a strong competitor. The conclusion is that the characteristic of the O_2-consumption of the food has to be known. The O_2-scavenger characteristic has to be adapted to the oxygen consumption characteristic of the food to make sure that the scavenger system incorporated in specific film structures is faster in chemically scavenging the residual oxygen than the food to be packed.

CONCLUSION

The first demonstrator films containing the novel developed active substances offer a promising potential for application in the area of food packaging for oxygen sensitive food and pharmaceutical products. They provide both high oxygen uptake and fast oxidation kinetics and simultaneously a visible oxygen detection functions. The oxygen scavenger and indicator materials can be applied by conventional film conversion techniques and can be adapted to different kinds of substrates. Further developments are planned to improve the scavenging capacity and kinetics and the determination of the indication threshold of the demonstrator films.

ACKNOWLEDGEMENTS

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MOISTURE BARRIER, MECHANICAL AND ANTIMICROBIAL PROPERTIES OF LOW pH PECTIN-SOY FLOUR EDIBLE FILM

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Key words: edible film, soy flour, pectin, mechanical properties, shelf-life

While both pectin and protein are materials used for edible film and coatings, little information is available on the properties of pectin-protein composite films.

Protein-protein cross linking in edible films we affected by heat denaturation conditions (Tapia-Blacido *et al.*, 2005; Perez-Gago and Krochta, 2001). It has also been reported that hydrophobicity of soybean proteins is maximal near the iso-electric region of pH (Elizalde *et al.*, 1996).

In the attempt to improve the water resistance of a pectin-soy film (Mariniello *et al.*, 2003), the effect of protein-solution pH on water vapour permeability, sorption isotherms and mechanical properties have been investigated.

Use of a low-pH film could to be a means of retarding surface microbial growth, thereby extending shelf-life. In addition, pectins have been reported to improve water retention in colloidal systems together with their stabilization (Baeva and Panchev, 2005). Accordingly, we used the pectin-soy film to control microbial growth on smoked salmon, and to retain the crispness of cookies covering ice cream.

When soy flour solution was adjusted to pH 3 (instead of 9, Mariniello *et al.*, 2003), water vapour permeability (WWP) decreased from $4.55\ 10^{-6}$ g m^{-1} h^{-1} Pa^{-1} (Mariniello *et al.*, 2003) to $1.28\ 10^{-6}$ g m^{-1} h^{-1} Pa^{-1}. The moisture adsorption data of the pectin-soy film were fitted to GAB model ($R^2 = 0.989$) and experimental monolayer value was 6.2 g water/100 g dry material.

Tensile strength (TS) increased from 6.8 (Mariniello *et al.*, 2003) to 12.1 MPa, whereas elongation break (EB) % decreased from 11.61 (Mariniello *et al.*, 2003) to 5.7. This was attributed to soy protein denaturation, probably resulting in tighter compact protein networks. The greater interaction of proteins may result in less elastic film structure. Effects of RH on TS, Young's modulus and EB were evaluated, ranging from 48 MPa, 63 N/mm, 6.12% at 25% RH to 1.2 MPa, 0.42 N/mm, 18.33% at 90% RH, respectively.

Crispness evaluation by a bending test showed that application of film on cookies covering ice cream retarded crispness decrease. Assessed on smoked salmon, the pectin-soy film was found effective in inhibiting the growth of *Enterobacteriaceae* and spoilage psycrotrophyc bacteria, resulting in shelf-life extension at salmon stored at 3°C.

PHYSICAL PROPERTIES OF EDIBLE FILMS BASED ON CASSAVA STARCH AS AFFECTED BY THE PLASTICIZER CONCENTRATION

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ABSTRACT

The aim of this work was to investigate the effect of glycerol contents on the physical properties of cassava starch films. Films were prepared from film-forming solutions with 2g cassava starch/100g water plus 0, 15, 30 and 45g glycerol/100g starch, and analyzed to determine its mechanical properties by tensile tests; the glass transition temperature (Tg) by differential scanning calorimetry (DSC); and the crystallinity by X-ray diffraction (XDR). The infrared spectra of the films were also recorded. The resistance values of the films decreased while those of the elasticity increased with the increase of the glycerol concentration, due to plasticizer effect of glycerol, which was also observed in DSC curves. The Tg of the films prepared decreased with the glycerol content. However, for samples with 30 and 45g glycerol/100 g starch, two Tg curves were observed, probably due to a phase separation phenomenon. According to the XRD diffractograms, films with 0 and 15g glycerol/100 g starch presented an amorphous character, but some tendency to show crystalline peaks were observed for films with 30 and 45 g glycerol/100 g starch. The results obtained with FTIR corroborated these observations.

Key words: edible films, mechanical properties, differential scanning calorimetry, X-ray diffraction, Fourier transform infrared

INTRODUCTION

Edible films are thin materials based on biopolymers, such as polysaccharides. Starch is a polysaccharide composed by the amylose, a linear or sparsely branched polymer, and the amylopectin, a highly branched polymer (Mali and others, 2005, Famá and others, 2005). This biopolymer could be interesting in the edible film technology because it is produced abundantly around the world, and could be considered as inexpensive.

An important starch source is the cassava (*Manihot esculenta* C.), which is a tropical root. Cassava starch is able to form transparent coatings (Vicentini and Cereda, 1999) and flexible films (Vicentini and others, 2005) without any previous chemical treatment, neither plasticizer addition.

However, for the production of edible films with good workability, a plasticizer such as the glycerol is usually used. The plasticizer modifies the interactions between the macromolecules, resulting in an increase in the chains mobility and consequently, causing a reduction in the glass transition temperature of the system (Sobral and others, 2001). Thus, plasticizers may affect all physical properties of films.

The aim of this work was to investigate the effect of glycerol content on the physical properties of cassava starch films, by means of mechanical analysis, X-ray diffraction, differential scanning calorimetry and Fourier-transform infrared spectroscopy.

MATERIAL AND METHODS

Cassava starch, supplied by a local industry (Flor de Lotus Co., Brazil), had the following characteristics (Vicentini and others, 2005): 14.9% moisture; 16.0% amylose, 0.21% soluble total sugars, 0.23% ash; 0.39% fiber; 0.24% total nitrogen; and 0.15% lipids.

For edible films production, film-forming solutions (FFS) were previously prepared with 2g of starch/100g of water and 0, 15, 30 and 45g of glycerol/100g of starch. The starch gelatinization was undertaken by thermal treatment at 70°C/1 min. in a water bath (Tecnal, TE 184). The FFS was poured in an acrylic plate and dehydrated in an oven with air circulation and renewal (Marconi, MA037), at 30°C/18-24 hours. Thus, transparent and flexible films with thickness around 0.070 mm were obtained.

Mechanical properties were determined by tensile tests, using a Texturometer (TA.XT2i, SMS), following the method suggested by Paschoalick and others (2003). Each test was done in quadruplicate. These tests were performed at room temperature (22-25°C) with samples previously conditioned at 25°C and 58% of relative humidity (NaBr saturated solution), for one week. However, for the subsequent analysis, samples were conditioned over silica gel.

The glass transition temperature of the films were measured using a differential scanning calorimeter (DSC-2010, TA Instruments), in duplicate. The samples were weighted (±0.01 mg, Analytical *Plus*, Ohaus) in aluminum pans, hermetically sealed and heated at a rate of 5°C/min (Sobral and others, 2001). The reference was a void pan.

The crystallinity of films was studied with an X-ray diffratometer (Rigaku), with Cu source, operating at room temperature, 40kV and 30mA. Rectangular samples were analyzed between $2\theta = 10^\circ$ and $2\theta = 30^\circ$ with a step size $2\theta = 4^\circ$.

Also, infrared spectra (FTIR) of films were recorded between 4000 and 600 cm^{-1} at 4 cm^{-1} of resolution, with a Spectrum One (Perkin Elmer) spectrometer, supplied with a universal attenuated total reflectance (UATR) accessory, as suggested by Vicentini and others (2005). For each spectrum, 25 scans were co-added. These analyzes were run in duplicate.

RESULTS AND DISCUSSIONS

As expected, the increase in the glycerol concentration on the FFS produced films less stiff and rigid, and more extendible, i.e., it caused a reduction on the tensile strength and increase of the elongation at break of films, respectively (Figure 1). These trends were probably due to the reduction in interactions between the biopolymers chains. This effect of the plasticizer concentration on the mechanical properties is well known and broadly discussed in the literature (Arvanitoyannis and Biliaderis, 1998, Sobral and others, 2001, Paschoalick and others, 2003, Mali and others, 2005).

The resulting trends of the mechanical properties of the films could be explained in a general manner, by the effect of the glycerol on the state properties of the films. It can be observed in the DSC curves of the films (Figure 2), that the glass transition temperature (Tg) decreased from 131.9 to 42.1°C, when the glycerol content increased from 0 to 45%, respectively, i.e, the films became less glassy with the addition of glycerol. This behavior was also observed by Mali and others (2002) working with yam starch based films, and by Forssell and others (1997), dealing with films based on barley starch, in both cases with glycerol. These latter authors also observed an endothermic peak as the one observed in this work (Figure 2) with samples without glycerol. In this case, this phenomenon, observed at a temperature of about 197°C in films without glycerol, was due to thermal degradation of starch, which shifted to higher temperatures by the increase of the glycerol

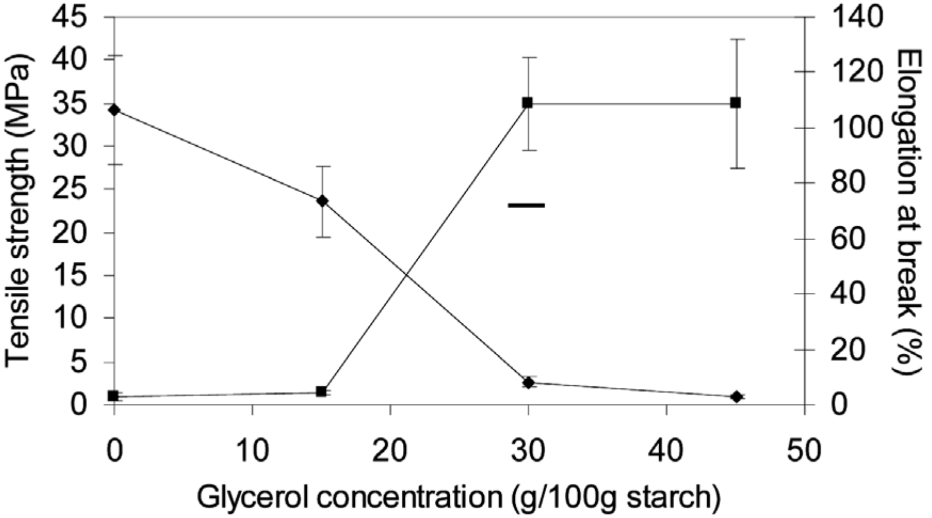

Fig. 1. Effect of the glycerol concentration on tensile strength and elongation at break of cassava starch films.

content, attaining 218°C in films with 45% of glycerol.

Also, it can be observed in DSC curves (Figure 2) of cassava starch films, that another glass transition was visible at very low temperature when glycerol concentration was 30% (Tg= -55.7°C) and 45% (Tg= -58.8°C). This behavior was also observed by Forssell and others (1997), whom suggested that a phase separation has occurred between a starch rich phase and glycerol rich phase. This phenomenon may explain the high standard deviation on data of elongation at break of films with 30 and 45% of glycerol. In fact, these films presented less workability than those with 0 or 15% of glycerol due to a "sticky" character.

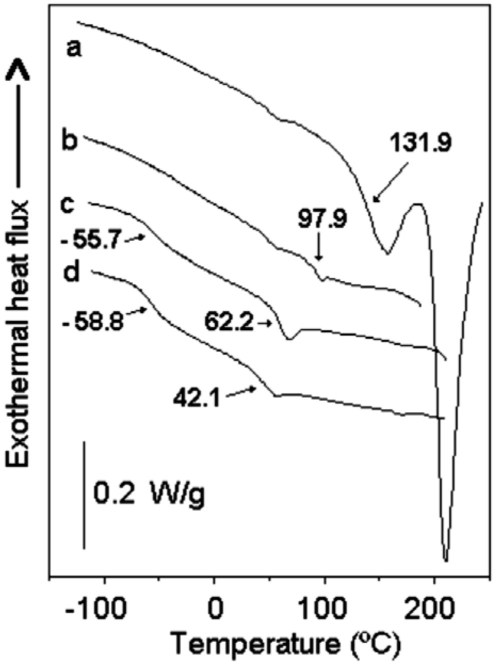

Fig. 2. DSC curves of cassava starch films containing (a) 0%; (b) 15%; (c) 30%; and (d) 45% of glycerol. Numbers indicated in the plots are values of glass transition temperature (°C).

Figure 3 shows the X-ray diffractograms (XRD) obtained with the films studied in this work. They can be related to complex structures like B-V type crystal structure, typical of tuber starches (Mali and others, 2002, Famá and others, 2005). According to these XRDs, films with 0 and 15g glycerol/100 g starch presented an amorphous characteristic, but some tendency to crystalline peaks, at $2\theta \approx 20°$, were observed for films with 30 and 45 g glycerol/100 g starch. Starch films could have amorphous character because the thermal treatment of FFS provoked starch gelatinization, causing disruption of the double helix conformations of the cassava starch. However, the increase in glycerol content in the films may have increased the macromolecular mobility, allowing the formation of microcrystalline junctions, i.e. some re-crystallization occurred. Mali and others (2002) observed that glycerol in FFS did not notoriously influence the X-ray pattern of yam starch films.

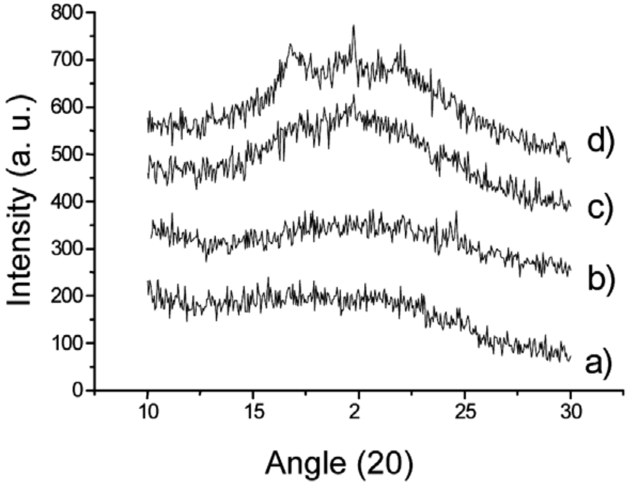

Fig. 3. X-ray diffraction pattern of cassava starch films containing (a) 0%; (b) 15%; (c) 30%; (d) 45% glycerol.

Some small differences in terms of band shape and intensity can be observed in the fingerprint of cassava starch in the FTIR spectra (Figure

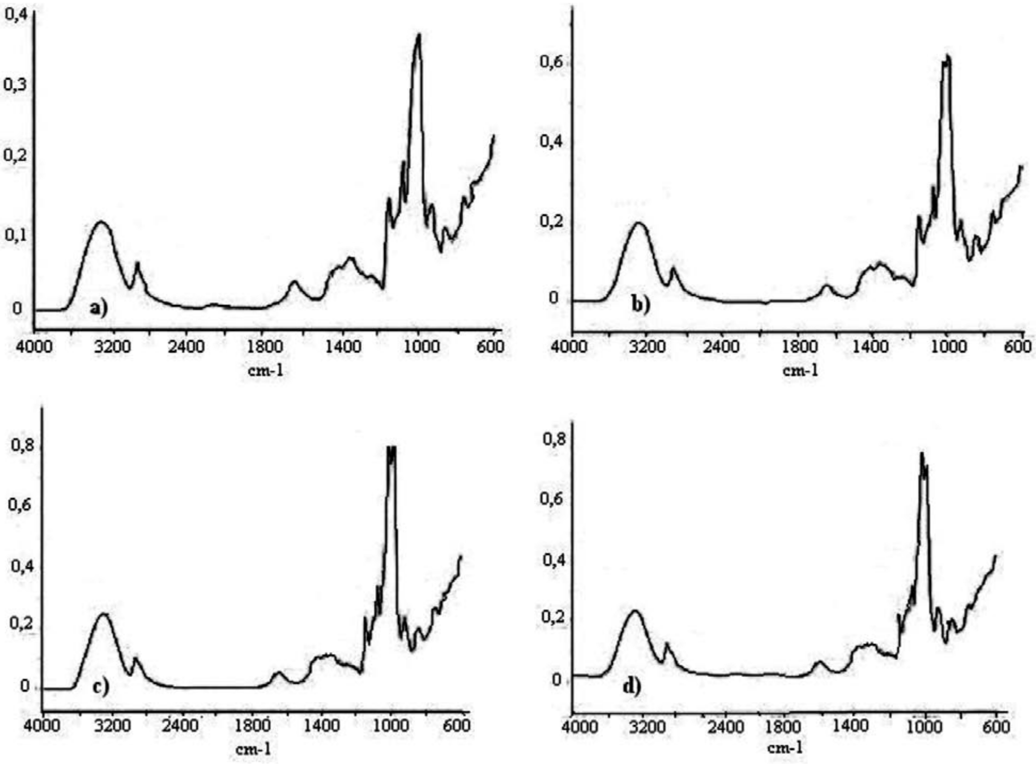

Fig. 4. FTIR spectra of cassava starch films containing (a) 0%; (b) 15%; (c) 30%; (d) 45% glycerol.

4), as a result of the glycerol content variation. The peak observed at 1011.8 cm^{-1}, that appeared as a shoulder in films without glycerol, became more prominent and presented a displacement to 1013.4, 1014.0 and 1014.8 cm^{-1}, for glycerol concentrations of 15, 30 and 45%, respectively. These peaks could be associated with COH bond vibrations or solvation, and could also be associated to changes from an amorphous to a semi-crystalline state (van Soest and others, 1995, Vicentini and others 2005). The displacement of the peak observed from 995.0 to 997.9 cm^{-1} when the glycerol content increased from 0 to 45%, could also be associated to the amorphous-crystalline transition in these films.

The glycerol also affected the peak normally associated to COC antisymmetric bridge stretching (van Soest and others, 1995). This peak, initially observed at 1148.6 cm^{-1} in films without glycerol, was displaced to 1149.6, 1150.2 and 1150.5 cm^{-1} when the glycerol concentration was 15, 30 and 45%, respectively. Thus, it can be suggested that the thin difference between these last two data may be explained by a phase separation, such as that observed in DSC curves (Figure 2).

CONCLUSIONS

The increase of glycerol content in cassava starch films, increase the macromolecular mobility, probably by solvation of COH bonds of the starch, which allowed some polymer re-crystallization. However, at the macroscopic scale, cassava starch films became less stiff and more flexible. In fact, the glycerol addition had two effects: it caused an increase in the mobility of amylose and amylopectin chains which overcame the opposite effect of the re-crystallinization. Also, the increase of the flexibility of the films with plasticizer could be attributed to a lubrication effect of glycerol, associated to a phase separation observed at the highest concentrations.

ACKNOWLEDGMENTS

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TASTE ADDITIVES TO PRODUCT AS DETERMINANT OF ITS SHELF-LIFE

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ABSTRACT

The purpose of the author's own research was to examine the extent of the impact which a taste additive added to a hygroscopic product with a high fat content had on the fluctuations of quality of the packaged product. The tested material consisted of crackers with no flavouring, sesame crackers and onion crackers.

The analyses performed during storage included the changes of the dry mass in the product, with the use of a type WPS 110S moisture balance, as well as the scope of variations in the colouring, texture, flavour and aroma of the product.

Key words: shelf-life, the dry mass of product, taste additives

INTRODUCTION

At the present moment, a violent growth of the confectionery sector is noted on the domestic market, the main manifestation of that being the emergence of a large number of competing companies who commercialise many new products. Given the rich assortment of supplied products and the diversity of the packaging materials used, the confectionery industry is particularly interesting. It supplies products which are very popular among the consumers. From the point of view of nutrition, the value of those products is rather low. Their essential feature is a high energetic value and flavours, thanks to which they occupy an important rank in the human diet (BAKER *et al.*, 1999).

The confectionery, just like in the case of other food products, ought to be characterised by a suitably high quality during the 'best before' period. The occurrence of adverse processes, leading to the deterioration of the quality of bakery products, can be significantly limited, or their course slowed down by using raw materials of suitable freshness, appropriate conduct of the technological process, using packaging acting as barrier for water steam and volatile substances, and ensuring appropriate storage conditions (BRODY *et al.*, 2000).

Among them the most important are the factors directly related to the physicochemical properties of the packaged product. Those include chemical composition, physical state, texture, porosity, and the storage time and conditions in which the product shall remain until consumed by the consumer. The knowledge of the processes (mechanisms) and factors stimulating the physical, chemical, biochemical and biological transformations occurring in the product during the storage and resulting in a lowered usability, is very important (MAN *et al.*, 2000; PORRETTA, 2004; UCHEREK *et al.*, 2005).

MATERIALS AND METHODS

Different kinds of crackers: crackers, sesame crackers and onion crackers were subject of this investigation.These products were packaged in bag with a side fold OPP/OPP which were moulded in v.f.f.h (vertical form-fill-seal) system - weight 180 gramme,

In order to determine the influence of the additives on the durability of the products packaged, the research was carried out in a special microclimate, using air

Table 1. Functions approximating the changes of the quality parameters of different kind of crackers.

Quality parameter	Regression equation	R^2 (%)
Crackers		
Water content (X)	$X= -0,0009t^4 + 0,0272t^3 - 0,258t^2 + 1,0956t + 3,2711$	96,01
ITSQ	$ITSQ = 0,0007t^4 - 0,0147t^3 + 0,0851t^2 - 0,5494t + 5,2306$	99,23
Colour (C)	$C = 0,0017t^4 - 0,0188t^3 - 0,0151t^2 + 0,2554t + 4,7500$	96,63
Texture (T)	$T = 0,0014t^4 + 0,0083t^3 + 0,0822t^2 - 0,9442t + 5,7778$	97,08
Flavour (F)	$F = 0,0019t^4 - 0,0346t^3 + 0,1919t^2 - 0,7716t + 4,9444$	97,43
Aroma (A)	$A = 0,0017t^4 - 0,024t^3 + 0,0632t^2 - 0,3277t + 5,3611$	97,88
Sezame crackers		
Water content (X)	$X = 0,0036t^4 - 0,0707t^3 + 0,4108t^2 - 0,3399t + 3,6856$	98,06
ITSQ	$ITSQ = 0,0007t^4 - 0,0147t^3 + 0,0851t^2 - 0,5494t + 5,2306$	99,23
Colour (C)	$C = 0,0014t^4 + 0,0319t^3 - 0,2714t^2 + 0,6787t + 4,5556$	93,45
Texture (T)	$T = 0,002t^4 - 0,0366t^3 + 0,1714t^2 - 0,4566t + 4,3889$	94,40
Flavour (F)	$F = 0,003t^4 + 0,0827t^3 - 0,7052t^2 + 1,6258t + 2,9722$	98,37
Aroma (A)	$A = 0,0018t^4 - 0,0356t^3 + 0,2265t^2 - 1,0527t + 5,9444$	98,31
Onion crackers		
Water content (X)	$X = 0,0007t^4 + 0,0252t^3 - 0,2623t^2 + 1,1568t + 2,9861$	94,46
ITSQ	$ITSQ = 0,0016t^4 - 0,0321t^3 + 0,1999t^2 - 0,7414t + 5,5389$	98,67
Colour (C)	$C = 0,0006t^4 + 0,0184t^3 - 0,2059t^2 + 0,6085t + 4,5556$	91,96
Texture (T)	$T = 0,0002t^4 - 0,0318t^3 + 0,407t^2 - 1,7276t + 6,3611$	98,76
Flavour (F)	$F = 0,0043t^4 - 0,0834t^3 + 0,5221t^2 - 1,6402x + 6,1667$	98,94
Aroma (A)	$A = 0,0015t^4 + 0,0253t^3 - 0,3788t^2 + 1,2626t + 4, 2144$	94,70

Source: own’s work.

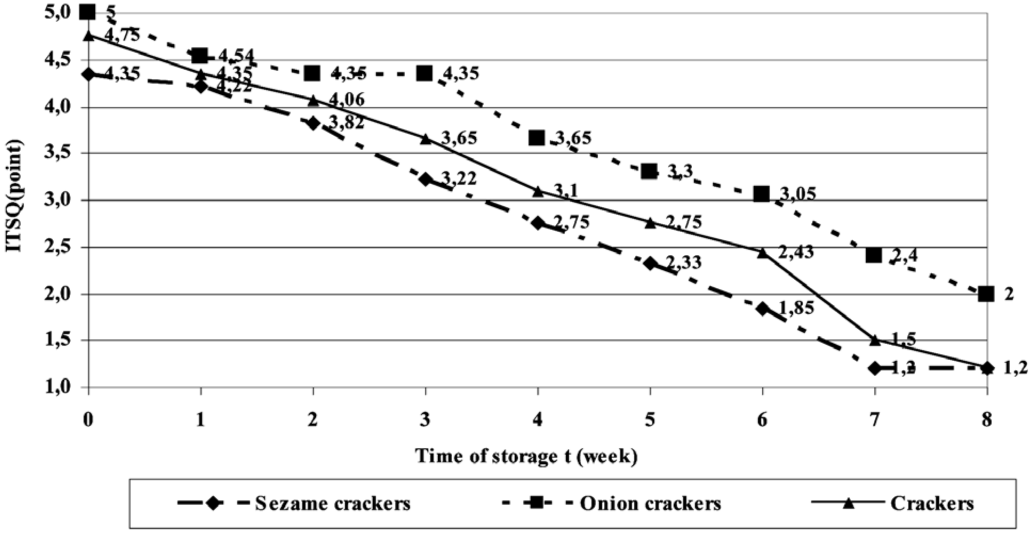

Fig. 1. Changes of the index of total sensorial quality (ITSQ) of the products: crackers, onion crackers and sezame crackers during storage (t) in an environment of accelerated ageing: T= 40°C ± 1°C, R= 80% ±2%. Source: own's work.

conditioning chamber, as following: T= 40°C ± 1°C, R= 80% ±2%. For the purposes of the storage research, a procedure of accelerated tests was used D/NI-04-04-01 "Crash Test"(D/NI-04-04-01, 1997).

The research material thus prepared was then used as a basis for the sensory evaluation of changes in the products and of the water content in the product. The water content was determined using a moisture balance and the method of drying until dry mass using Electronic Weighting Equipment, Type WPS 110S Radwag: mod E1(SERVICE MANUAL OF ELECTRONIC WEIGHTING EQUIPMENT, 2002).

The sensory tests were performed in a special sensory laboratory with separate testing and product- preparation areas. Products were assessed by a trained panel consisting of 6 persons (ISO 8586, 1987). The scoring method applied to sensory estimation of product was a 5 point scale. Finally, an index of total sensorial quality (ITSQ) was determined (ISO 4121, 1987).

RESULTS AND DISSCUSION

The statistical analysis of the obtained results was carried out using the appropriate modules of the Statistica 6,0 software. The average values, calculated on the basis of the results showing the changes of specific quality parameters of the analyzed products, were used to calculate the functions mapping the development of such changes during storage. The function equations were calculated using the method of the smallest squares, and the "Non-linear estimation" package. The approximating model was selected on the basis of the observation of the spread of the empirical points on the plane established by two coordinates (t = time of storage; Y = value of a specific quality parameter) (ZELIAS *et al.*, 2004).

The equations of the calculated approximating functions are shown in table 1. An analysis of the changes X in the examined products pointed to a lesser influence of the additives to the scope of the quality changes in the course of the storage. Because of that, the changes ITSQ (fig. 1) were analysed in detail,

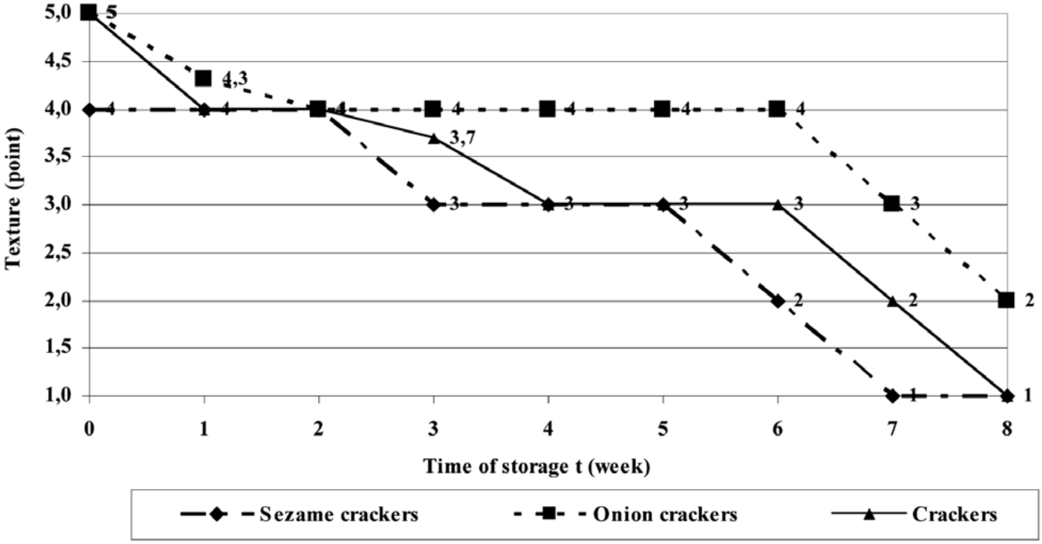

Fig. 2. Changes of the texture (T) of the products: crackers, onion crackers and sezame crackers during storage (t) in an environment of accelerated ageing: T= 40^{0}C ± 1^{0}C, R= 80% ±2%. Source: own's work.

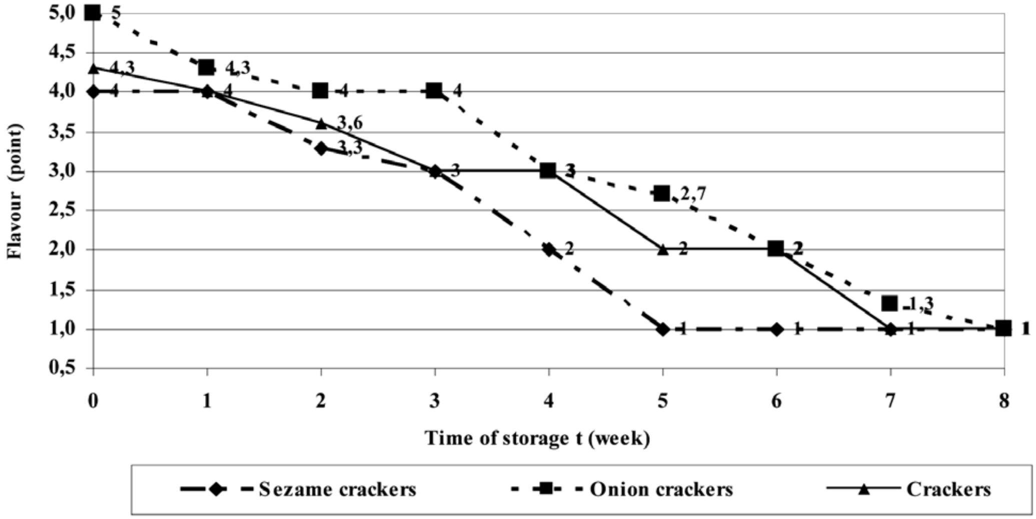

Fig. 3. Changes of the flavour (F) of the products: crackers, onion crackers and sezame crackers during storage (t) in an environment of accelerated ageing: T= 40^{0}C ± 1^{0}C, R= 80% ±2%. Source: own's work.

focusing on the most dynamical sensorial features, i.e. texture, flavour and aroma (fig. 2-4).

An addition of sesame and onion to the crackers resulted in the differentiation of the intensity of the aroma in the product, which has found its reflection in the preliminary evaluation of the aroma (fig. 3) and analogously ITSQ (fig. 1).

An addition of sesame to the product made a significant impact on the intensity and scope of the changes of all the quality properties, compared to the crackers without additives. That was caused by a higher content of fat in the product with sesame, and as a consequence by specific kinetics of the process of fat auto oxidation.

The onion added to the product resulted in a lower kinetics of the adverse changes of the sensorial quality expressed by ITSQ (fig. 1) and more particularly of the aroma and texture (fig. 2 and 4).

With respect to the flavour, significant changes of evaluation, for the benefit of the onion crackers, occurred up to the 5 week of storage. In the following weeks, the adverse changes in the flavour as a result of the auto oxidation of the fat present in the product were obvious (fig. 3).

CONCLUSION

The sensorial properties play a key role in the evaluation of the quality of a product. They are principally the result of the selection of appropriate ingredients, including additives, of the kind and course of technological processes, and the physico-chemical properties.

As a result of the operation of adverse factors, changes occur in the sensorial properties, and to a lesser extent, in the physico-chemical profile of the product.

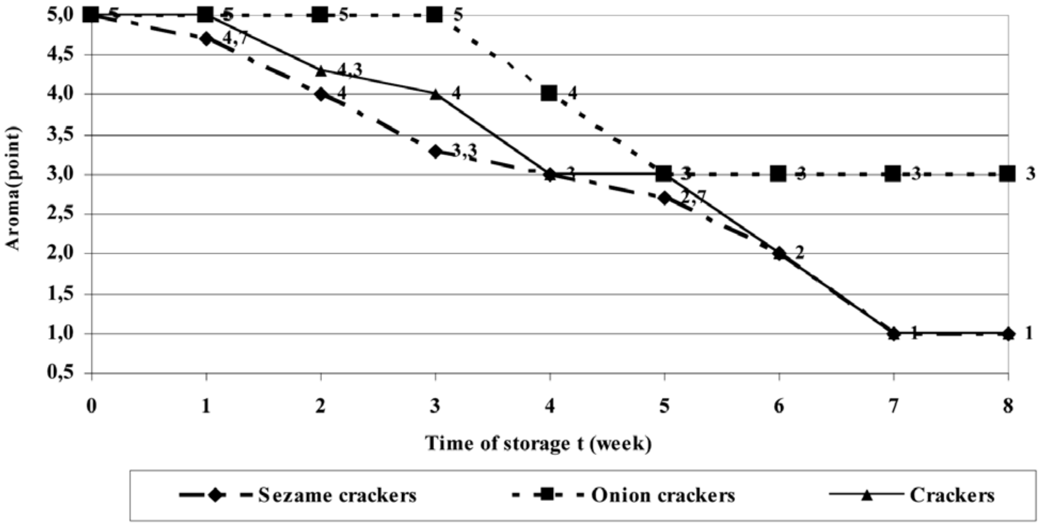

Fig. 4. Changes of the aroma (A) of the products: crackers, onion crackers and sezame crackers during storage (t) in an environment of accelerated ageing: T= 40⁰C ± 1⁰C, R= 80% ±2%. Source: own's work.

The deterioration of the sensorial quality of the confectionery/bakery is the result of the changes in the texture, flavour and aroma of the product.

The most important physico-chemical changes are related to the transformations in the lipid fraction of the product. The fat auto oxidation reaction is particularly dangerous for the quality. Its products have unpleasant flavour and smell, and their accumulation may lead to the disqualification of the products.

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ECONOMIC ASPECTS OF THE EXAMINATION OF SHELF-LIFE OF PRODUCT IN MODIFIED ATMOSPHERE PACKAGING

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ABSTRACT

Packaging products in a modified atmosphere is one of the range of specific packaging systems ensuring that the shelf-life of products is extended. Using the method of packaging in a mix of gases might bring a lot of benefits to the producers, distributors and potential purchasers, however it is associated with certain expenditure, which is related principally with the original level of quality of the packaged products, with the appropriate choice of the packaging materials and formats, and with the parameters of the packaging process.

While determining the shelf-life of the products packaged in modified atmosphere one has therefore to take into consideration many factors directly affecting the durability period of the packaged products. Thus, the purpose of the study is to develop a model of the path of costs for the MAP packaged products.

Key words: modified atmosphere packaging, costs of shelf-life

INTRODUCTION

From an economical perspective, the issue of the durability of packaged products ought to be analysed first of all with respect to the costs incurred and directly influencing the obtained economically justified durability of food.

Extending the durability of the packaged products as a rule entails an increase of the costs of production related mainly to the initial level of quality of the raw materials, kind of packaging materials used, packaging construction solutions, and packaging systems. Thus a problem arises: to what extent the financial outlay is cost-efficient and to what level it can be increased. It entails the necessity of using an economic optimisation calculation with respect to costs sustained for the increased durability of the packaged products, which is of a multi-criterion nature. The costs ought to cover all the phases of the product's life cycle, starting from the product development phase, through the production phase, lab tests, distribution and sale, to the usage phase involving among other things the disposal of the packaging waste (KUBERA, 2003; LISIŃSKA-KUŚNIERZ *et al.*, 2003).

THE COST PATH OF THE PACKAGED PRODUCTS

An example of the cost path of the MAP packaged products, influencing the durability of the packaged products, is shown on fig. 1.

The decision-making process pertaining to the specific elements in the presented simplified model of the cost path is closely associated with the knowledge of the factors determining the durability of products packaged in a mixture of gases.

The initial level of the product durability ought to be established so as to facilitate the meeting of specified requirements and expectations of the recipients, and guarantee optimised costs related to the development, production, storage and sale of the packaged products. Such condition can be fulfilled only when technical, economical, ecological and other factors related to the phases of production, distribution and consumption of the packaged product are taken into account during the selection of the initial level of durability.

However, an extension of a food product durability leads first of all to increased costs at the stage of production and packaging, with reduction of costs during the distribution, sales and utilisation. The total cost involved in the ensuring of a required level of durability reaches a minimum, which determines an economical optimum of the durability level.

The assessment of the economical optimum of food durability ought to be of a comprehensive nature. Besides the costs of production of the packaged product, its distribution and sale, and the costs related to the utilisation, the assessment should take into account (DWILINSKI, 2000; LISIŃSKA-KUŚNIERZ *at al.*, 2003; MAN, 2000; ROBERTSON, 2000):

- costs of durability extension arising in the development of the packaged food,
- economic effects of the extension of durability pertaining to the entire life cycle of the product,
- calculation of the costs and economic effects, taking into account the time and site of their generation,
- confrontation of the aggregate costs with the obtained economic effects,
- confrontation of several alternative solutions (e.g. various kinds of packaging) in the light of achieved durability period of the packaged products.

At the time the decisions are taken concerning the extension of the product durability, the technical and organisational aspects are taken into account in the first order. It should be noted however, that the technical issues related to the selection of raw materials, packaging system and kind of packaging material are examined only after the company sets the main directions of their policy in respect of the quality assurance, and as a result also in respect of the durability of packaged products.

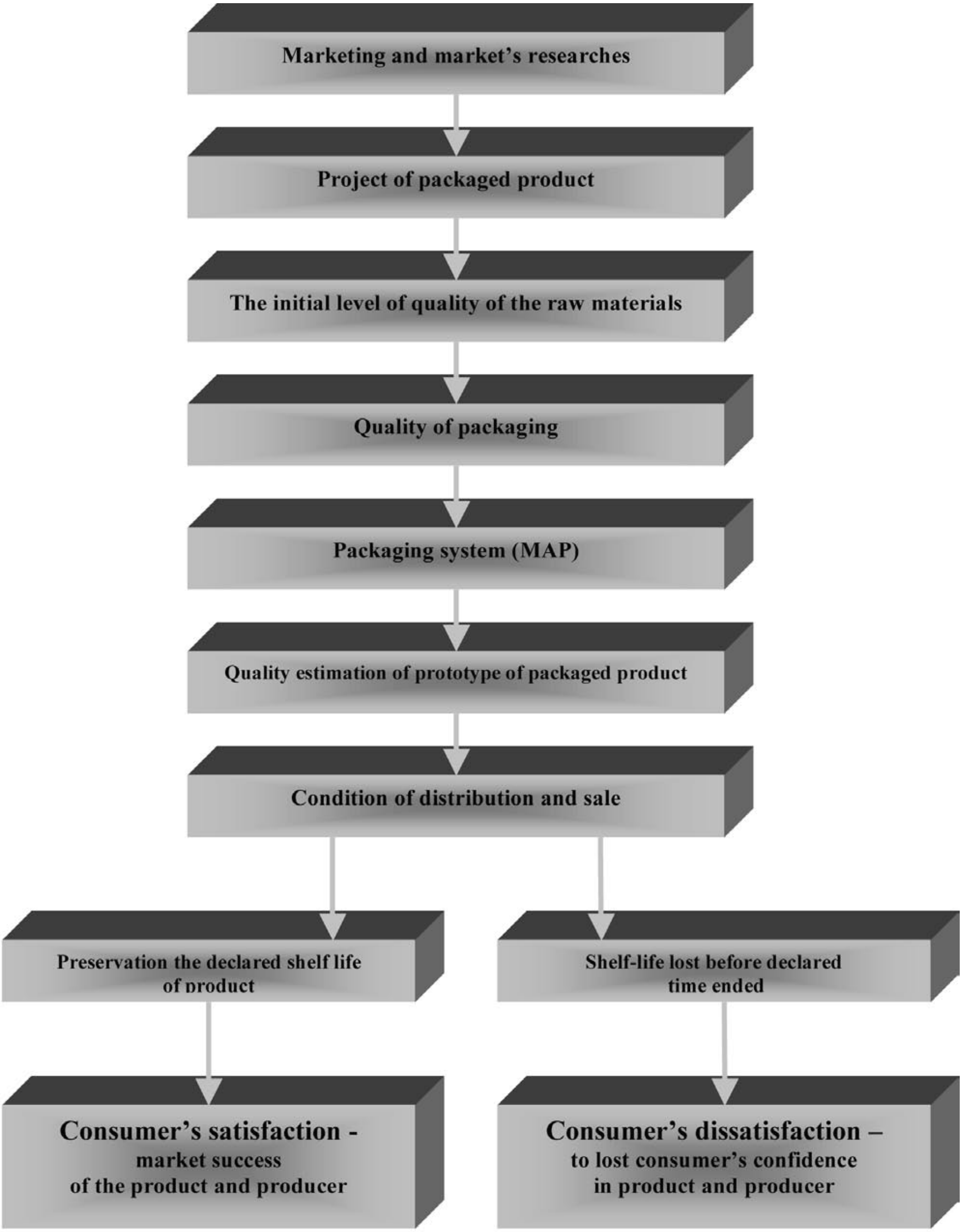

Fig. 1. The cost path of the MAP packaged products.
Source: own's work.

In many cases the major reason of the quality deterioration during the storage, distribution and sale of food, besides the initial quality of the product, are the properties of the packaging material and the quality of the packaging. For that reason, the construction of most packaging solutions needs to be optimised with respect to its barrier properties, mechanical resistance and thermal sealability. That is important not only because of the necessity to ensure the best possible protection of the products. It is also a means to achieve tangible economic results, avoiding the so-called 'over-packaging' (LISIŃSKA-KUŚNIERZ *et al.*, 2000).

The determination of the durability period of food products is a an important issue in the field of the science of goods, which has its economic consequences connected also with the uncertainty of the predictions of the product 'best before' date. That aspect has a material influence on the volume of sales and profit of the company. Thus, from the point of view of the durability policies, the methodologies of durability testing and the related costs become particularly important.

Conducting durability tests requires appropriate planning and organisation of the research. The researchers should take into account an efficient use of the technical possibilities of measuring the selected quality parameters, and a cost-efficient realisation of the assumed scope of the research. It is worth noting however, that the prevailing criterion in such selection is the content-related value of the results obtained, constituting a basis for an evaluation of the durability of the packaged product. In order to guarantee an economically justified test of the durability, the method selected should include modelling and assessing a wide spectrum of issues, and facilitate a reliable quality and quantity assessment of the packaged product (DWILINSKI, 2000).

The plan of the research and the size of the sample should be determined not only on the basis of statistical criteria, but also of the cost of information collected, costs of the posts and products used for the research, costs of the consumed raw materials, consumables and energy, as well as the costs of organising the research. If the sample is increased beyond a certain limit, the costs grow dramatically while the statistical credibility of the results is increased only slightly. While setting the schedule of data collection, the researcher should also pay attention to the fact that certain data can be used also for purposes other than the currently focused on, which should lead to the selection of the most cost-efficient solution (KUBERA, 2003).

While analysing the foundations of the economic optimisation of the durability of packaged products, one could not avoid the impact of the ecological requirements on the choice of packaging materials and packaging, and as a consequence, on the costs of packaging. The commercialised packaging have to meet the environment protection requirements and be consistent with the rules governing the use and disposal of the packaging and packaging waste, in compliance with the principle of sustainable development. The essential responsibility of the entrepreneur is to ensure the recovery, and practically a specific level of recycling of packaging waste generated after the products are commercialised. That level depends from the kind of the packaging material (UCHEREK, 2003).

CONCLUSION

Packaging products in a modified atmosphere is one of the range of specific packaging systems ensuring that the shelf-life of products is extended. Using the method of packaging in a mix of gases might bring a lot of benefits to the producers, distributors and potential purchasers, however it is associated with certain expenditure, which is related principally with the original level of quality of the packaged products, with the appropriate choice of the packaging materials and formats, and with the parameters of the packaging process (UCHEREK, 2004; 2006).

Thus, achieving an assumed level of the economically justified durability of food requires specialised knowledge in such fields as science of goods, economics and ecology, supported by a familiarity with the pertinent requirements and regulations currently in force, and by suitable experience and financial outlay.

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ANTIMICROBIAL ACTIVITY OF LEMON EXTRACT

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ABSTRACT

A quantitative investigation on the antimicrobial activity of lemon extract against some food spoilage microorganisms is presented. Growth kinetics and dose-response profiles were obtained by using experimental data from turbid metric technique. Non-Inhibitory Concentration, as sensibility to the active compound and Minimum Inhibitory Concentration, as microbial resistance, were calculated. Results prove that the extract exerts good effectiveness on the growth of selected bacteria and yeasts.

Key words: antimicrobial properties, lemon extract, natural compound

INTRODUCTION

In recent years the use of natural compounds instead of synthetic food additives increased considerably. Natural agents, especially obtained from plants, such as thymol, eugenol and carvacrol, have been studied as food preservatives, due to the antimicrobial effect of their volatile oil fraction and their potential safety for human consumption (Dorman and Deans 2000; Skandamis 2001). The antifungal properties of origanum oil (Manohar *et al.*, 2001), as well as thyme, bay and clove were far back assessed (Smith-Palmer et al. 1998; Hammer *et al.*, 1999). An important source of bioactive compounds, in particular, flavonoids and vitamin C, is represented by citrus fruits (Schieber *et al.*, 2001). One of the most promising citrus extracts is the lemon one, very rich in terpenes (Fernandez-Lopez *et al.,* 2004). It was used by Belletti *et al.,* (2004) with the aim to assess the antimicrobial activity of the essence against a *Saccharomyces cerevisiae* strain in relation to its composition, as well as to verify the role of vapour pressure on its bioactivity. The aim of this study was to investigate the activity of lemon extract against typical spoilage yeasts, lactic acid bacteria and spore-forming bacteria, in order to promote the use of the natural compound as food preservative.

MATERIALS AND METHODS

Selected lactic acid bacteria, *Lactobacillus curvatus* and *Lactobacillus plantarum*, yeasts, *Pichia subpelliculosa* and *Candida lusitaniae* and *Bacillus licheniformis* were isolated from fresh and spoiled foods; *Bacillus cereus*, *Bacillus subtilis* and *Saccharomyces cerevisiae* were purchased from public collection. All microorganisms were grown on slants of appropriate media and stored at 4°C as stock cultures. A spore suspension (10^7-10^8 spores/ml) of *B. cereus*, as enumerated on PCA, was also obtained and stored at 4°C until use.

Sterile solutions of pure lemon extract, kindly provided from Spencer Food Industria (Amsterdam), were prepared in distilled water and added to microbial suspensions in different concentrations from 5 to 1000 ppm (v/v). Each liquid medium, with an appropriate concentration of active compound, was inoculated (1:10) with standardized cultures and spores to yield active tests (AT) with a concentration of 10^5 ufc/ml. Inoculated media without lemon extract were used as positive controls (PC) and not inoculated media containing only active solution were also used as negative controls (NC). Measures on spectrophotometer at 420 nm were acquired to monitor microbial growth. Each experiment was replicated twice.

The experimental data were modeled by fitting a modified version of a Gompertz function to calculate the integral curve. The time necessary to reach the stationary phase was set to 283 hours, for all tested microorganisms. The obtained values of the area for each growth curve were used to calculate the growth index (GI) (Lambert, 2001). A rearranged form of an exponential decay model (Lambert *et al.,* 2001; Lambert and Pearson 2000) was fitted to all GI_{283} data as function of Log of lemon concentrations to obtain, for each microorganism NIC and MIC values, as well as the inhibition profile.

RESULTS AND DISCUSSION

The results on the antimicrobial activity of the investigated natural preservative were discussed in terms of NIC and MIC. All obtained values, with their standard deviation, were reported in the Table 1. NIC values, defining microbial sensitivity to the active compound, ranged from 9 to 20 ppm, whereas MIC vales, defining microbial resistance, ranged from 27 ppm to 170 ppm. The spores of *B. cereus* exerted NIC and MIC values lower than that related to all other vegetative bacteria and yeast cells, probably due to their hydrophobic nature. Furthermore, the results demonstrated that the two lactic acid bacteria were more resistant to the action of lemon extract than spore-forming bacteria. These results are in agreement with that obtained by Falcone *et al.* (2005), related to the antimicrobial properties of the thymol, used in the same concentrations of the lemon extract, against the same

Table 1. MIC and NIC values along with their standard deviations.

Microorganisms	NIC [ppm]	MIC [ppm]
Bacillus cereus	8.4346 ΔNIC = 1.6119	53.8654 ΔMIC = 19.7578
Bacillus cereus spores	6.8499 ΔNIC = 8.2908e-3	24.5132 ΔMIC = 6.5934e-2
Bacillus subtilis	13.1017 ΔNIC = 1.6015	76.4256 ΔMIC = 33.3821
Bacillus licheniformis	10.0632 ΔNIC = 2.4227	100.8502 ΔMIC = 67.7127
Lactobacillus curvatus	13.9823 ΔNIC = 2.6651	149.9234 ΔMIC = 55.2737
Lactobacillus plantarum	9.9335 ΔNIC = 2.2101	169.9887 ΔMIC = 69.1050
Saccharomyces cerevisiae	20.3858 ΔNIC = 0.3031	27.7943 ΔMIC = 0.3427
Candida lusitaniae	14.0841 ΔNIC = 1.7837	133.1858 ΔMIC = 36.9080
Pichia subpelliculosa	12.7184 ΔNIC = 1.7860	45.6208 ΔMIC = 10.7097

food spoilage bacteria and yeasts. With thymol all microorganisms showed NIC levels under 100 ppm, except *L. curvatus* and *L. plantarum*, which were characterized by the greatest resistance against thymol. However, both NIC and MIC levels in presence of thymol are higher than the correspondent values obtained by using lemon extract. It's worth noting that an high value for the MIC level does not necessarily correspond to an high value of the NIC level and vice versa. In fact, in the case of the *S. cerevisiae*, the higher NIC value was detected, suggesting that, among the tested microorganisms, this is the less sensitive to the action of the investigated antimicrobial compound. However, contrarily to what one would expect, the threshold value over which the lemon exerts complete inhibition is very low. As in the case of the previous yeast, also the *L. plantarum*, exerts the higher resistance to the active extract but the value under which no effect on microbial growth can be observed is very low. In order to quantify the differences in microbial susceptibility to lemon extract, a new parameter [MIC-NIC] was calculated. As can be inferred from the new values the two acid lactic bacteria are the more resistant, whereas *S. cerevisiae* is the more susceptible yeast. The *Bacillus* specie seems to exert an intermediate behavior between lactic bacteria and yeasts with a susceptibility to the lemon in the following order: spores of *B. cereus* < *B. cereus* < *B. subtilis* < *B. licheniformis*.

Considering the results obtained from the work, the lemon extract represents an interesting natural compound to apply to food products because it exerts great activity even if it is used in low concentrations. The high effectiveness of this compound allows decreasing the amount of additive to be used in the industrial applications with interesting effects on both economical and safety aspects involved in the food preservation.

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EFFECTS OF EDIBLE COATINGS ON DEEP FAT FRIED CHICKEN NUGGETS

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ABSTRACT

Deep fat frying is a widely used operation for preparing foods because of the desirable and unique sensorial characteristics of fried products, such as flavor, appearance and texture. The aim of this study was to evaluate the effect of edible coatings based on methylcellulose (MC) and cassava starch (CS) to reduce oil uptake and improve water retention of chicken nuggets. Edible coatings were prepared with 1g of MC/100g solution and 4g of CS/100g solution, both with 25 or 55g glycerol/100g biopolymer. These solutions were applied on nuggets samples before battering. Pre-fried (180°C/30seg.) and fried (180°C/3min.) nuggets were analyzed to determine oil and water contents. Also, color and texture were measured in the fried nuggets. Coatings based on CS with 25% of plasticizer were the best effective for reduction of lipid content and oil uptake in the whole samples after frying. During pre-frying, the samples coated using MC with 55% of plasticizer presented the lowest lipid content. The coated samples were darker and presented brighter yellowish color when compared with the control. Also, a significant decrease in the peak force of the fried coated samples was verified, indicating a reduced hardness of these samples.

Key words: edible coatings; deep fat frying; oil uptake; water retention, chicken nuggets

INTRODUCTION

Deep fat frying remain a popular food prepare because of the taste and texture of fried products. The high temperatures and high content of lipids originated from frying result in a dry food, crispy at the crust and soft inside. Typical examples are french-fries and nuggets which stand out in the fast food business. On the other hand, the demand for products with low fat content is increasing because the excess fat consumption is considered as a key dietary contributor to high blood cholesterol and high arterial pressure, i.e., cardiovascular diseases (Albert and others, 2002).

Normally, hydrocolloids are added to food products in order to control and improve functional properties such as viscosity, water holding capacity, and emulsification. Some hydrocolloids, mainly polysaccharides, can be applied as edible coatings with the aim of lowering oil uptake during deep fat frying (Williams and others, 1999). Coatings based on cellulose derivatives are the most utilized as a barrier to fat absorption during the frying process (Albert and others, 2002, Garcia and others, 2002). Another applicable hydrocolloid is the cassava starch which is a cheap and abundant (Vicentini and others, 2005) and has not been applied in the studies on oil uptake.

Therefore, the aim of this study was to apply coatings based on cassava starch and methylcellulose to reduce oil uptake in chicken nuggets during deep fat frying.

MATERIALS AND METHODS

The coatings were prepared from solutions containing 4g of cassava starch (CS) (Vicentini and others, 2005) or 1g of methylcellulose (MC) (Garcia and others, 2002) per 100g solution. Plasticizer effect of glycerol was analysed, and the concentrations were 25g and 55g glycerol/100g biopolymer. The chicken nuggets were prepared according to Trindade et al. (2001). The samples were dipped in coating suspensions at 40°C for 5s. After that, the nuggets were breaded, pre-fried at 180°C for 30 s, and frozen.

The frozen pre-fried samples were fried at 180°C for 3 minutes. As a control, nuggets without coatings were fried. Hydrogenated vegetable oil was used in the pre-frying and frying process.

Raw, pre-fried and fried nuggets were analyzed for determination of water and lipids contents on the crust (breading system), on internal portion and on the whole sample, according to AOAC (1997). The water loss and oil uptake during frying were calculated by the difference between the water and lipids contents before and after frying.

Whole samples of fried nuggets were analyzed to determination of the surface color, shear and compression force. The shear force was also determined with the internal portion of nuggets. Colorimetric measurements were carried out with a portable colorimeter (mod. Miniscan XE, manufacturer HunterLab), and expressed using the color standards of the CIELab – “Comission Internationate de L’Eclairage” system: lightness (L*) and chromaticity parameters a* (red–green) and b* (yellow–blue).

For texture characterizations, a texture analyser TA.XT2*i* (SMS) was used. The internal portion (1cm width x 2cm length) was sheared using the Warner-Bratzler probe and the whole samples were sheared using the Knife blade probe. Cylindrical samples (20mm diameter) were compressed until a deformation of 50% with a cylindrical probe (20mm diameter). In all the tests, the probes moved at 500mm/min.

The experimental results were submitted to analysis of variance (ANOVA) and Duncan mean comparison test. The significance level used was 0.05 (SAS, 1989).

RESULTS AND CONCLUSIONS

After frying, lipids content in nuggets coated with CS and 25% of plasticizer was about 14% inferior to that of the control (Figure 1A). CS coatings with 25% plasticizer had been effective to reduce oil uptake during deep fat frying (Table 1). The others coatings did not show noticeable differences with regard to oil uptake during frying (Table 1). On the other hand, during pre-frying, the lower lipid content was observed in the nuggets coated with MC and 55% of plasticizer, which was about 15% lower than the lipid content of the control sample (Figure 1A).

It could be observed that the relatively high values of lipid content in the fried nuggets (~13-14%) were due to a high oil uptake by the crust of the nuggets (Table 1), which was not protected by hydrocolloid coatings, once these were applied before the breading system. Although the crusts were not protected, they showed different values of lipid content (Figure 1A), probably due to the water migration of hydrocolloids towards the crust, which prejudiced the oil uptake during frying, in certain cases. Also, the internal portion of the nuggets showed relatively low lipid content during frying (7-8%) as well as during pre-frying (6-7%), although significant differences in relation to the uncoated nuggets had not been observed.

A contradictory behavior with the oil uptake can be observed in the water content of the nuggets (Figure 1B). Although a lower water loss had been observed in the products coated with CS and 55% plasticizer (Table 1) during frying process. It could still be observed in Table 1, that the higher water loss during frying occurred in the crust and not in the internal portion of the nuggets. These behaviors were expected because basically, the physical effect of the frying process consisted of the substitution of water for oil, and due to this reason, it could be considered as a process of dehydration (Saguy and others, 1998).

These results were not according to those observed in the literature, where the protecting effect of coatings based on methylcellulose in the frying of diverse products, such as, biscuits prepared with commercial mixture (Albert and others, 2002), potatoes (Garcia and others, 2002), and pastry mix (Williams and Mittal, 1999) was evident. However, this behaviors may be considered as complex, because the degree of oil uptake during deep fat frying is significantly affected by many factors such as process conditions (temperature, time), pre-treatment and physico-chemical characteristics of food, oil origin and oil composition (Rimac-Brncic and others, 2004). A possible explanation for the non-effectivity of the filmogenic solutions in this work is that the lipids were absorbed principally by the breading material, but the hypothesis of the loss of the coatings integrity cannot be discarded.

Surface color parameters (L*, a*, b*) of the fried coated nuggets were slightly darker than the uncoated, that is, they showed lower values of L*, and a yellow coloring (high values of a*) without clear effect of the polysaccharide coatings (Table 1). From the technological point of view, this could be interesting because the consumer would not notice any difference due to the presence of these coatings.

Another interesting technological information was the coatings effect on the textural properties of the coated nuggets. In a general manner, the coated nuggets, independent of treatments, were softer than uncoated nuggets. It could be observed in Table 1 that the shearing force of the coated samples varied between 57 and 65N, while the control samples showed higher values in the order of 78N. In the compression tests, these differences were even higher; the control showed compression force values of 40N, while in the coated samples, these values varied between 20 and 27N.

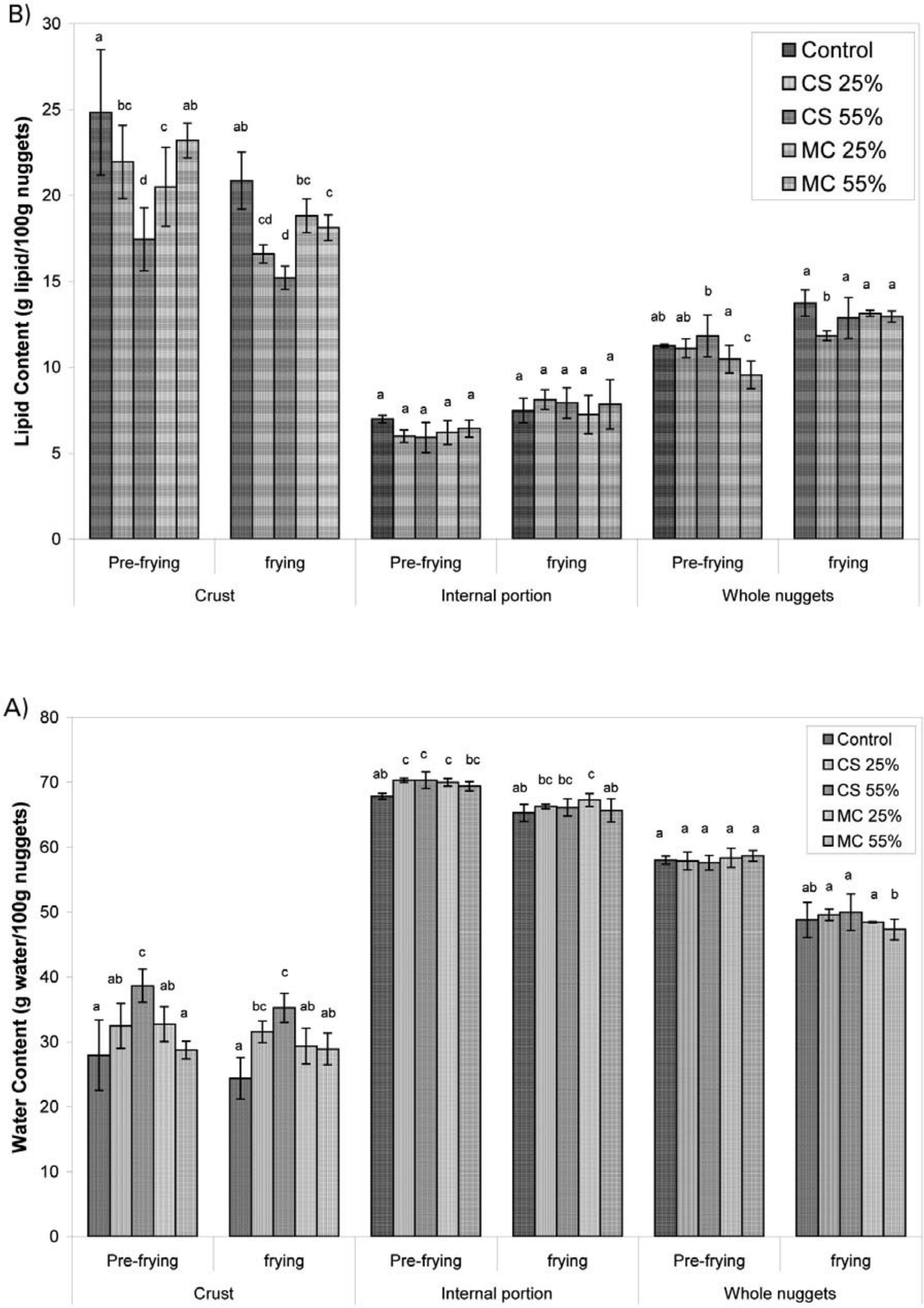

Fig. 1. Water (A) and lipids (B) contents (g/100g sample) of the nuggets coated and control (uncoated), determined from the crust, internal portion and whole nuggets. Coatings formulation: CS-cassava starch (4g CS/100g of solution), and MC-methylcellulose (1g MC/100g of solution), with different concentrations of plasticizer: 25g and 55g of glycerol/100g of biopolymer.

Table 1. Water loss (WL, g moisture/100g of sample) and oil uptake (OU, g lipids/100g sample) from the crust, internal portion, and whole nuggets during frying of coated and control (uncoated) nuggets; surface color parameters (L* a* b*), shearing force of the whole nuggets (Fcis), shearing force of the internal portion of the nuggets ($Fcis_{interno}$), and compression force at 50% (F_{Comp}). Coatings formulation: CS-cassava starch (4g CS/100g of solution), and MC-methylcellulose (1g MC/100g of solution), with different concentrations of plasticizer: 25g and 55g of glycerol/100g of biopolymer.

	Treatment	Control	CS25%	CS55%	MC25%	MC55%
WL (g water/100g sample)	Crust	24.7 ± 3.2^{a}	25.0 ± 1.7^{a}	25.2 ± 2.2^{a}	19.2 ± 2.8^{b}	19.2 ± 2.4^{b}
	Portion	6.4 ± 1.3^{a}	5.5 ± 0.4^{a}	6.6 ± 1.3^{a}	5.6 ± 1.0^{a}	7.7 ± 1.8^{a}
	N whole	17.1 ± 2.7^{ab}	17.1 ± 0.9^{ab}	14.5 ± 2.8^{b}	17.6 ± 0.1^{a}	17.6 ± 1.6^{a}
OU (g lipids/ 100g sample)	Crust	19.0 ± 1.7^{ab}	15.9 ± 0.5^{cd}	14.3 ± 0.7^{d}	18.3 ± 1.0^{ab}	17.4 ± 0.8^{bc}
	Portion	3.5 ± 0.7^{a}	3.3 ± 0.6^{ab}	2.8 ± 0.9^{ab}	1.8 ± 1.1^{b}	3.7 ± 1.4^{a}
	N whole	9.5 ± 0.8^{a}	8.2 ± 0.3^{b}	9.9 ± 1.2^{a}	9.5 ± 0.2^{a}	9.3 ± 0.3^{a}
Color	L*	62.7 ± 0.8^{a}	56.2 ± 2.3^{b}	57.9 ± 2.8^{bc}	57.8 ± 1.3^{bc}	60.0 ± 1.5^{c}
	a*	10.5 ± 0.7^{a}	13.9 ± 2.0^{c}	12.5 ± 1.0^{b}	11.8 ± 0.9^{ab}	11.2 ± 0.5^{ab}
	b*	40.5 ± 3.6^{a}	39.5 ± 3.8^{a}	40.5 ± 2.9^{a}	40.1 ± 1.9^{a}	40.1 ± 2.7^{a}
TEXTURE	FCIS (N)	77.8 ± 11.3^{A}	$57.8. \pm 9.6^{B}$	69.6 ± 7.0^{AB}	60.4 ± 5.4^{B}	$65.1. \pm 7.5^{AB}$
	$FCIS_{INT}$ (N)	8.6 ± 1.4^{A}	8.6 ± 1.8^{A}	7.8 ± 0.9^{A}	7.6 ± 1.6^{A}	7.7 ± 1.5^{A}
	F_{COMP} (N)	39.6 ± 5.1^{A}	23.1 ± 4.1^{B}	24.4 ± 9.9^{B}	26.56 ± 9.0^{B}	19.8 ± 6.5^{B}

$^{a-d}$ Means in the same line with different superscripts are significantly different ($p \leq 0.05$).

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STRAWBERRY PRESERVATION BY IRRADIATION AND NATURAL ANTI-MICROBIAL COMPOUNDS

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ABSTRACT

During the past decade the emphasis in post-harvest fruit protection has shifted from using chemicals to various alternative techniques, including biological control, cultural adaptations, controlled atmosphere and irradiation. We can today add to the list natural antimicrobial compounds such as phenolic acids and essential oils.

To keep under control strawberry post-harvest decay, we screened: UV-C irradiation, and terpens in different doses and combinations as well as different modalities in packaging. For 8 days we checked the fruit deterioration and the sensory characters (visual aspect, smell, taste and firmness).

We found out that: the use of essential oils as anti-microbial compounds prolong strawberry shelf-life even without UV pre-treatment; the anti-microbial compounds have to remain, for whole the storage period, into the packaging atmosphere; the natural anti-microbial compounds maintain the fruit sensorial characteristics; the strawberry shelf-life is related to the packaging modality.

Key words: sensory analysis; strawberries shelf-life; terpens; UV-C irradiation

INTRODUCTION

Microbial safety and stability as well as the sensory and nutritional quality of most foods is based on an application of technologies for a gentle but effective preservation of foods. In order to achieve mild but reliable preservation effects the use of combined processes and factors ("hurdles") have been studied and is still under study. Hurdles technology involves a wide range of food products, it is particularly appropriate for fresh vegetable and fruit shelf-life (Leistner, 2000).

During the past decade the emphasis in post-harvest fruit protection has shifted from using chemicals to various alternative techniques, including biological control, cultural adaptations, controlled atmosphere and irradiation (Marquenie, 2002; Ross *et al.,* 2003; Vincente *et al.,* 2003). We can today add to the list natural antimicrobial compounds such as phenolic acids and essential oils (Burt, 2004; Roller and Seedhar, 2002; Smid, 1999). Low temperatures are also one of the tools to avoid, at least partially, mould growth and fruit senescence. Low temperatures used to increase strawberry shelf-life can induce small changes in some of the quality parameters. The aim of researchers is to find a good compromise between extended shelf-life and maintenance of nutritional value.

To keep under control strawberry post-harvest microbial spoilage maintaining quality attributes at room temperature (20°C), we screened: UV-C irradiation, and terpens in different doses and combinations as well as different modalities in packaging.

MATERIAL AND METHODS

Strawberries were purchased from a retailer. Fruits having same size, same ripening stage (90% full red colour) and undamaged were randomly distributed into 8 lots of 8 fruits and processed to UV-C treatment. A UV-C lamp (λ = 254 nm) with a normal power output of 30 W and 0-36 A was used. The fruits were irradiated with a predetermined 63,2 kJ/m^2 doses (data not showed). The dose of UV-C treatment was subdivided into 4 smaller sub-doses and fruits were individually rotated four times to expose 4 separate sides of the same fruit to the lamp. Then they were packed in transparent polystyrene baskets (500 ml capacity) and sealed. The lid (110 cm^2) bear no hole or 13 2 mm-diameter holes (to prevent CO_2 saturation and O_2 depletion in the atmosphere and avoid the terpen impact on strawberry flavour). To carried out the terpen antimicrobial tests a sponge disc (4 mm diameter; 2 mm thick) after immersion for 5 min with 20 or 200 ppm terpens solution has been inserted into the basket before its closure. After 4 days of exposure the sponges were removed from the bag to avoid over exposure of the fruit to terpens.

Strawberry decay was examined at 2, 4, 6, 8 days of storage. The undamaged strawberries sensory characters (visual aspect, smell, taste and firmness) were evaluated at 8 days of starvation according to AFNOR by a panel of 8 sensory assessors. The panelists were asked to discriminate in bright, in colour, in strawberry flavour, in sur-ripe, in firmness and in spongy and to evaluate in a scaling test by a 10 point scale (0 = no strawberry alteration 10 = intense alteration).

Terpen solution was constituted with linalool (15% v/v); alpha terpineol (10% v/v); thymol (25% v/v); 1,4 cineole (5% v/v); carvone (40% v/v); trans-caryophyllene (5% v/v) purchased from Extrasynthese (Genay, France).

The evaluation of fruits decay was expressed in percentage of damaged surface.

The experimental data were analysed statistically by SPSS software version 11.0.

Table 1. Percentage of contaminated fruit during storage.

Perfored packaging	uv				no uv			
	2 days	4 days	6 days	8 days	2 days	4 days	6 days	8 days
terpens 20 ppm	25	87.5	100	100	25	100	100	100
terpens 200 ppm	12.5	100	100	100	62.5	100	100	100
no terpens	25	100	100	100				
No perfored packaging	**uv**				**no uv**			
	2 days	4 days	6 days	8 days	2 days	4 days	6 days	8 days
terpens 20 ppm	12.5	12.5	50	50	12.5	25	62.5	75
terpens 200 ppm	0	0	25	25	12.5	25	25	75
no terpens	20	90	95	100				

RESULTS AND DISCUSSION

The % of contaminated fruit in each test is reported in table 1. The contamination level differs among the sets of treatments. The lowest occurrence of contamination is in strawberry in close package and none decay is noted up to 4 days in UV fruit pre-treated and stored at 20°C in presence of 200 ppm of antimicrobial compounds.

The percentage of damaged surface differs according to the various types of treatments (fig. 1). By comparing UV-C irradiation pre-treatment, in perforated package without terpens or with terpens in different doses, we can see the microbial spoilage of strawberries rise after two days of preservation; it is more evident in the strawberries stored with terpens without UV pre-treatment (from 50-to 60% surface damaged). The contamination goes on: we can see an high contamination level (80-100% damaged surface) in all the tests on the 8th day of storage and the highest average contamination 100% was observed in strawberry with only UV pre-treatment.

The packaging without hole reduces the contamination

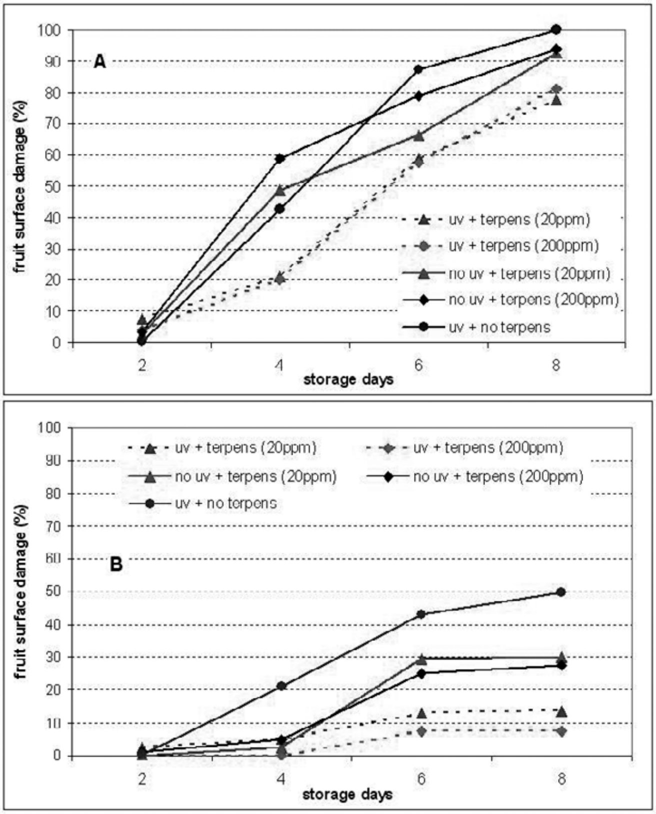

Fig.1. Contamination level (% of strawberry damaged surface) according to the various types of treatments. A – Perforated package; B – Non perforated package.

from 50% to about 90% when compared to holed package.

In closed package the microbial spoilage of strawberry rises on 4th day after sponge disc removal. The decay of the strawberries UV pre-treated and stored with 20 or 200 ppm terpens reaches respectively 8 and 15% fruit damage surface on 8th day. An higher contamination about 30% is observed when the fruits are not UV pre-treated but stored with 20 or 200 ppm terpens. The only UV treatment is not able to reduce significative the contamination even in the close package (50% fruit damage surface).

By comparing exposed and non exposed fruits to antimicrobial compounds, it is evident that the presence of 200 ppm terpens reduces significatively the damage on the fruits (Student test: t = -2.06).

Table 2. Strawberry decay vs storage time. Duncan test ($p \leq 0.05$).

A - Perforated package	df	F	Sig.
uv + terpens (20 ppm)	28	18.77	0.000
uv + terpens (200 ppm)	28	40.31	0.000
uv + no terpens	28	70.62	0.000
no uv + terpens (20 ppm)	28	29.65	0.000
no uv + terpens (200 ppm)	28	24.63	0.000
B - Not perforated package	df	F	Sig.
uv + terpens (20 ppm)	28	0.44	0.729
uv + terpens (200 ppm)	28	3.82	0.021
uv + no terpens	28	38.22	0.000
no uv + terpens (20 ppm)	28	1.84	0.163
no uv + terpens (200 ppm)	28	1.17	0.337

The presence of holes in the packaging engraves the deterioration of the fruits independently of the concentration of anti-microbial compounds: the strawberry deterioration is much higher in the perforated packaging.

The statistical analysis of the results show that the storage time intervenes significatively on the fruit contamination when using or not perforated packaging (table 2).

Duncan test underline that the time is not significant in fruit degradation when strawberries are in closed package (table 2 – B). The time is significative in strawberry decay only in perforated package even if antimicrobial compounds are present and UV pre-treatment is applied (table 2 – A).

Our results show that to prolong the shelf-life of the strawberry, the anti-microbial compounds have to remain, for whole the storage period into the packaging atmosphere.

Our results agree with Marquenie et al. (2002) and Reddy et al. (1998) that carried out respectively a positive effect of UV-C-heat treatment and essential oil exposure on strawberry decay.

The sensory analysis of non contaminated strawberries shows significant differences in visual and taste characters among the strawberries stored into holed and not holed packaging (fig. 2).

PCA analysis regarding sensorial characters explains 99.8% of the total variance. It permits to distinguish clear-

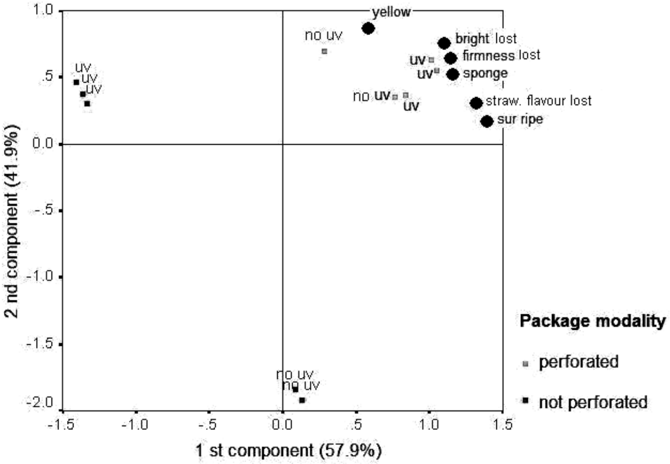

Fig. 2. PCA analysis of fruit sensorial characters. Samples are labeled on the basis of UV treatment and package modality.

ly fruits maintained (closed package) or not (holed package) in an atmosphere with antimicrobial compounds. Strawberries exposed to terpens are less yellow, show better firmness, flavour and brightness and have less sponge and sur-ripe taste.

Sensory parameters of fruits conserved into closed package have been analyzed separately (fig. 3). The factorial plane, explaining 98.3% of the total variance, groups the samples on the basis of UV pre-treatment. The UV pre-treated fruits, both with and without exposure to 20 ppm terpens are less bright and firm, more yellow and spongy, but do not show differences compared to the not-treated samples regarding sur-ripe and strawberry-like flavour characters. UV pre-treatment with 200 ppm terpens exposure preserves the sensorial characters of the strawberries.

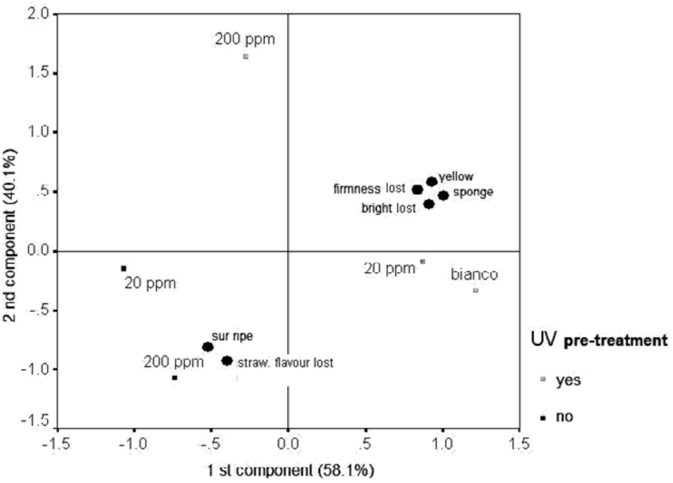

Fig. 3. Closed package - PCA analysis of fruit sensorial characters. Samples are labeled on the basis of UV treatment and terpen concentrations.

CONCLUSION

The contamination level (% of damaged surface) differs according to the various type of treatments. The presence of holes in the packaging engraves the deterioration of the fruits independently of the concentration of anti-microbial compounds: the strawberry deterioration is much higher in the perforated packaging.

In closed package the use of essential oils as anti-microbial compounds prolongs strawberry shelf-life even without UV pre-treatment.

By comparing exposed and non exposed fruits to antimicrobial compounds, it is evident that the presence of 200 ppm terpens reduces significatively the damage on the fruits.

In closed package the UV irradiation with 200 ppm terpen exposure is effective in reducing the strawberry microbial contamination: none fruit decay is up to 4 days at 20°C. Moreover it preserves the sensorial characters of the strawberries.

Our results show that to prolong the shelf-life of the strawberry, the anti-microbial compounds have to remain, for whole the storage period, into the packaging atmosphere.

The use of natural anti-microbial compounds to better strawberry shelf-life is full of promise and new study are been carried out for its optimization.

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EDIBLE FILMS FOR FOOD APPLICATION: INFLUENCE OF COMPOSITION ON NETWORK FORMATION

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ABSTRACT

Edible films with different compositions were submitted to dynamic mechanical analyses and microstructure analyses. The aim was to gain information on the composition on network formation by means of the Friedrich-Heymann model. Results prove that the film network organization is reasonably well described by the simplified Friedrich-Heymann model and that the oscillatory tests might be a useful tools for screening among the film components for product and process design purpose.

Key words: biopolymers, edible film, film network, mechanical properties, modelling

INTRODUCTION

Edible film are a mixture of a high molecular weight polymers, solvent and a plasticizer. Several ingredients such as antimicrobial compounds antioxidants, flavours, colouring and others can be incorporated into there (Krochta *et al.* 1994). An edible film must act as an efficient barrier against moisture, gases, flavours or solutes. Barrier properties of edible films can be affected by polymer structure, plasticizer concentration, solvent and other factors related to film dissolution, permeability and diffusion properties (Chen, 1994). Recently, many authors have extensively studied films constituted by polysaccharides and/or proteins because of their barrier and mechanical properties. Some of these investigations have demonstrated that the mechanical and the barrier properties to carbon dioxide of protein-based films are better than those possessed by the polysaccharide-based films (Park and Chinnam, 1995) whereas the latter show a more effective barrier to oxygen (Gennadios and Weller, 1991). However, the functional properties of the edible films, in the present state of the art, are still inferior to those of synthetic plastics. This way, more research is necessary in order to improve the characteristics of edible films. A possible solution to improve the performance of these films is blending. The polymer blending to obtain a new material with better performances than the single components is a quite common practice when dealing with polymers of synthetic origin and may represent an interesting way to explore for biopolymers. Besides, proteins and polysaccharides generally form films with good mechanical properties but they form poor moisture barriers, because of their hydrophilicity. Improved film performances are obtained with a multi-component system where hydrocolloids (proteins or polysaccharides) form a continuous and cohesive network, and the hydrophobic substances (lipids) provide the moisture barrier properties (Krochta *et al.* 1994). However, polymeric films produced by physical methods often result in poor mechanical and permeability properties when compared with those obtained through chemical reactions. On the other hand, chemical cross-linking agents frequently induce toxicity or confer other undesirable effects to these materials. Therefore, the possibility to use enzymatic methods both to prepare polymeric films and to improve their features has been object of extensive studies (Lim *et al.* 1999; Jun-Hyun *et al.* 2004). In a recent paper the potential use of transglutaminase as biotechnological tool for preparing pectin-soy protein films through an enzymatic cross-linking reaction was examined (Mariniello *et al.* 2003). The objective of this study was to determine the influence of composition on film structure formation. For this purpose the mechanical and micro-structural properties of pectin and pectin-soy protein films obtained in presence or in absence of TGase were detected. Information concerning the three-dimensional film structural network was derived by using the Friedrich-Heymann model.

MATERIALS AND METHODS

Pectin from citrus fruits and soy flour was purchased from Sigma (Milano, Italy).

Two different type of films were investigated: films made from pectin and films made by blending pectin and soy protein. Pectin were dissolved in acidified water (pH 2) at a concentration of 16mg/ml. To obtain films with the desired amount of pectin (2.5, 3.8, 5.8mg/cm^2), different volumes of solutions were pipetted into 28.26 cm^2 polystyrene Petri dishes and dried at 35°C and 50% RH overnight under air circulation. To obtain films from mixtures of soy proteins and pectin, soy flour and pectin were first dissolved in water obtaining two different suspensions with concentration equal to 13mg/ml and 16mg/ml respectively and then mixed in the appropriate ratio. These films were prepared in absence or in presence of microbial TGase.

Film thickness was measured using a micrometer model HO62 with sensitivity of ± 2 μm (Metrocontrol Srl, Casoria (Na), Italy). Film strips were placed between the jaws of the micrometer and the gap reduced until the first indication of contact. Mean thickness (μm) of films were determined from the average of measurements at 10 locations.

Dynamic mechanical analyses (DMTA V, Rheometrics Inc. Piscataway, USA) were performed on rectangular film specimens (50 x 7 mm). The sample was cut whit scissors and mounted on grips so that its length was 10 mm. All measurements were conducted in dynamic mode. Before any measurements were taken, samples were rested for 3 min, allowing the stress induced during sample loading to relax. The linear viscoelastic region was determined by performing a strain sweep test at a given frequency of 1 rad/s. Then, the frequency sweep tests were conducted by applying an oscillation amplitude of 0.01% (within the linear region) over a frequency range between 10^0 and 10^3 rad/s. The parameters used for this study were the storage modulus (E') and the loss modulus (E").

For microscopy analysis, dried strip fragments of films were mounted on specimen stubs with cross-section oriented up and coated with a thin layer of gold by DC sputtering (AGAR B7340). Digital images of film cross-section were collected by using a LEO EVO 40 scanning electron microscope (Zeiss, Germany) with a 20kV accelaration voltage.

Data analysis

Friedrich-Heymann model (1988) was used to describe the frequency sweep curves as described by Moresi *et al.* (2001). Model parameters were estimated by non linear regression method by considering the α parameter of this model, in which the relaxation function was assumed to be independent of the relaxation time and G_∞ equal to zero. The estimated parameter (α) were submitted to analysis of variance t ($p \leq 0.05$) by means of SPSS v10.1 package.

RESULTS AND DISCUSSION

Figure 1 shows, as sake of example, E' curves relative to pectin film at different concentration (a) and pectin soy and proteins film with and without TGase (b), in the range of frequency 1-1000 rad/s. The dots represent the experimental data and

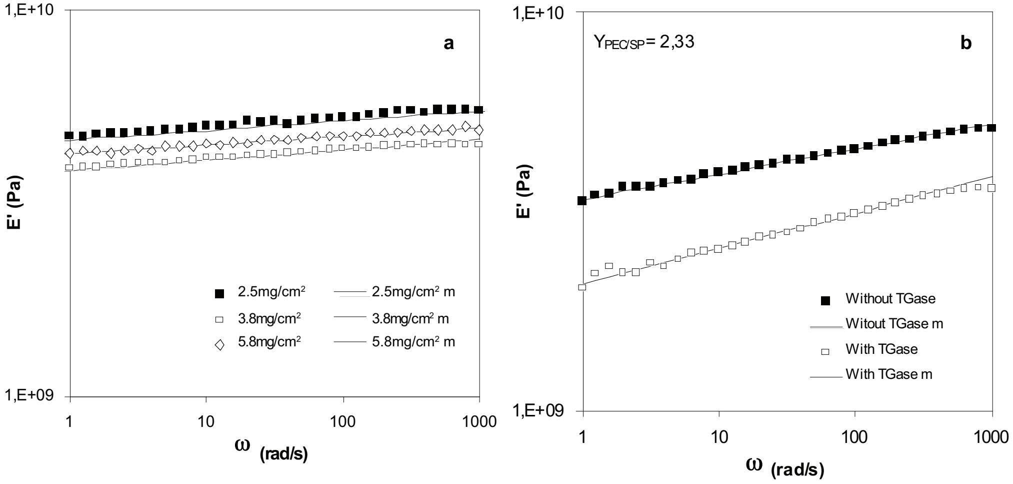


Fig.1. E' (Pa) as function of ω (rad/s) for pectin film at different concentration (a) and pectin and soy proteins (b) film with and without TGase.

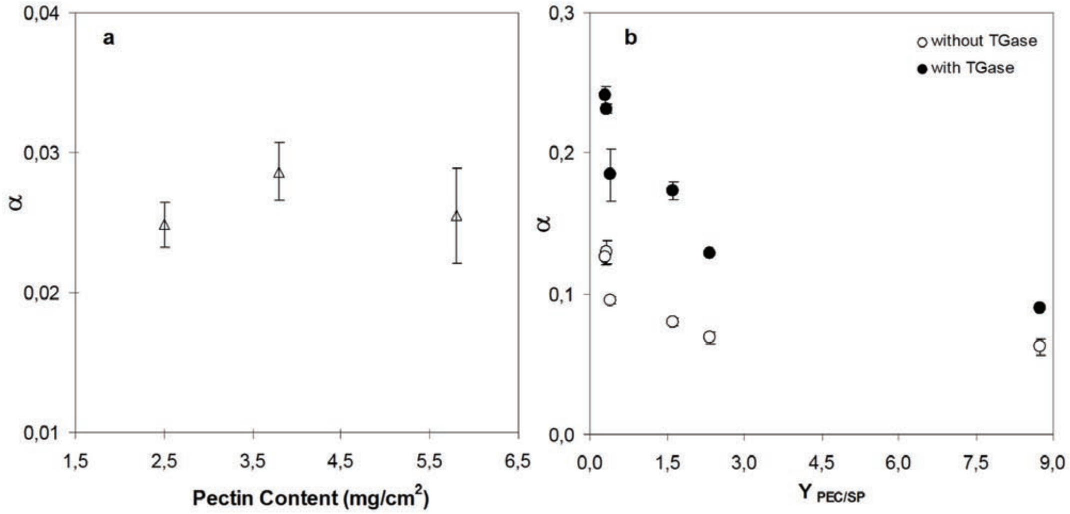
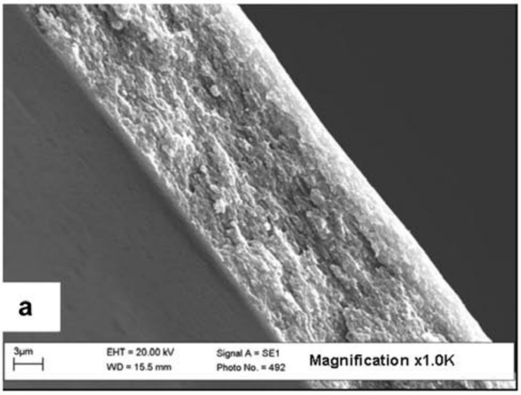


Fig.2. The α parameter as function of pectin content for the pectin films and pectin/soy protein ratio ($Y_{P/SP}$) for the pectin-soy flour films.



the solid line the storage and loss modulus curves as predicted by the Friedrich and Heymann model. The agreement between the model and the experimental data is very satisfactory ($R^2>0.99$). Figure 2a shows the α parameter of the model as a function of pectin concentration. ANOVA proves that the α parameter is independent from the pectin concentration ($P>0.05$), on the other hand, the pectin/soy protein ratio and the TGase have a significant effect on the α parameter ($p<0.01$). α decreases as the PEC/SP

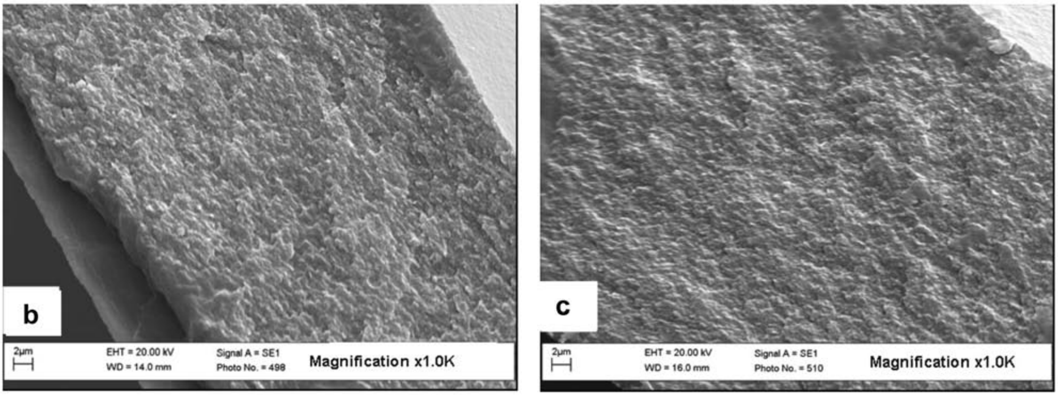


Fig. 3. SEM micrographs of pectin films with different pectin content: a) 2.5 mg/cm², b) 3.8 mg/cm², c) 5.8 mg/cm².

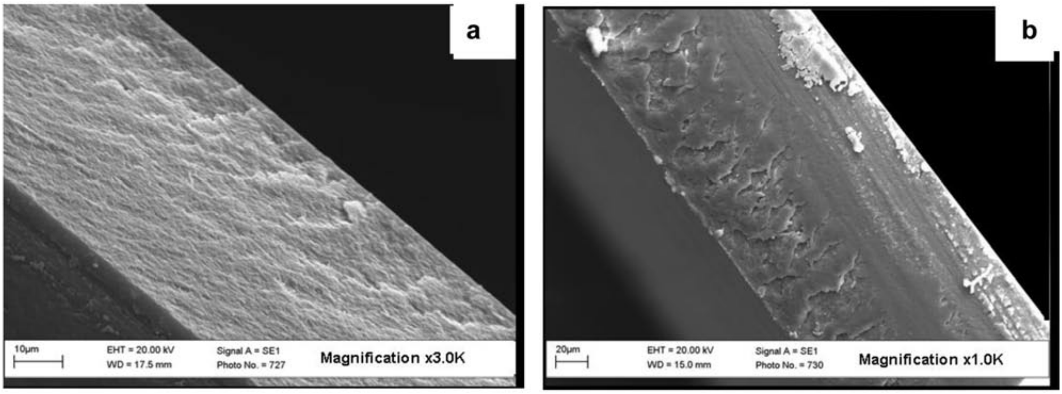


Fig. 4. SEM micrographs of pectin-soy flour film: a) without TGase, b) with TGase.

ratio increases and increases in presence of the enzyme TGase (Figure 2b). In order to evaluate the effect of composition on the film microstructure, the films were analyzed by SEM. One can observe, from the micrographs shown in Figure 3, that by varying the pectin content in the film-forming suspension the microstructure of the film does not changes. This is in agreement with the fact that the α parameter was independent from pectin concentration. The pectin films were characterized by the absence of a homogeneous structure and the film structure appears to be the result of pectin clusters packed in a more or less tied way. In particular, the films with pectin content of 5.8 mg/cm^2 presents a more compact network than the one with the lowest pectin content (2.5 mg/cm^2). When pectin and soy flour proteins are blended together an improvement in the network formation of the film is observed (Figure 4a). Besides, the films cross-linked by TGase show a more compact and homogeneous structure then the corresponding film without TGase addition (Figure 4b). These results are in according with α parameter values.

CONCLUSIONS

The results obtained in this study suggest that in film made by a pectin suspension the pectin held together as a result of physical entanglements that establishes when the solvent in which pectin are dissolved is removed. Therefore, by increasing the pectin content in the film-forming suspension the network link density does not changes. By adding soy proteins a more dense structure forms with link density that depend on both pectin and soy protein content. The film obtained in presence of TGase exhibits a even more compact and homogeneous structure.

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ISOLATION AND CHARACTERIZATION OF A NOVEL BACTERIOCIN PRODUCED BY *LACTOCOCCUS LACTIS* RUC9 WILD STRAIN

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ABSTRACT

The aim of the present work was to characterize the antimicrobial compound, produced by *Lactococcus lactis* wild strain RUC 9, previously isolated from minimally processed vegetables products. The antimicrobial peptide was screened by the agar spot assay and identified by using different proteases. In order to establish the molecular size of the peptide, SDS-PAGE analysis was applied. The bacteriocin exhibits a broad inhibitory spectrum against *Listeria innocua* DSMZ 20649 and several LAB strains used as target microrganisms. Moreover the peptide was stable over a wide range of pH (3-11) and at different temperatures.

Key words: minimally processed vegetables products, bacteriocin, lactic acid bacteria

INTRODUCTION

The lactic acid bacteria (LAB), present in several ecosystems, may produce antimicrobial compounds, including ribosomally synthesized proteins or peptides, collectively known as bacteriocins. In general, these compounds are proteins which show activity against genetically closely species and are also effective against foodborne pathogens, such as *Listeria monocytogenes* and many other gram-positive spoilage microrganisms (Tagg *et al.*, 1976). Bacteriocins are divided in three different classes for their biochemical and genetic caracteriristics. Class I contains the bacteriocins, called lantibiotics, as nisin (Kanatani *et al.*, 1995). Lantibiotic are small peptides contaning unusual amino acids, such as lanthionine and 3-methyl.lanthionine. Class II bacteriocins are peptides of low weight, heat-stable, hydrophobic. Members of class III are large, heat-labile proteins and a fourth class consists of complexes bacteriocins (Klaenhammer, 1993). Recently a new class of bacteriocins, class V, has been proposed to include bacteriocins consisting of ribosomally synthesized non modified head-to-tail-ligated cyclic antibacterial peptides (Kemperman *et al.*, 2003). Bacteriocins generally exert their anti-microbial action by interfering with the cell wall or the membrane of target organisms, either by inhibiting cell wall biosynthesis or causing pore formation (O'Sullivan *et al.*, 2002). Production of these compounds by lactococci has been extensively studied. Nisin, produced by *Lactococcus lactis* subsp. *lactis*, is the best known lantiobiotic because of its importance and approved use as a food preservative, by the US Food and Drug Administration.

The aim of the present work was to partially characterize antimicrobial compound, produced by *Lactococcus lactis* wild strain RUC 9, previously isolated from minimally processed vegetable products.

MATERIALS AND METHODS

Several lactic acid bacteria strains, isolated from minimally processed vegetable products and previously identified, were screened for their capacity to produce bacteriocin-like-activity by agar spot assay. The selected active strain RUC9 was cultivated in MRS medium (2% v/v) at 32°C for 24 h; the culture was centrifuged (10000g for 20 min) and the supernatant (CFS: *cell free supernatant*) filtered using a membrane filter with 0.2 micron porosity, to exclude fagic nature of antimicrobial activity, and stored at 4°C until use. The presence of antimicrobial activity was visualized on Petri dishes by the presence of the inhibition ring. The latter was verified after 24-48 h of incubation at optimal temperature of target microrganism. In particular, antimicrobial activity of RUC 9 wild strain was tested against the following strains: *Listeria innocua* DSMZ 20649, *Lactobacillus fermentum* DSMZ 20052, *Lb. reuteri* DSMZ 20016, *Lb. brevis* DSMZ 20556, *Lb. casei* subsp. *casei* CR 609, *Lb. plantarum* CR 7A, *Lb. delbruecki* DSMZ 20081, *Lb. hilgardi* DSMZ 20176, *Leuconostoc mesenteroides* CCR 310, *Pediococcus acidilactici* DSMZ 20238, *Enterococcus faecalis* CCR 300, *Enterococcus hirae* CCR 303, *Enterococcus sulfurans* CR 316, *Streptococcus thermophylus* CCR 314, *Staphylococcus aureus* C7016 Oxoid, *Escherichia coli* C7085 Oxoid.

The antimicrobial compound titre was determined by the serial two-fold dilution method previously described by Mayr-Harting *et al.*, (1972). Activity was defined as the reciprocal of the highest dilution corresponding to maximum inhibition and expressed as AU/ml (arbitrary unit).

Preliminary characterization of antimicrobial compounds

To verify the proteic nature of antimicrobial compound, the proteolytic enzymes (proteinase K, papain, trypsin) were added to CFS. For preliminary characterization of bacteriocin, CFS was tested at 37°C for 5 h using several chemical substances at different concentrations (acetone, chloroform, dimethylsulfide, EDTA, trichloroacetic acid, tween 80, DTT and triton X-100). To analyze thermal stability of bacteriocin, CFS was exposed to temperature ranging from 4 to 100°C for different times. The activity of bacteriocin was evaluated at different pH values from 3 to11 pH.

Partial purification of the bacteriocins

Partial purification of bacteriocin was carried out by adding solid ammonium sulphate until saturation. The precipitated was collected by centrifugation at 3800 rpm for 40 min, the pellet was dissolved in 3mL 10mM TRIS-HCl buffer and exhaustively dialysed through membranes of 1000Da cut-off for 24 h against the same buffer.

Molecular size approximation

Molecular weight of bacteriocin was determined by electrophoresis onto Tris-Tricine-SDS-PAGE, using the Mini-Protean III electrophoresis system (Schagger and Jagow, 1987), comparing the molecular weight with wide range standards (Sigma). The bacteriocin was loaded more times on the same gel so that after electrophoresis one-half of the gel was stained with Silver-staining method, while the second half of the gel was assayed for antimicrobial activity by the direct method of Bhunia *et al.* (1987).

RESULTS AND CONCLUSIONS

One hundred and nineteen strains of LAB were screened for bacteriocin production. Only *Lactococcus lactis* RUC 9 was found to have antimicrobial activity against *Listeria innocua* DSMZ 20469, *Lactobacillus reuteri* DSMZ 20016, *Lb. fermentum* DSMZ 20052. *Lb. hilgardi* DSMZ 20176, *Enterococcus faecalis* CCR 300, *Enterococcus hirae* CCR 303, *Enterococcus sulfurans* CCR 316 and *Streptococcus thermophylus* CCR 314.

The value of antimicrobial activity against *Listeria* was, at 32 °C overnight, corresponding to 80 AU/mL.

Preliminary characterization of antimicrobial compounds

In the table 1 were showed the results of effects of proteolytic enzymes, pH, temperature and chemical substances on bacteriocin activity. Complete inactivation of antimicrobial activity of this compounds was detected with all proteolytic enzymes tested. The bacterio-

Table 1. Sensitivity of bacteriocin to chemical substance, enzymatic, pH and heat treatments.

Treatment	Activity
TWEEN 80 1% (v/v)	+
Triton X-100 1% (v/v)	+
Trichloroacetic acid 1% (p/v)	+
DTT 1% (p/v)	-
Dimethyl sulfoxide 0.1% (v/v)	+
EDTA 0.1, 2, 5, 10mM	-
Chloroform 10% (v/v)	+
Chloroform 50% (v/v)	-
Acetone 10% and 50% (v/v)	+
Papaina 1 mg/mL	-
Trypsin 5 mg/mL	-
Proteinase K 0.5 mg/mL	-
60°C for 30 min	+
75°C for 20 min	+
80°C for 10 min	+
100°C for 5 min	+
100°C for 30 min	Low
110°C for 5 min	Low
121°C for 15 min	-
3.25 pH	+
6.00 pH	+
9.30 pH	+
11.36 pH	Low

cin was stable at 60, 75, 80, 100 °C respectively for 30, 20, 10, 5 min, while its activity was lower when incubated at 100 °C for 30 min and 110 °C for 5 min. After the treatment at 121 °C for 15 min the activity of the bacteriocin was completely absent. The bacteriocin was active in a pH range of 3-9 and less active at pH 11. Moreover the bacteriocin lost its activity after treatment with DTT, EDTA and high concentration of chloroform.

Molecular size approximation

In tricine SDS-PAGE the antimicrobial compound migrated with an apparent molecular weight of about 64 kDa (Fig 1). An inhibition zone surrounding the peptide band showed the position of the active bacteriocin band (data not skown).

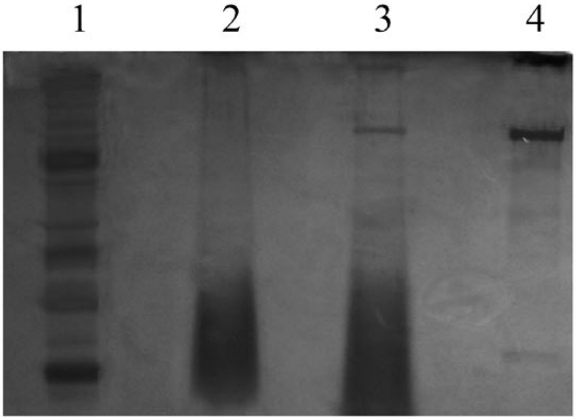

Fig. 1. Molecular weight of bacteriocin produced by *Lactococcus lactis* RUC 9 estimated by SDS-PAGE. 1, molecular wide range marker (Sigma); 2, MRS; 3, partially purified sample; 4 albumina marker.

The results of the present study highlighted that this bacteriocin, produced by *Lactococcus lactis*, could be a novel compound, different by Nisin.

ACKNOWLEDGEMENTS

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THE USE OF AN INERT GAS DURING THE OLIVE OIL BOTTLING: SHELF LIFE EVALUATION

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ABSTRACT

The extra virgin olive oil has precious characteristics that may be lost during the commercialization because oxidative and hydrolytic reactions may occur. It is possible to obtain a reduction of the oxidative process by storing oil in dark and closed bottles.

In this paper we have studied the effect of the composition of the bottles' headspace and its influence on the shelf life of the product. The oils that have origin from different industrial storage conditions were bottled and the headspace filled with air and with nitrogen. Then the samples were stored under conditions similar to those found in supermarkets for six months. The oils were examined before bottling and after 30, 60, 90, 120, 150 and 180 days on the shelf. Each sample was examined for free acidity, peroxide value, specific extinction coefficients K_{232} e K_{270}, total phenol content. Its stability was measured periodically using a Rancimat apparatus at 120 °C (20 l/h). The best results were obtained from the oils stored in industrial refrigerated tanks saturated with nitrogen and then the headspace of bottles was filled up all the way with nitrogen.

Key words: virgin olive oil, nitrogen gas, shelf life, total phenol content, the stability of the olive oil

INTRODUCTION

The characteristics of virgin olive oils are aroma, taste, colour and the nutritive properties and these distinguish it from other edible vegetables oils. It is therefore a matter of great concern for the olive industry to preserve its product without loss of these positive attributes. (Gutièrrez and Fernàndez, 2002) Virgin olive oil provides a rich source of natural antioxidants. The phenolic compounds, are considered to be the main antioxidant compounds in virgin olive oil. These compounds play an important role in extending the shelf-life of olive oil (Baldioli, Servili, Perretti e Montedoro, 1996). In fact the presence of the natural anti-oxidants such as phenol compounds prevents the auto-oxidation phenomena and the formation of "free radicals" caused by the presence of reactive oxygen species. In fact the shelf life of a bottled vegetable oil is limited by the auto-oxidation of unsaturated fatty acids with the formation of hydroperoxides. The decomposition of hydroperoxides gives rise to different compounds, some of which are volatile and responsible for the sensory degradation of the oil (Frankel, 1998). The factors that may favour the formation and the decomposition of hydroperoxides are exposure to air, light and high temperature. In this experiment, to exclude the influence of light on the oxidative process dark bottles were used, to realize conditions similar to those found in supermarkets the bottles were stored at ambient temperature from 18 to 24 °C. To verify the effect of the atmosphere on the composition of the headspace of the bottles and its shelf life, the oils that have origin from different industrial storage conditions were bottled employing both air and nitrogen. Then the samples were stored under conditions similar to those found in supermarkets for six months. The oils were examined before storing and after 30, 60, 90, 120, 150 and 180 days on the shelf. They were evaluated for free acidity, peroxide value, specific extinction coefficients K_{232} e K_{270}, total phenol content and the induction time.

MATERIALS AND METHODS

Samples: Olive fruits (*Olea europaea* L.) of two varieties: Leccino and Cellina di Nardò, grown in the Apulia Region (Italy) and harvested during the crop 2004-2005. The olives randomly picked at industrial optimum ripening stage, according to their skin colour. Harvesting was done by hand, using rakes. The olives were put into 30 kg boxes and immediately taken to an industrial oil mill. The oil samples were extracted using a industrial plant equipment with a hammer crusher a mixer and a triple-phase decanter. After crushing, the olive paste obtained was malaxed for 45 min at 30°C.

Storage experiments: The virgin olive oils immediately after the extraction process were stored in three industrial tanks (capacity of 250 litres). The stainless tanks were almost completely filled (3% headspace). In the first phase, of the experiment the oils were stored in three different conditions: (*A*) saturating the headspace of the tank with air at ambient temperature; (*B*) saturating the headspace of the tank with nitrogen at ambient temperature; (*C*) saturating the headspace of the tank with nitrogen and maintaining the temperature under 20°C. After six months of storage in tanks, the oils of the three tanks were bottled under different conditions: (*a*) saturating the headspace of the dark bottle (250 ml) with air at ambient temperature; (*b*) saturating the headspace of the dark bottle (250 ml) with nitrogen at ambient temperature. The oil stored in the tank under condition *A*, was bottled using both (*a*) and (*b*) bottling conditions. The oil stored in the tank under condition *B* and *C* were bottled under bottling condition (*b*). The industrial bottling equipment was equipped with an automatic system to fill the headspace of the bottle with nitrogen. Dark bottles of the 250 mL were used. The total number of the thesis at this step of the experimentation were four: A (a), A (b), B (b) C (b). All the samples (24 bottles) were well taped and stored at room temperature and under diffused light to simulate the typical home storage conditions for six month. They were periodically rearranged to equalize exposure. We analyzed all samples each 30 days. The oils were sampled before the first bottling operation and after 30, 60, 90, 120, 150 and 180 days of storage. The oil samples were analyzed for free acidity, peroxide value (PV), specific extinction coefficients K_{232} e K_{270}, total phenol content. The stability was measured using a Rancimat apparatus at 120 °C (20 l/h). The samples were run in duplicate and analyses were done in triplicate. The flux diagram of the experimental procedures are shown in figure 1.

Acidity value, peroxidase index and specific extinction coefficient at 232 and nm (K_{232} and K_{270}): Acidity value, peroxide index and ultra-violet light absorption (K_{232}, K_{270}) were carried out according to the European Official Method of Analysis (EEC/1989/2003 of the Commission Regulation).

Oxidative stability: The oxidative stability was evaluated according to an accelerated automatic test using the Rancimat apparatus, (Metrohm AG, Herison, Switzerland) using an oil sample of 2,5 g, warmed to 120°C and a purified air flow rate of 20 l/h. Stability was expressed as the oxidation induction time (h). In this process, the volatile oxidation products were stripped from the oil and dissolved in the water, increasing the water conductivity. The time taken until there is a sharp increase of conducibility is termed the induction time, and it is expressed in hours (Halbault, Barbè, Aroztegui, & De La Torre, 1997).

Total phenol content: Phenolic compounds were isolated from a solution of oil in hexane by triple-extraction with water–methanol (60:40 v/v). Total phenols, expressed as gallic acid equivalents (ppm), were determined with a UV visible spectrophotometer (Beckman) at 765 nm using the Folin-Ciocalteau reagent (Swain & Hillis, 1969).

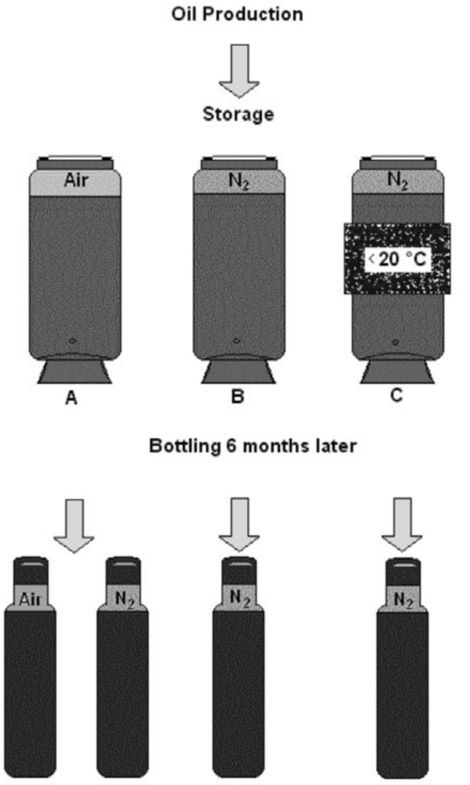

Fig. 1. Flux diagram of the experimental procedures.

Statistical Analysis: Experiments to de-

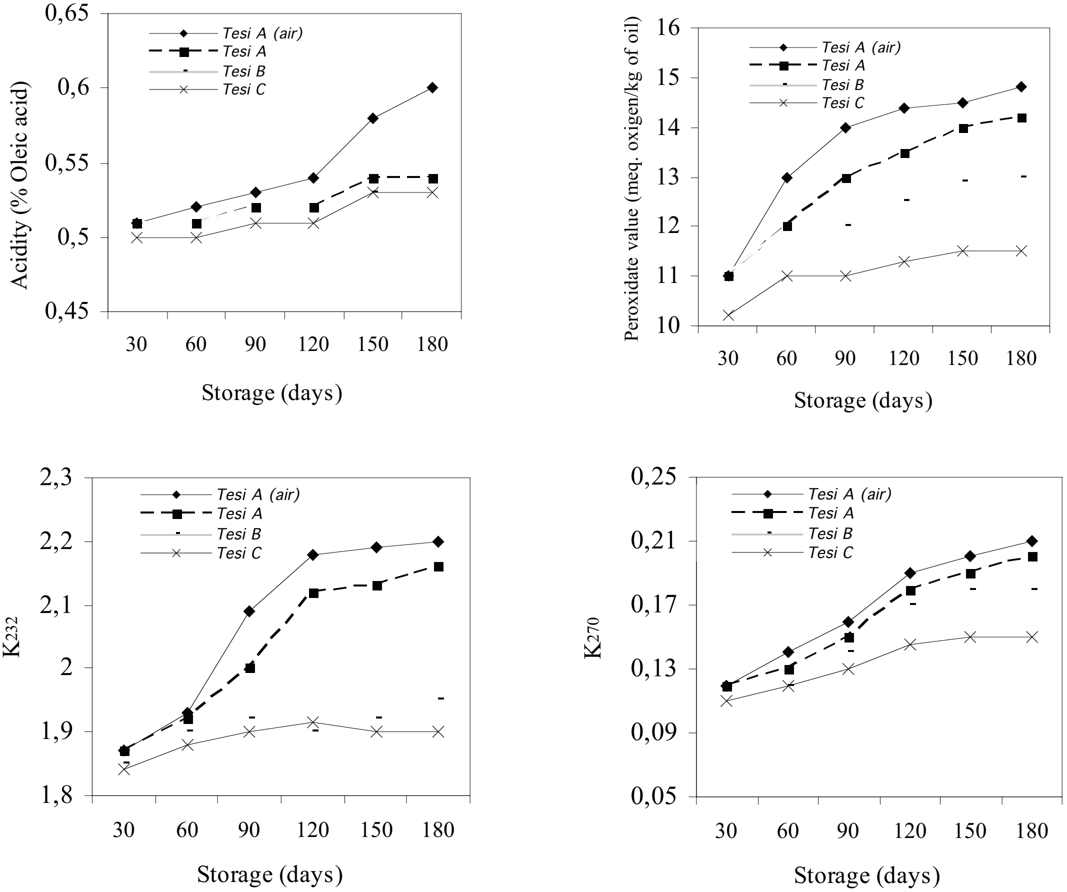

Fig. 2. Changes in the free acidity, peroxidase value and specific extinction coefficient at 232 nm and 270 nm of oils stored in dark bottles for 180 days. Each data point represent mean af three replicates ± SD.

termine the different olive oil parameters were performed in five times, the result are expressed as means ± standard deviations. One-way analysis of variance (Anova) were used. Differences between groups were considered statistically significant at $p<0,05$.

RESULTS AND DISCUSSIONS

The paper focuses on the change in olive oil quality during the storage of the bottles under different headspace atmosphere compositions, monitoring the oxidation progress when/while the antioxidant content decreased. Standard quality indicators of olive oil samples are shown in Figure 2. The acidity, increased above all in the bottles corresponding to the conditions *A* (*a*). That is, the bottling from the tank which headspace was saturated with air and there wasn't a control of the temperature and bottling was done without nitrogen gas.

As can be observed, increase in peroxide value, and in primary and secondary oxidation product indices (K_{232} and K_{270}) was moderate and these values remained within the limits for extra virgin olive oil quality.

The values of oxidative stability for these samples together with the minor compounds studied, encompass a considerable range of stability. Stability, is useful to provide information about the hypothetical shelf life of an oil (Cinquanta *et al.*, 2001). The amount of phenolic compounds is an important factor when evaluating the quality of virgin olive oil, because of their involvement in its resistance to oxidation and its sharp bitter taste, (Morelló *et al.*, 2004) this trial also aimed at determining the changes in the phenolic fraction of oils after a six month storage period in bottles under different storage conditions. The loss of polyphenolic substances was less rapid in bottles corresponding to the conditions B (b) and C (b), which were bottled with nitrogen. Morover, the antioxidant activity of poliphenol extracts showed to be positively correlated with the oxidant stability as measured with Rancimat. The correlation is show in figure 3. These result, confirm the importance of polyphenols content in the improvement of oxidate stability of the oils (Baldioli *et al.*, 1996). The major changes were found in the bottles without nitrogen, increasing the temperature from 12 to 25°C and increasing the duration of the storage from 30 to 180 days we found that diminishing the phenolic componds, the stability of the oil also reduced.

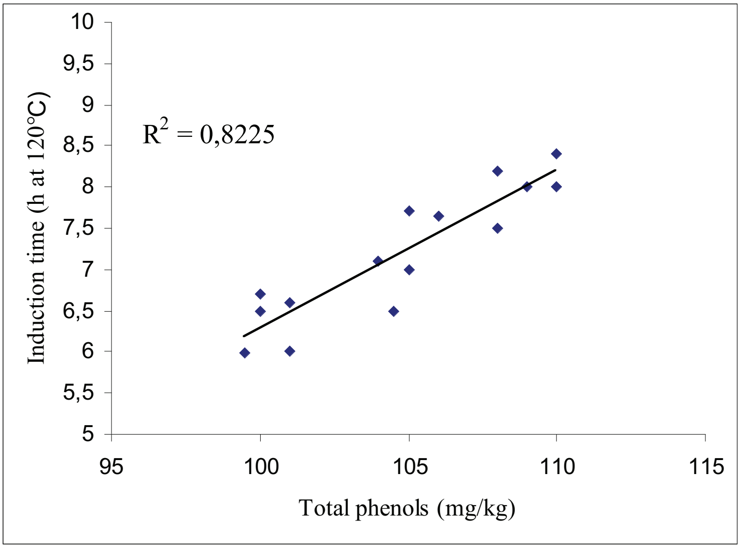

Fig. 3. Correlation between total polyphenols and induction time in the samples before the bottling.

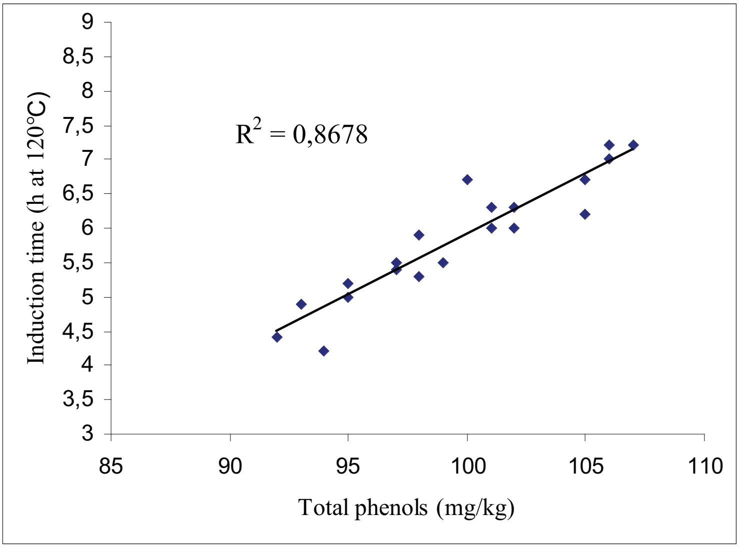

Fig. 4. Correlation between total polyphenols and induction time in the samples after the bottling.

Samples A (a) and A (b), despite differences in their initial peroxidate and acid values, show similar deterioration.

CONCLUSION

The probability of the packaged olive oil not reaching the end of its shelf life after a certain time period, was investigated. Special interest was given to the influence of nitrogen gas on the examinated parameters. Only in the final phases of the storage, when the level of antioxidant was reduced, we have observed an increase of the oil's peroxide number in the samples without inert gas packaging. The presence of air could stimulate the oxidative degradations of the olive oil resulting in a sharp reduction of its shelf life. Finally, we found that storage of olive oil using nitrogen gas under dark conditions offers a significantly high possibility for a prolonged shelf life of even beyond 12 months.

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NEW MIXER EQUIPPED WITH CONTROL ATMOSPHERE SYSTEM: INFLUENCE OF MALAXATION ON THE SHELF LIFE OF EXTRA VIRGIN OLIVE OIL

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ABSTRACT

To study the influence of operative conditions adopted during the malaxation of pastes on the shelf life of resulting oils we compared acidity percentage, peroxidase index, phenol compounds and oxidative stability, of the oil extracted from homogeneous batches of olive fruits from *Coratina* cultivar by using different malaxation atmosphere compositions.

Results reported in this research show a higher concentration of total phenols and a higher oxidative stability, in extra virgin olive oil using hermetically closed mixer with modified atmosphere in confront of the olive oil obtained mixing without modified atmosphere.

Finally from the analytical point of view, the process in inert atmosphere has demonstrated that there haven't been evidences of meaningful variations in the acidity percentage and peroxide number.

Key words: virgin olive oil, industrial storage conditions, total phenol content, olive oil stability

INTRODUCTION

The aim of this paper is to verify the infuence of the technological innovations of the mixer on the quality of oils with particular reference to the increment of the shelf-life of the product. During the olive oil process it is important to improve the reologichal property of the olive paste in order to obtain a good yield of the oil during the centrifugal separation stage. This phase is the malaxing phase. Malaxing is an extremely important phase in olive oil extraction. It is used to balance olive oil quality and extraction performance. During the malaxing phase the paste endures a slow mixing with the aim to break off the emulsions that have been formed during the crushing process and to facilitate the phenomenon of the adequate coalescence (Di Giovacchino L. *et al.,* 1994). The olive paste is then ready for the following operation of the centrifugal extraction. During the malaxing it is necessary to heat the paste for 45-60' to diminish the viscosity and stimulate the enzyme activity to increase the extraction yields (Amirante P. *et al.,* 2005).

This heating and stirring allows the oil/water emulsion to coalesce. Microscopic oil droplets join together into larger drops that can be extracted using a centrifuge. This must be done without affecting the biochemical structure of the olive paste, as this would affect the flavour, shelf life and nutritional properties of the oil.

It's also important to maintain the aromatic aspects because they influence the taste of the oil.

The main aim of this research consists in planning and fine tuning the olive paste mixer using modified atmosphere and implementing an on line automatic control of the composition of the atmosphere in the head space. This implementation reduces the contact between the oil and oxygen and decreases the phenomena of oxidation during the malaxing stage.

During the malaxing phase there are optimal conditions for the development of various enzymatic reactions. Simultaneously there are remarkable variations of the reologichal property of the olive paste.

The aim of this work was to study the influence of operative conditions adopted during the malaxing of the olive pastes. We compared two different malaxing process (with and without inert atmosphere in the head space) and estimated the compositions of the oil extracted using olive fruits (*Olea europaea* L.) from *Coratina* variety.

MATERIALS AND METHODS

Extraction experiments: the aim was to estimate the influence of the inert gas during the malaxing operation on quality of the olive oil. In the first phase we have planned, constructed and fine tuned (in collaboration with Alfa Laval Company) the malaxing machine, which consisted of two sections 350 kg each with a control system, the atmosphere for a space head control system. The malaxing machine has covered holding tanks with hollow walls, in which warm water flows to heat the olive oil paste to a carefully monitored temperature, while it is slowly but continuously stirred using a specially designed set of blades.

In fact this machine can also work with an inert gas (nitrogen) under light pressure.

The malaxing machine is equipped with an opening in transparent PVC in order to inspect the inside of the sluice.

The liquid nitrogen that was in bottles (inert gas) was connected with PVC. The malaxer machine was equipped with a safety valve which will avoid pressure problems.

Before the introduction of the olive paste in the malaxer, the machine was injected with nitrogen. After the machine has been loaded with the paste, we continued the injection of the nitrogen and also during the stirring of the paste with capacities of 10-20 l/min. until no oxygen remained.

In order to verify the saturation of the malaxer head space we used an instrument with an oxygen probe (Abiss type PAK 01P) to measure the oxygen in the tanks. This instrument allowed us to estimate on-line the oxygens percentage in the atmosphere malaxer. In all the tests the presence of the oxygen (21% approximately in atmosphere) has been reduced to values oscillating between 0.3-0.5 % for the whole duration of the mixing/stirring of the olive paste.

In all the tests that were done during the malaxing phase the temp was 27°C and the duration was of 45 minutes (Angerosa F. *et al.*, 2001).

The experiment to control the nitrogen always has been followed with an experiment without the usage of nitrogen, but with equal conditions in the headspace's atmosphere.

In all the tests we used olives of the Coratina variety turning colour, produced in Ostuni (BR) Apulia Region (Italy), and milled 7 hours after the harvest in an mill with an Alfa Laval continuous centrifugation system.

Samples: at the end of the extraction 4 bottles have been collected. These were the test conditions.

- The preparation of the olives: the division of the olives in to homogenous tanks of 500 kg each.
- The de-leafing and washing of the olives.
- The crushing of the olives: a disk crusher has been used.
- The malaxing phase: a mixer saturated with nitrogen, loaded with olive paste and the oxygen percentage in atmosphere was controlled. Time and temperature of the stirring was: 27°C for 45', with nitrogen in the head space, or without nitrogen (comparison tests).
- The separation and centrifuging: an 2-phase decanter Alfa Laval Oliver 500.
- The clarification: a centrifuge separates the oil from any remaining water and olive residue.

Acidity value, peroxidase index and specific extinction coefficient at 232 and nm (K_{232} and K_{270}): Acidity value, peroxide index and ultra-violet light absorption (K_{232}, K_{270}) were carried out according to the European Official Method of Analysis (EEC/2568/91 of the Commission Regulation, 1991).

Oxidative stability: The oxidative stability was evaluated according to an accelerated automatic test using the Rancimat apparatus (Metrohm AG, Herison, Switzerland) using an oil sample of 2,5 g, warmed to 120°C and a purified air flow rate of 20 l/h. Stability was expressed as the oxidation induction time (h). In this process, the volatile oxidation products were stripped from the oil and dissolved in the water, increasing the water conductivity. The time taken until there is a sharp increase of conducibility is termed the induction time, and it is expressed in hours (Halbault, Barbé, Aroztegui, & De La Torre, 1997).

Total phenol content. Phenolic compounds were isolated from a solution of oil in hexane by triple-extraction with water–methanol (60:40 v/v). Total phenols, expressed as gallic acid equivalents (ppm), were determined with a UV visible spectrophotometer (Beckman) at 765 nm using the Folin-Ciocalteau reagent (Swain & Hillis, 1969).

Statistical Analysis. Experiments to determine the different olive oil parameters were performed in five times, the result are expressed as means ± standard deviations. One-way analysis of variance (Anova) were used followed by Duncan's multiple range test with a significance level of $p < 0,05$.

RESULT AND DISCUSSION

The results have been estimated in terms of acidity values, peroxidase index and specific extinction coefficient at 232 and 270 nm (K_{232} and K_{270}), oxidative stability and total phenol content.

Acidity value, peroxidase index and specific extinction coefficient at 232 and nm (K_{232} and K_{270}).

Acidity percentage: in all the tests the percentage was between 0,3-0,4% and there haven't been meaningful variations between the tests with or without nitrogen (Tab. 1).

Peroxidase index: these values increased in the tests done without nitrogen compared to the test done with nitrogen (Tab. 1).

Specific extinction coefficient: the primary and secondary oxidation product indices (K_{232} and K_{270}) have been moderated and these values remained within the limits for the extra virgin quality (Tab. 1).

Total phenol content

The total phenol content of the oil from the test without nitrogen is increased from the value of 282 mg/kg to 380 mg/kg of the oil from the test with nitrogen.

These results have been confirmed by numerous studies that have demonstrated that the nitrogen indirectly acts on the activity of endogenous enzymes of the olive, as the poliphenoloxidized (PPO), perossida (POD) and lipossigenasi (LPO). The effect, consists in a clean reduction of the oxygen content in the malaxing pastes. The consequence of this phenomenon is the inhibition of the oxidative enzymatic complex, which the phenolic oxidation catalyzed from PPO and POD is considerably reduced.

Therefore the continuous nitrogen flow during the malaxing allows at the same time a reduction of the phenomenon of oxidation of phenolic compounds (Fig. 1).

Oxidative stability

The values of oxidative stability Rancimat test has been increased in the tests with nitrogen in the malaxing phase passing from value of 13,6 h (tests without nitrogen) to 18,2 h (tests with nitrogen) (Tab. 1).

In the following figure (Fig. 2) is indicated the correlation

Table 1. Standard quality parameters in two different mixing techniques.

	Mixing techniques			
	MWN		MWoN	
	m	*sd*	*m*	*sd*
A	0,3	0.03	0,4	0.02
PV	4,2	0.04	4,8	0.05
K_{232}	1,71	0.02	1,68	0.01
K_{270}	0,12	0.00	0,12	0.00
IT	18,03	0.73	13,93	0.54
TP	383,97	29,73	261,29	24,00

Data represents mean (*m*) and Standard Deviation (*ds*).
Abbreviation: MWN: Mixing with nitrogen; MWoN: Mixing without nitrogen; A: Acidity (% of oleic acid); PV: Peroxide value (meq of oxygen/kg of oil); IT: induction time (H at 120°C); TP: Total phenols (mg/kg).

between total phenols and induction time.

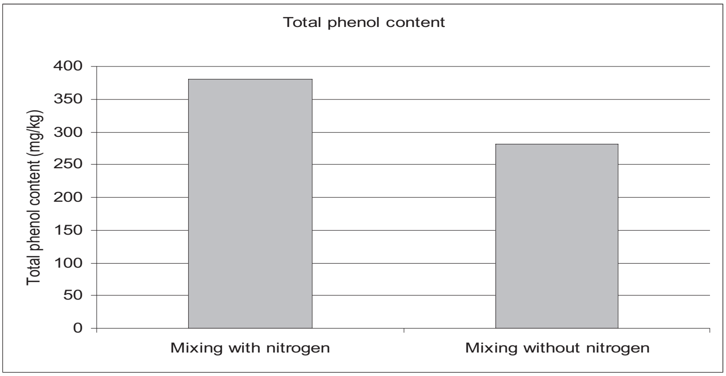

Fig. 1. Total phenol content in olive oil from test with/ without nitrogen in mixing phase.

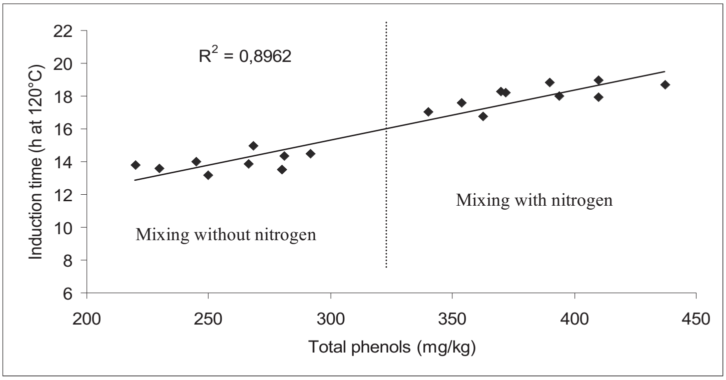

Fig. 2. Correlation between total phenols and induction time.

CONCLUSION

The individuation of technical solutions aimed to elevate the qualitative standards of the extravirgin olive oil and its shelf life has been the objective of this research program. The reduction of the oxidation phenomena, in the malaxing phase, represents an important objective for the qualitative implications.

From the analytical point of view, the process in inert atmosphere has demonstrated meaningful lessening of the peroxide number, consequently a drastic reduction of the oxidation phenomena during the malaxing phase of the extraction process is seen. Moreover an increase of the total phenol content has been evidenced in the extra virgin olive oil produced in the process with inert atmosphere.

There haven't been evidences of meaningful variations in the acidity percentage.

Finally the easiness of an adaptation of the malaxer and the low cost of nitrogen confirm the possibility to adopt this system and to produce extra virgin olive oil with elevated standard parameters, for the quality and longer shelf life.

ACKNOWLEDGEMENTS

The authors have contributed to the same extent to the present study.

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EFFECT OF THE CHEMICAL TREATMENT ON THE MECHANICAL PROPERTIES, WATER VAPOR PERMEABILITY AND SORPTION ISOTHERMS OF GELATIN-BASED FILMS

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ABSTRACT

Proteins contain hydrophilic groups which can bind to the water molecule through hydrogen bridges resulting in water adsorption. The increase of the cross-linking degree can be an alternative to improve the cohesiveness force and functional properties of protein based films. Thus, the objective of this work was to evaluate the effect of chemical treatment of gelatin with formaldehyde and glyoxal on the mechanical properties, water vapor permeability and water vapor sorption characteristics of gelatin based films. Films were produced using gelatin, with and without chemical treatment. The chemical treatments caused a reduction on the water vapor permeability, however, only the formaldehyde allowed a significant increase on the tensile strength. The GAB and Halsey models could be used to model the sorption isotherms of films. It was observed that the increase of the temperature produced a decrease in water sorption and the chemical modifications caused a slight reduction on the monolayer moisture content.

Key words: physical properties, protein, cross-link, formaldehyde, glyoxal

INTRODUCTION

Gelatin has been widely used in the production of edible and/or biodegrable films in the last years (Sobral and others, 2001; Carvalho and Grosso, 2004; Vanin and others, 2005). However, the gelatin, as well as other proteins, contain hydrophilic groups that can bind to the water molecule through hydrogen bridges resulting in water adsorption and increase on the diffusivity coefficient, originated by the rupture of intermolecular interactions. Because of that, gelatin based films presents a limited barrier to water vapor (Sobral and others, 2001).

Chemical modification of proteins has been normally used for improving the functional properties of their films. The increase in the degree of cross-linking can bring about a decrease of the water vapor permeability through the reduction in water diffusivity and an improvement of mechanical characteristics as a function of the increase of the cohesiveness force in polymeric matrix. Formaldehyde and glyoxal are chemicals frequently used for protein cross-linking. These agents react with some amino acid side chains, particularly with the lysine ε-NH_2 group, forming similar bonds to those in the formation of the Schiff base (Fraenkel-Conrat and others, 1947). Marquié and others (1995) verified that chemical treatment with formaldehyde, glutaraldehyde and gossypol, provoked a significant decrease in solubility and an increase in the puncture force of cottonseed proteins based films. Thus, the objective of this study was to evaluate the effect of the chemical treatment of gelatin with formaldehyde and glyoxal on the mechanical properties, water vapor permeability and sorption characteristic of gelatin-based films.

MATERIALS AND METHODS

Bovine hide type B gelatin (bloom= 270.0g; protein content= 88.92±0.02%) donated by Gelita South America (São Paulo, Brazil); formaldehyde solution (HCHO, 35%), glyoxal solution (HCOCHO, 40%) and glycerol from Merck (São Paulo, Brazil) were used for film production. The salts used were: LiCl, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, NaBr, KCl, NaCl (Sinth, São Paulo, Brazil).

The gelatin based films (without modification - NF; formaldehyde modified film - FMF and glyoxal modified film -GMF) were produced by the casting technique with 10g of gelatin/100mL of filmogenic solution and 4.5g of glycerol/100g of gelatin according Carvalho and Grosso (2004). The film thickness was kept constant at 0.080±0.004 mm through the mass/area relationship and determined using a digital micrometer (Mitutoyo Corp., Tokyo, Japan). Before the mechanical and water vapor permeability analysis, the samples were conditioned for 72h in a desiccator containing $Mg(NO_3)_2$ saturated saline solution at 25°C (relative humidity of 50%).

The tensile strength (TS) and elongation at break (E) of the films, average of five determinations, were determined using a texturometer TA.XT2 (TA Instruments, New Castle, U.S.A.) according to method ASTM D882-95 (ASTM, 1995a). The initial distance of separation and the velocity were fixed at 50 mm and 1 mm/s, respectively. The water vapor permeability (WVP) of the films was determined gravimetrically, in triplicate, according to the method ASTM E96-95 (ASTM, 1995b). The relative humidities (RH) were 50% (outside RH) and 0% (inside RH). In both tests, the temperature was 25°C.

The sorption isotherms for gelatin based films were determined gravimetrically at temperatures of 15, 25 and 35°C. Films samples were equilibrated in hermetically sealed containers with saturated saline solutions to various water activities between 0.11 and 0.85. The equilibrium moisture content was determined at 105°C (24h). The values were fitted to the GAB (Guggenheim-Anderson-De Boer), BET (Brunauer-Emmett-Tetter), Halsey and Oswin models (Table 1) using Statistica®

Table 1. Sorption isotherm equations.

BET	GAB
$\frac{a_w}{(1-a_w)}\frac{1}{X}=\frac{1}{X_M C'}+\frac{a_w(C'-1)}{X_M C'}$	$X=\frac{X_M C_G\ K\ a_w}{(1-K\ a_w)(1-K\ a_w+K\ C_G\ a_w)}$
Halsey	Oswin
$a_w=\exp\left(\frac{-A}{RT\theta^r}\right)$	$X=m\frac{a_w}{1-a_w}^{n}$

A_w = water activity; X_m = value of the moisture content in the monolayer; C', C_G, K, m, n = models constants; A, r = equations parameters; $\theta = X_m/X$; X = water content, dry basis.

software. The fit of model were evaluated according to the values of correlation coefficient (R) and the mean relative deviation (DMR).

RESULTS AND DISCUSSION

The values of the mechanical properties and water vapor permeability (WVP) are shown in Table 2. It was verified that the treatment with formaldehyde provoked a significant increase in TS and a decrease in the WVP as compared to the other films studied (NF and GMF). The chemical modifications were carried out in an excess of the formaldehyde and glyoxal with respect to the available lysine content of the gelatin used (Carvalho and Grosso, 2004). The formaldehyde is a low molecular weight molecule, when compared to glyoxal, and could easily migrate between the protein chains and establish covalent bonds with the lysine and others reactive groups (cystine and hystidine groups) of the proteins.

The elongation of films treated with formaldehyde and glyoxal, respectively, did not vary significantly compared to NF. Rhim and others (2000), working with soybean-isolate protein-based films modified with glutaraldehyde, did not verify significant variations in the tension strength of the modified films in relation to the unmodified films; however, the elongation increased significantly.

Table 2. Tensile strength (TS), elongation (E) and water vapor permeability (WVP) for gelatin based films: whitout treatment (NF), formaldehyde modified films (FMF) and glyoxal modified film (GMF).

Film	TS (MPa)	E (%)	WVP (g.mm/m2.h.KPa)
NF	15.2 ± 0.8a	39.2 ± 0.8a	0.198 ± 0.003^a
FMF	23.1 ± 1.3b	37.7 ± 1.9a	0.155 ± 0.008b
GMF	15.0 ± 0.5a	38.1 ± 0.4a	0.174 ± 0.007c

Note: different letters in rows represent significant differences ($p < 0.05$) between averages obtained through the Tukey test.

In relation to WVP, Ghorpade and others (1995), studying soybean protein-based films modified with formaldehyde, observed a reduction in water vapor permeability from 0.84 to 0.79×10^{-9}g/m.s.Pa when compared to the unmodified films. Micard and others (2000) also observed

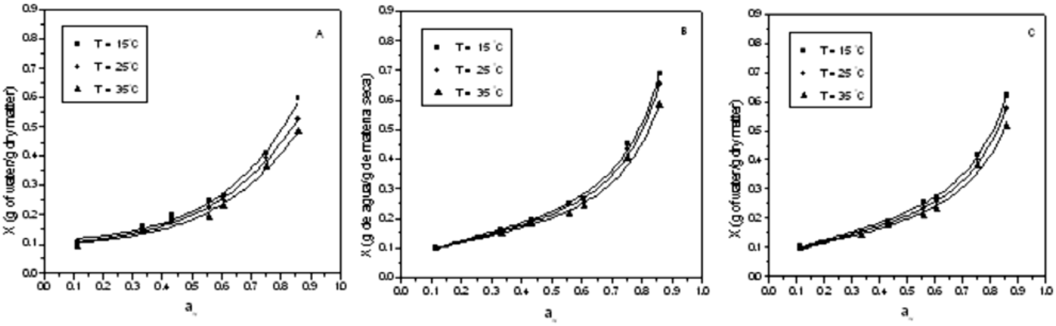

Fig. 1. Sorption isotherms for gelatin based films without treatment (A); formaldehyde modified film (B) and glyoxal modified film (C) with fit lines from GAB equation at temperatures 15, 25 and 35°C.

Table 3. Parameters of the GAB equation (Xm, CG and K) for gelatin based films (NF, FMF and GMF) at 15, 25 and 35°C.

	NF			FMF			GMF		
T (oC)	Xm	CG	K	Xm	CG	K	Xm	CG	K
15	12.52	23.37	0.93	12.20	20.46	0.96	12.46	19.70	0.93
25	12.18	21.05	0.92	11.62	23.37	0.98	11.84	20.90	0.95
35	11.70	21.80	0.92	11.14	30.13	0.98	11.47	21.73	0.94

a reduction in water vapor permeability from 7.6 to 6.7x10^{-12} g mol/m.s.Pa for gluten protein-based films treated with formaldehyde, in relation to the unmodified films.

Concerning the sorption isotherms, all models provided a high R value (>0.99) for all studied temperatures, however, only the GAB and Halsey models provided a MDR <5.0%. Thus, the GAB and Halsey models were considered the most appropriate equations for modeling the moisture sorption characteristics of the gelatin based films, but only the GAB was used for modeling isotherm, because it allows the determination of the monolayer value (Xm). The fit lines using GAB models, for NF, FMF and GMF at different temperature can be observed in Figure 1, and the values of the GAB parameters are shown in Table 3. The shape of the isotherm of the NF, FMF and GMF was similar to that of gelatin (Sobral and Habitante, 2001) and whey protein film (Coupland and others, 2000). It was observed that the increase of the temperature caused a decrease in water sorption. This behavior has been observed for different biopolymers (Cruz and others, 2001). The chemical modifications induced a slight reduction on the monolayer moisture content (Table 2).

CONCLUSION

Formaldehyde treatment of gelatin produced films more resistant mechanically and less permeable to water vapor than glyoxal. The GAB and Halsey models were considered the most appropriate equations for modeling the moisture sorption characteristics of the gelatin based films and the chemical modifications caused a slight reduction on the monolayer moisture content.

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EFFECT OF SOME ESSENTIAL OILS AS NATURAL FOOD PRESERVATIVES

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ABSTRACT

This work studied the effects of some essential oils against the growth of some microorganisms potentially danger to the safety of foods. These were *Escherichia coli*, *Staphylococcus aureus* and *Listeria innocua.* The effect and the minimum concentration of the essential oils were observed. The essences tested were: lavender (*Lavandula officinalis*), lemon-balm (*Melissa officinalis*), juniper (*Juniperus* communis), lemon verbena (*Lippia citriodora*), rosemary (*Rosmarinus officinalis*) and cypress (*Cupressus sempervirens*). All tested essential oils were purchased from a factory that obtained them through vapour distillation. Before use they were preserved in dark sealed glass containers kept at 4 °C. The most bacteriostatic essential oils, as lemon verbena, cypress and lemon-balm, were tested on grated carrots inoculated with bacteria and stored at 8°C until 8 days as they are sold in market. The most resistant of the three strains against all the tested oils was *E. coli*, while *Listeria* strain was the most easily influenced. The 1% *Cupressus* oil in plastic bag had the most inhibitory effect on all the tested strains.

Key words: *Escherichia coli*, essential oils, *Listeria innocua*, shelf-life, *Staphylococcus aureus*

INTRODUCTION

Due to the increasing interest of consumers in natural ingredients and to adopt more natural alternatives for the maintenance of product shelf-life, there is great interest in the potential use of essential oils extracted by many spices and herbs as antimicrobial additives. Microorganisms are involved in the formation of many diseases and also cause deterioration of many products. The beginning of examination about the growth-inhibitory effects of substances from essential oils on microorganisms lies long in the past (Pauli, 2001), in fact, many authors have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties (Hirasa *et al.*, 1998) by spices and essential oils and, in some cases, a direct food-related application has been tested (Madsen *et al.*, 1995). Moreover, the problem of preserving food is becoming more complex, due to the fact that the new products being introduced in the market require an ever longer shelf-life and a higher degree of protection against pathogenic microorganisms (Marino *et al.*, 2001). Unfortunately, only a few studies have focused on the mechanism by which spices or their essential oils inhibit microorganisms (Tassou *et al.*, 2000). Recent studies suggest that this mechanism of action may be more complex: the spices oils can inhibit enzyme activity and some of theme disrupt the cell's membranes (Gill *et al.*, 2002). Naturally, their action depends on the type, composition and concentration of the spice or the essential oils, the type and concentration of the target microorganism, the composition of the substrate, the processing and the storage conditions (Pandit *et al.*, 1994; Skandamis *et al.*, 2000). The aim of this work was to evaluate antimicrobial activity such as minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC) of some essential oils on survival and growth of *E. coli*, *S. aureus* and *L. innocua* strains, and to investigate their application on fresh carrots. *L. innocua* generally share the same habitat that *L. monocytogenes* (Cattai *et al.*, 2002) and so we have tested this species instead of the pathogenic one.

MATERIALS AND METHODS

CG-MS analysis of the essential oils. The essential oils (Polichimica, Bologna) were analysed by CG/MS using an Hewlett–Packard 6890 gas chromatograph equipped with a Hewlett–Packard 5973 quadrupole mass selective spectrometer. The separation was achieved by a HP-5 fused-silica capillary column (30 m x 0.2 mm, film thickness 0.25 μm). The column temperature was programmed from 50°C (5 min) to 280°C at 4°C/min. The flow rate of helium as a carrier gas was 1 mL/min kept constant for all the chromatographic run. 0.2 μL neat oil was injected in split mode with a split ratio of 1:50 and at a temperature of 250°C. The sample components were identified by matching their mass spectra with those of the John Wiley & Sons library, or those of pure standard components and confirmed by their GC retention times.

Microorganisms and cultures. Three species of bacteria, *Escherichia coli* (C 7085, Culti-Loops, Oxoid), *Staphylococcus aureus* (C 7016, Culti-Loops, Oxoid) and *Listeria innocua* (DSM 20649, Germany), were studied. The 24 h-cultures of each bacterium, activated in Tryptic Soy Broth (TSB CM129, Oxoid), were nebulized on grated carrots. Bacterial populations of every inoculum were determined by surface plating duplicate samples on TSA. The plates were incubated for 24 h at 32°C before colony counts were obtained.

Carrots as a model food for antimicrobial study. Effect of essential oils as antimicrobial agents was studied on commercial carrots procured from local stores and were kept frozen (4°C) until the use. Samples of grated carrots (60 g each) were placed into food containers and then sprinkle inoculated with every single strain (1 mL), to obtain an initial level of 10^8 CFU/g, and with the solution of different concentrations (5% and 1% v/v) of several essential oils. The samples were shaken for 1 min to evenly distribute the inocula, packed both in the plastic vessel covered superficially with a PVC film (15 μm) and sealed plastic bag and finally stored in a refrigerator at 8°C until 8 days.

Microbiological analyses. For enumeration of *E. coli*, *S. aureus* and *L. innocua* from carrots at 2, 5, 8 days, 10 g of the single sample was transferred into sterile stomaching bags (BagFilter P/10, 400 mL, Interscience), with the aid of a sterile stainless-steel spatula, combined with 90 mL of sterile ringer solution (BR0052G, OXOID) and then pummelled in a Stomacher (BagMixer 400, Interscience) at medium speed for 2 min. Serially dilution of samples were prepared and analysed in the solid selected media, Rapid E. coli 2 Agar (Target Diagnostica) for *E. coli*, Baird Parker Agar added with RPF supplement (Target Diagnostica) for *St. aureus* and TSB broth (CM129 Oxoid) for *L. innocua*, and incubated at 37°C for 24 h.

Chemical analyses. On fresh grated carrots and after 8 days of test, water activity was measured by an Aqua lab (Decagon devices Inc., Washington) apparatus, while the dry matter content was determined by oven drying at ~105°C for 48 h.

RESULTS AND CONCLUSIONS

Preliminary tests were performed using ten essential oils and measuring the MIC and MBC. The most effective essential oils (lemon verbena, cypress and lemonbalm) were tested in this work on grated carrots.

The analysis of the carrots sprinkled with the three essential oils has shown results, different for each of the three inoculated strains. In the Table 1 the results

Table 1. Enumeration of *E. coli*, *S. aureus* and *L. innocua* in grated carrots sprinkled with 1 and 5 % of the three essential oils (B: bag; V: vessel).

Essential Oils Concentration	Packaging	*Escherichia coli*			*Staphylococcus aureus*			*Listeria innocua*		
		time (days)			time (days)			time (days)		
		2	5	8	2	5	8	2	5	8
Control	B	8,00	5,10	4,23	6,91	1,15	2,00	3,39	2,00	0
	V	8,00	8,33	6,65	7,39	5,17	8,59	5,70	2,00	3,30
5% Lippia	B	8,00	5,00	2,59	6,98	0	0	3,57	0	0
	V	8,00	8,22	6,61	7,54	7,08	6,46	5,91	0	0
1% Lippia	B	8,00	6,22	1,24	6,69	3,31	0	4,00	0	0
	V	8,00	8,00	6,98	7,52	6,20	5,91	5,00	2,42	0
5% Cupressus	B	8,00	6,70	3,04	7,25	2,28	0	2,83	0	0
	V	8,00	8,00	6,48	7,60	6,74	4,02	4,92	0	0
1% Cupressus	B	7,35	7,38	0	6,45	1,15	0	1,42	0	0
	V	8,00	8,43	7,12	7,80	6,21	3,83	5,37	0	0
5% Melissa	B	8,00	5,25	1,64	6,13	0	0	2,10	0	0
	V	8,00	8,30	8,16	8,08	5,80	3,44	5,00	0	0
1% Melissa	B	8,00	5,00	3,61	6,71	1,15	0	0	0	0
	V	8,00	8,32	7,84	7,15	4,39	0	5,75	0	0

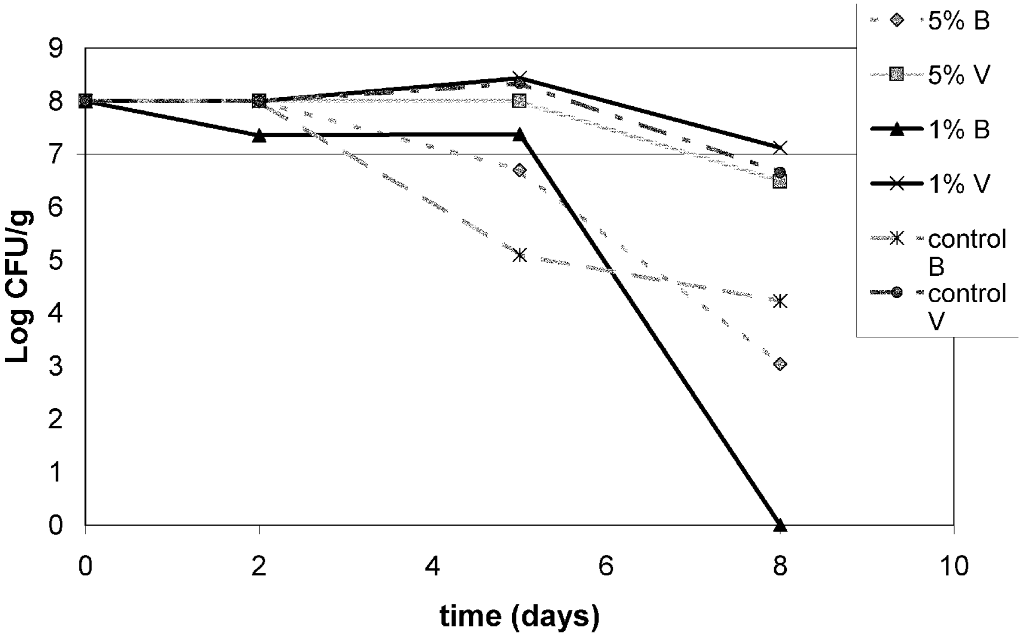

Fig. 1. Inhibitory effect of 1 and 5 % Cupressus essential oil on *E. coli* in grated carrots stored until 8 days (B: bag; V: vessel).

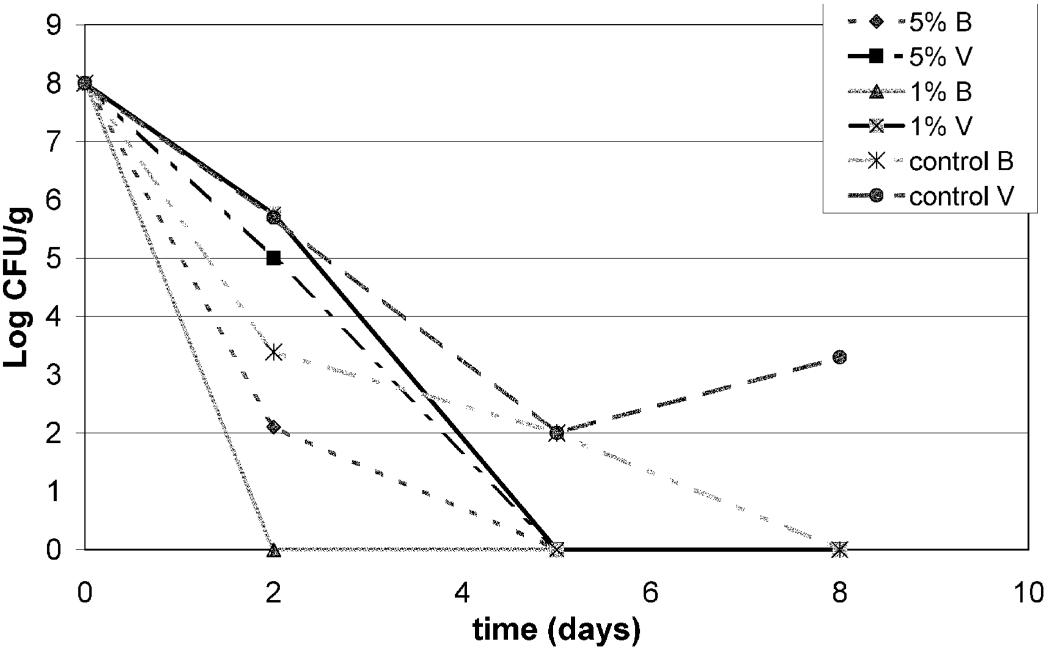

Fig. 2. Inhibitory effect of 1 and 5 % Melissa essential oil on *L. innocua* in grated carrots stored until 8 days (B: bag; V: vessel).

of the microbial growth are reported. At time zero, all the three bacteria showed a population of around 10^8 CFU/g. In the Figure 1 are showed the inhibitory effect of *Cupressus* essential oil on *E. coli*, that is revealed itself the most resistant of the three strains against all the tested oils. In this case, it is observed as the 1% oil concentration has been more effective than that at 5%. Nevertheless, we must underline that the sample in plastic bag has shown a strong inhibition towards *E. coli*, and this fact is repeated in other cases (see Tab. 1).

The gas inside the bags filled with grated carrots become poor of oxygen and increase the carbon dioxide. Moreover, the metabolic products of the microorganisms naturally preset on the surface of the carrots, has an influence on the growth of the three inoculated bacteria.

In general, the tested essential oils showed the most bacteriostatic activity in the plastic bags than in the plastic vessels, this fact points out that the bag-oil synergy has probably been conclusive. Probably, the volatile compounds of the essential oils in plastic bags are more retained than in vessels that are covered by a PVC film.

The behaviour of *L. innocua* in presence of *Melissa* essential oil is showed in Figure 2. This tested *Listeria* strain was the most easily influenced by oils in comparison to the other two bacteria. In fact, after only two days in plastic bags, it has not already survived, while the same result in all bags after five days with every essential oil was obtained. While in vessels the same results were obtained with 5% of *Lippia*, 5 and 1% of *Melissa*, 1% of *Cupressus*. There were no *Listeria* survivors neither in bags neither in vessels with every oils after eight days. The behaviour of the tested *S. aureus* was differently influenced by the three oils, but also this strain was more inhibited in bags than in vessels at the end of the test.

The results of the present work prove the effectiveness of the essential oils against the microbial population present in food. Nevertheless also the packaging is shown conclusive in this sense. Then, to analyse thoroughly the study and the experiments in the application of the essential oils it would interesting. Their application as food preservatives that would extend the fresh food shelf-life, and the synergistic effect between the plastics films and the essential oils, must be deepen.

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ANALYSIS OF VOLATILE OXIDATION COMPOUNDS IN BREAKFAST CEREALS. EFFECTS OF THE EMPLOYMENT OF NATURAL ANTIOXIDANTS

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ABSTRACT

The aim of this work was to look over the volatile oxidation compounds, responsible of off-flavours, in corn flakes and to verify the effectiveness of the employment of natural antioxidants. The headspace analysis by SPME/GC/MS technique was carried out. The results showed significantly higher levels of volatile oxidation compounds in the control than in the samples added with tocopherols. The obtained data are encouraging with regard to the employment of such natural products in order to increase the shelf-life of breakfast cereals.

Key words: breakfast cereals; natural antioxidants; SPME/GC/MS analysis; volatile oxidation compounds

INTRODUCTION

Cereals, due to their high starch contents, are a particularly suitable raw material for the extrusion-cooking process, which founds on a severe thermal and mechanical treatment with the purpose to get products with new physical and structural characteristics. Because of its limited duration, extrusion-cooking is not able to determine an immediate oxidative degradation of fats; nevertheless, it makes the lipid fraction more susceptible to such alteration during the storage of the product (Artz *et al.*, 1992). In this phase the lipid fraction runs into oxidative phenomena that compromise the nutritional and sensorial quality of the product and reduce its shelf-life. Such rancidity processes lead to the formation of volatile compounds responsible of off-flavours (Palamand and Dieckmann, 1974; Frankel, 1982; Ullrich and Grosch, 1987; Kinderlerer and Johnson, 1992; Neff *et al.*, 2000). The aim of this work was to verify the effectiveness of the employment of natural antioxidants in order to slow down the oxidative phenomena which lead to the formation of such undesirable compounds.

MATERIALS AND METHODS

The production tests were carried out at an industrial establishment, adding to the basic mixture – consisting of corn meal, sugar, salt, malt and water (control) – natural tocopherols in proportion of 0.6% w/w on the total fat. Three samplings were carried out. The flakes obtained were packed in plastic bags weighing 500 g, made of a coupled film (co-extruded polypropylene / printed polypropylene cast). The samples were stored for 180 days at room temperature and in normal conditions of brightness, in order to simulate the shelf conditions. The analysis of the volatile compounds was carried out by means of SPME/GC-MS technique. Ten grams (± 0.05 g) of minced sample were weighed in a 50 ml glass vial fitted with a PTFE septum and sealed with an aluminium crimp seal. After equilibration for 30 min in a thermostatic oven at 40 °C, a SPME manual holder (Supelco, Bellafonte, PA, USA), equipped with a fiber assembly (50/30 µm, Divinylbenzene/Carboxen/Polydimethylsiloxane, Supelco) was inserted through the PTFE cap. The fibre coating was exposed for 30 min to the sample headspace. The volatile compounds were desorbed by directly inserting the fibre for 6 min into the injection port of the GC (splitless mode) maintained at 250 °C. Gas chromatographic analysis was carried out using a gas-chromatograph Fisons GC 800 and MD 800 mass spectrometer (MS) detector. Helium was used as the carrier gas with a flow rate of 0.8 ml/min. The components were separated on a SPB-624 capillary column from Supelco (30 m length x 0.25 mm i.d., 1.4 µm film). The column was initially maintained at 40 °C for 2 min; subsequently, the temperature was increased to 230 °C at a rate of 5 °C/min, and was then held at 230 °C for 10 min. The volatile components were detected by mass spectrometry with electron impact ionization at 70 eV. Compounds were identified by matching mass spectra with the Nist and Wiley libraries of standard compounds. The analysis was carried out in duplicate. The obtained results were submitted to one way analysis of variance.

RESULTS AND DISCUSSION

In Table 1 the results of the headspace analysis of corn flakes volatile compounds are reported together with the results of the statistical analysis. Thirty-five compounds associated to lipid oxidation were identified (Palamand and Dieckmann, 1974; Frankel, 1982; Ullrich and Grosch, 1987; Kinderlerer and Johnson, 1992; Neff *et al.*, 2000; Heiniö *et al.*, 2002).

The control emphasized significantly higher levels for a large number of the compounds under examination, than the samples with antioxidants, denouncing an intense oxidation. Aldehydes were the most represented class of compounds with significantly higher levels (p <0.05) in the control (integrated areas of the peaks amounting to a total of 173 millions) than in the samples added with tocopherols (area of 79 millions). The most important compound was found to be hexanal, a product of the oxidation of linoleic acid, that is the most represented fatty acid in corn meal lipids. Hexanal reached a mean value of integrated area equal to 124 millions in the control while was 63 millions in the samples added with tocopherols (p <0.05). The differences found between the levels of 2-hexenal, decanal (p <0.05), heptanal, octanal, 2-octenal (p <0.01) resulted significant, too with higher values in the control than in the samples with antioxidants. The volatile compound 2-heptenal was detected exclusively in the control. A very significant difference (p <0.001) was pointed out in the mean values of integrated area for the organic acids – pentanoic and hexanoic acid, deriving from further oxidation of the aldehydes pentanal and hexanal, respectively – that constituted 19% in the control and only 5% in the samples with tocopherols. Hexanoic acid showed an integrated area 9 times higher in the control (p <0.001), while pentanoic acid reached the values of 3.7 millions in the control and only 0.2 millions where antioxidants were employed (p <0.01). Four different lactones were also identified: γ-hexalactone, δ-hexalactone, γ-octalactone and γ-nonalactone, deriving from further oxidation of the corresponding organic acids. Also in these cases we found significant differences between the two typologies of samples under examination, still indicating lower levels of oxidation in the samples containing antioxidants (where δ-hexalactone and γ-octalactone were not detected). Three ketones were detected: 2-heptanone, 3-octen-2-one (both with significantly higher levels in the control, at p <0.01), 3,5-octadien-2-one; while, out of seven alcohols identified, five (1-pentanol, 1-hexanol, 1-heptanol, 1-octen-3-ol, 1-octanol), showed more consistent peak areas in the control. Moreover, also two furans were identified: 2-butylfuran and 2-pentylfuran, the first deriving from the

Table 1. Volatile oxidation compounds of corn flakes.

compounds	control		control + tocopherols		significance
	area[a]	SD	area[a]	SD	
aldehydes					
pentanal	12.02	*4.52*	4.81	*1.13*	
hexanal	124.16	*34.07*	63.13	*8.63*	*
2-hexenal	0.51	*0.17*	0.13	*0.02*	*
heptanal	3.01	*0.06*	1.65	*0.24*	**
2-heptenal	3.43	*0.34*	n.d.	-	***
benzaldehyde	0.32	*0.02*	0.25	*0.07*	
octanal	2.49	*0.28*	0.87	*0.27*	**
2-octenal	4.84	*0.36*	1.71	*0.60*	**
nonanal	8.27	*1.07*	5.23	*3.41*	
2-nonenal	0.48	*0.11*	0.25	*0.19*	
decanal	2.09	*0.24*	0.74	*0.50*	*
2,4-nonadienal	0.34	*0.48*	0.12	*0.05*	
2,4-decadienal	0.00	*0.00*	0.11	*0.06*	
	173.77	***54.15***	**79.26**	***7.15***	*
acids					
pentanoic acid	3.72	*1.10*	0.20	*0.13*	**
hexanoic acid	46.59	*0.45*	5.17	*3.64*	***
	50.31	***1.55***	**5.38**	***3.73***	***
lactones					
ã-hexalactone	2.78	*0.85*	0.56	*0.37*	*
ä-hexalactone	0.49	*0.21*	n.d.	-	*
ã-octalactone	0.40	*0.13*	n.d.	-	**
ã-nonalactone	0.29	*0.16*	0.13	*0.14*	
	3.97	***1.34***	**0.69**	***0.52***	*
ketones					
2-heptanone	1.56	*0.28*	0.41	*0.08*	**
3-octen-2-one	3.01	*0.04*	1.26	*0.30*	**
3,5-octadien-2-one	0.75	*0.10*	1.09	*0.42*	
	5.31	***0.41***	**2.76**	***0.75***	*
alcohols					
2,3-butanediol	5.74	*2.10*	7.96	*2.16*	
butanol	1.45	*0.73*	1.03	*0.10*	
1-pentanol	14.43	*1.07*	3.53	*0.93*	**
1-hexanol	0.32	*0.01*	0.09	*0.04*	**
1-heptanol	1.04	*0.20*	0.16	*0.28*	*
1-octen-3-ol	3.85	*1.90*	1.48	*0.24*	*
1-octanol	1.84	*0.23*	0.29	*0.30*	**
	28.66	***3.92***	**14.54**	***3.36***	*
furans					
2-butylfuran	0.36	*0.16*	0.07	*0.02*	*
2-pentylfurann	1.72	*0.34*	0.66	*0.41*	
	2.08	***0.19***	**0.73**	***0.42***	*
hydrocarbons					
pentane	0.70	*0.28*	0.52	*0.25*	
hexane	1.48	*0.03*	0.72	*0.30*	*
heptane	4.33	*3.12*	0.67	*0.86*	
octane	0.79	*0.19*	0.32	*0.06*	*
	7.29	***3.24***	**2.23**	***1.43***	
total area	**264.89**	***58.70***	**103.67**	***12.28***	*

[a] = integrated area (x 10^6)
*= $p < 0.05$; **= $p < 0.01$; ***= $p < 0.001$

oxidation of linolenic acid, the second from the oxidation of linoleic acid. For both 2-butylfuran and the sum of the areas of the two compounds, significantly greater values were detected (p <0.05) in the control than in the samples with tocopherol. Finally, with regard to the hydrocarbons, only octane showed a significant difference (p <0.05) between the integrated peak areas.

Numerous studies in literature point out the odor implication of the compounds under examination. Neff et al. (2000) indicated hexanal, nonanal, 2-nonenal among the compounds responsible of negative odors in heated trilinolein. Jeleń et al. (2000) observed that, among oils of different sensory quality, those with lower levels of aldehydes resulted preferable and considered, particularly, the aldehyde 2-heptenal correlated to the "oxidized" descriptor in vegetable oil. High levels of alcanals and alcanoic acids were reported also by Kinderlerer and Johnson (1992) as present in rancid hazelnut kernels. Hexanal, 2-nonenal and 1-octen-3-ol are, according to Ullrich and Grosch (1987), among the most potent aroma compounds in oxidized linoleic acid.

It is possible to conclude that the employment of natural tocopherols, in the amounts adopted for the present study, results effective to slow down the oxidation processes of low fat foods, and to limit the levels of off-odor compounds. Useful information are furnished regarding to the oxidation compounds of linoleic acid, especially hexanal, and the volatile compounds that originate from its further oxidation.

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STUDY OF MASS TRANSPORT PROPERTIES OF A MICROPOROUS BIODEGRADABLE FILM

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Key words: biodegradable, water sorption, barrier properties

Biodegradable materials obtained from natural or synthetic sources have attracted interest in recent years due to the fact that they can reduce environmental pollution by lowering solid disposal waste. Polycaprolactone is one of the synthetic polymers that meets the ASTM definition of biodegradability. It has actracted attention of researchers due to its lack of toxicity and low cost if compared to other biodegradable polyesters. Due to its high degree of crystallinity (about 50%), it is considered an hydrophobic material. Enhancement of hydrophilicity can been achieved by blending the polymer with suitable additives such as polyethyleneglycol. In this work a microporous PCL membrane was obtained by leaching out water-soluble polymeric additive, PEG, using a solvent-casting leaching method. The structure of the obtained membranes was studied; from SEM micrograph, it was shown that the pores were spherical and had identical size, in particular, the micropores obtained by leaching PEG 600 were larger than those obtained using PEG 4600.

As well known, permeability of gases (oxygen and carbon dioxide) and vapours (water and organic compounds) is a critical property of polymers in their application as food packaging materials. For many food product, the mass exchange must be restricted to guarantee their quality and safety. For many others, gas and vapor permeation is needed to maintain their physiological activities. The knowledge of the correlations between the structure and transport properties of polymer materials is of great relevance in order to be able to properly design new food packaging materials and to improve the packed food quality. In this work we studied the mass transport properties of the obtained microporous membrane and the results were compared with those ones of the non porous PCL film. Water vapour sorption kinetics and water sorption isotherm at 25°C were investigated using an electronic microbalance (Intelligent Gravimetric Analyser, Hiden). Water vapour transmition rate was measured at 25°C using a Mocon Permeabilimeter. It was possible to determine the dependence of permeability coefficient from the water activity at downstream side of the film, ranging from 0.35 to 0.90. A significant increase in water sorption and water permeability was revelead. As far as permeation of organic aromatic compounds is concerned, permeation of hexanal, trans-2, eptenal and octanal through the PCL membranes was studied by means of a CC/MS.

GAS BARRIER PROPERTIES OF POLYMERIC FILMS WITH HYBRID ORGANIC/INORGANIC COATINGS FOR FOOD PACKAGING APPLICATIONS

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ABSTRACT

The permeability and diffusivity of oxygen in films of PET coated with a thin layer of an hybrid organic/inorganic (ceramer) material have been determined at 65°C. The permeability data are collected using a closed-volume permeation apparatus and the diffusivity has been calculated with the time-lag method. The addition of coating dramatically improves the oxygen barrier properties of the substrate, the apparent permeability of the multilayer film being up to 3 orders of magnitude lower than that of pure PET. The pure coating properties are opportunely calculated by taking into account the effect of the different diffusion resistances in the various layers. The effect of ageing has also been investigated, by comparing the oxygen permeability before and after immersion in water of the films, that are suspected to be degraded by water.

Key words: ceramers, hybrid organic/inorganic materials, barrier properties

INTRODUCTION

In the last few years a new class of materials with nanophasic morphology, the ceramers, has been proposed and developed because of its peculiar and in-

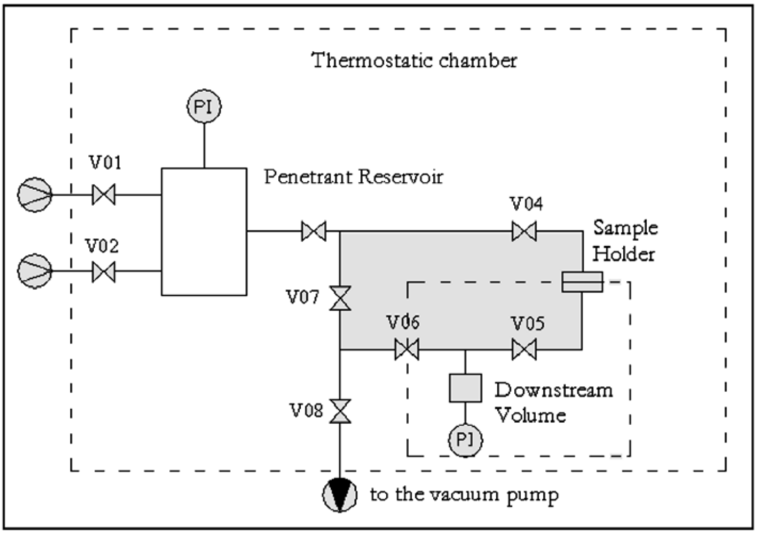

Fig. 1. Closed volume permeometer.

teresting features. Ceramers are hybrid materials composed of an organic and an inorganic phase, generally based on silica: thin films of these materials have been coated, in this work, on common polymeric films which serve as substrates. The aim of combining organic and inorganic compounds is to optimize the properties of the polymer composing the organic phase and those of the inorganic materials. In particular, the organic phase guarantees tenacity, flexibility and adhesion to the polymeric substrate of the final material and the addition of an inorganic phase can confer toughness, thermal and chemical resistance, resistance to flame and abrasion and, what is of interest in our case, barrier properties to gases which is a desired property in many food packaging applications (Schottner).

MATERIALS

The samples tested in this work were multilayer films in which a thin layer of ceramer coats a common food packaging material as PET. The organic-inorganic hybrid coating was prepared through sol-gel methodology and dip-coated on the substrate.

The samples were prepared in the framework of a National Italian Project PRIN whose main aim is to investigate the possibility of modifying the properties of polymeric packaging films, with particular regard to the gas barrier properties, through the addition of organic-inorganic hybrid coatings with nanocomposite structure.

Methods

Oxygen permeability in the films was investigated by using a closed-volume manometric apparatus, especially designed to characterize ultra barrier films (Fig. 1) (Pizzi et al. 2005). A constant pressure difference is maintained across the sides of the sample and the amount of mass permeated is calculated measuring the pressure increase in a closed volume of known dimensions at constant temperature. The value of the Oxygen Transfer Rate, *O.T.R*, which is defined in terms of the steady state gas flux, J_{SS}, the thickness of the membrane, l, and the pressure difference, Δp, is then calculated from the experimental data as:

$$O.T.R. \overset{DEF}{\equiv} \frac{J_{SS}}{\Delta p} = \left(\frac{dp_d}{dt}\right)_{t\to\infty} \cdot \frac{V}{R \cdot T \cdot A} \cdot \frac{1}{\Delta p} \qquad \text{Eq. 1}$$

where A is the area of the sample, V is the downstream volume, R is the universal gas constant, and p_d is the downstream pressure. The O.T.R. value in this work is expressed in $cm^3(STP)/(cm^2 \cdot d \cdot atm)$.

The characteristic time of the diffusion process can be evaluated using the time lag value (τ_l), that is the intercept on the t axis of the p_d vs. t curve after steady state, represented by a constant value of the slope, has been reached. If p_d is negligible compared to the upstream pressure, as it is the case of our experiments, and the film is initially gas-free, the time lag value is related to the diffusivity, D, of the gas in the material, through the following equation (Crank 1956):

$$\tau_L = \frac{l^2}{6 \cdot D} \qquad \text{Eq. 2}$$

The small size of the downstream volume and the high resolution of the manometer (full scale value 10 mbar, accuracy equal to 0.15% of the reading) allows for a high sensitivity of the apparatus.

The experiments on the multilayer films were carried out only at 65°C because, at room temperature, the flux was too small to be detected in reasonable time; the upstream pressure was maintained at about 4 bar while vacuum ($<10^{-2}$ mbar) was kept on the other side.

In order to estimate the permeability of each layer the series resistance formula was used:

$$\frac{l_{TOT}}{P_{TOT}} = \sum_i \frac{l_i}{P_i} \qquad \text{Eq. 3}$$

where l_i and P_i are the thickness and the permeability of layer i. The permeability P_i can be defined through the following relationship and the first part of Eq. 1:

$$P_i \equiv O.T.R._i \cdot l_i \qquad \text{Eq. 4}$$

It must be noticed that, when evaluated for multilayer materials, the values of P and D are effective quantities depending also on the sample geometry.

RESULTS

In Table 1, the oxygen transport properties in the materials studied are reported, in terms of oxygen transfer rate, time-lag, permeability and diffusivity, at 65°C. The data presented are the average values obtained from at least two permeation experiments, and the permeability of the hybrid coating has been calculated with eq.(3): the error bar on P_c represents the effect of a ±0.1 μm variation on the value of the coating thickness.

As one can see, the permeance of PET is reduced by more than 2 orders of magnitude after addition of the hybrid coating, that also allows to slow down remarkably the permeation process, the time-lag value of the coated material being almost 500 times higher than that of pure PET at 65°C. The efficient permeability of the multilayer material is less than 1/100 of that of pure PET: from Eq. 3 the permeability of the hybrid coating can be evaluated, leading to a value that is 3 orders of magnitude lower than that of pure PET. The effective diffusivity of the

Table 1. Transport properties of pure O_2 at 65°C.

65°C	O. T.R.×10^3 [cm^3(STP)/(cm^2 d atm)]	Permeability ×10^3 (Barrer [a])	Diffusivity ×10^9 (cm^2/s)	Time Lag (s)
PET (36 μm)	18.3	100	23	95
PET+ Hybrid coating (38 μm)	0.12	0.7	0.052	4.6 E+04
Hybrid coating (2 μm)		0.04±0.005		
PET+ Hybrid coating treated (liq.)	0.83	4.82	0.2	1.2 E+03
PET+ Hybrid coating treated (vap.)	0.77	4.46	-	-

[a] 1 Barrer= 10^{-10} (cm^3(STP)·cm)/(cm^2·s·cm Hg)

multilayer material, calculated with Eq. 2, is equal to 5.2×10^{-11} cm^2/s, while that of pure PET is 2.3×10^{-8} cm^2/s.

Due to the hydrophilic nature of the organic phase of the hybrid, exposition to humidity or water can degrade the material: the effect of ageing on the gas transport properties was therefore studied, by comparing the transport properties of the samples before and after immersion in both liquid and saturated vapour of water for 4 days. The data for the treated materials are also listed in Table 1: the oxygen permeability increases, but not dramatically, after immersion in liquid water, and to a lower extent when the sample is immersed in water vapor. The effective diffusivity, that could be evaluated only for the sample treated in liquid water, also shows an increased value with respect to the untreated material, but is still ten times lower than that of pure PET. The results show that, as it could be expected, the materials loose part of their barrier capacity after treatment in water, in particular when they are kept in liquid water. However, the transport behavior, especially if evaluated in terms of permeability, is still excellent for packaging applications: the satisfactory stability of the hybrid coating with respect to water is believed to be due to presence of the inorganic phase that hinders the mobility of the hydrophilic polymeric chains.

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LEMON EXTRACT AS PRESERVATIVE FOR MALOLACTIC FERMENTATION

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ABSTRACT

The antimicrobial effect of lemon extract on the growth of *Oenococcus oeni* and *Lactobacillus plantarum*, as malolactic bacteria, was assessed. Growth tests were run at 30°C using laboratory media. Carbon dioxide concentration in the vial headspace was used as metabolic activity index of the investigated microorganisms. For each of the investigated microorganisms, Minimum Inhibition Concentration (MIC) and Non Inhibiting Concentration (NIC) were calculated. Results suggest that lemon extract is active on the growth cycle of both investigated two malolactic bacteria.

Key words: antimicrobial properties, lemon extract, malolactic fermentation, natural compound

INTRODUCTION

In recent years the interest in natural compounds as alternatives to food additives has notably increased (Belletti et al. 2004). Oilseeds and spices have been used either to enhance the aroma of foods, to increase their shelf-life (Dorman and Deans 2000). Rosemary oil and thymol are some of the most studied active agents with good antimicrobial properties, due to their high content in phenol derivatives (Lanciotti et al. 2004; Olasupo et al. 2003). Little works has been published on the effects of natural compounds on growth of malolactic bacteria. The malolactic fermentation (MLF) is generally desirable for some dry white wines but it is not favourable for all wines; in fact, it is not recommended for sweeter wines because it affects both flavour and biological stability of the product (Versari et al. 1999). It is generally accepted that MLF is sensitive to SO_2, ethanol concentration, residual glucose, short chain fatty acids, lysozyme, biotin and thiamine contents, phenol compounds, presence of bacteriophages, some yeast strains, low temperatures and low pH values (Vivas et al. 1997). The effects of several wine-associated phenol acids, as well as the influence of acid pH, lyophilisation, temperature and ethanol concentrations on the growth and viability of malolactic bacteria were investigated (G-Alegria et al. 2004; Campos et al. 2003). In this work the effectiveness of an active agent produced by citrus fruits, lemon extract, against *Oenococcus oeni* and *Lactobacillus plantarum*, as responsible bacteria for MLF, was studied.

MATERIALS AND METHODS

The selected strains of *Lactobacillus plantarum* and *Oenococcus oeni* were purchased from DSMZ (Germany), revitalized in specified media and stored a 4°C as stock cultures.

The malolactic activity of each strain was measured by valuating the increase over time of the CO_2 (%) in the headspace of vials containing the microorganism (Guerzoni et al. 1992) by using a detector analyser (Dansensor, PBI). Pure lemon extract, kindly provided by Spencer food (Industrial bv, Amsterdam) was added in different amounts (0 ppm, 100 ppm, 200 ppm, 300 ppm and 500 ppm) in vials containing 9.9 ml of medium broth deprived of glucose and supplied with L-malic acid. The medium in the vials was separately inoculated with about 10^5 CFU/ml of each malolactic bacterium, obtained from decimal dilutions of 48 hours cultures of the two strains an then incubated at 30°C.

The experimental data (i.e., the carbon dioxide concentration in the vial headspace during time) were fitted by a modified version of a Gompertz equation and the value of the integral curve was calculated after each fitting. The time necessary to reach the stationary phase was set to 283 hours, for both tested microorganisms. The obtained values of the area for each CO_2 curve were used to calculate growth index (GI). A rearranged form of an exponential decay model (Lambert et al. 2001; Lambert and Pearson, 2000) was fitted to all GI_{283} data as function of Log of lemon concentrations and the inhibition profile was obtained for each microorganism. Growth and no-growth status of microbial suspensions containing lemon extract were expressed as NIC, representing a threshold value under which no effect on microbial growth can be observed, and MIC, indicating a threshold concentration over which the active compound exerts complete inhibition.

RESULTS AND DISCUSSION

With the aim of evaluating the potential biostatic or biocidal activity of lemon extract on the investigated microorganisms, kinetics of their metabolic activity and dose-response profiles were obtained and analyzed. Carbon dioxide concentration in the vial headspace was monitored during time as an index of microbial activity. The curves relative to this evolution over time, for both investigated microorganisms, at different concentrations of lemon extract, were reported in Figure 1. As expected, the gas concentration increased with time following a sigmoidal trend and at a fixed contact time, the carbon dioxide was a decreasing function of the

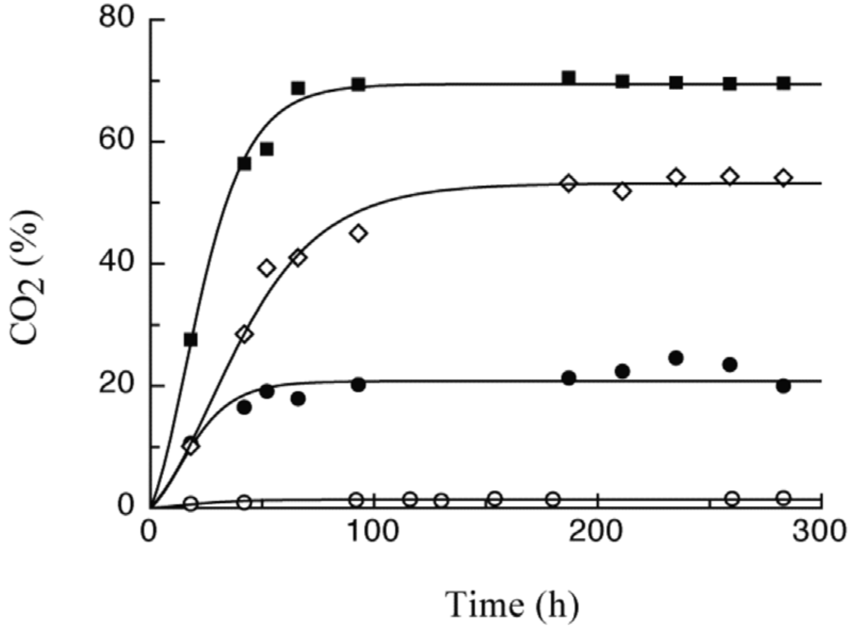

Fig. 1. CO_2 kinetics (%) produced by both microorganisms at different concentrations of lemon extract. The curves are the fitting to the experimental data. (■) CO_2 (%) produced by *L. plantarum* without lemon extract; (●) CO_2 (%) produced by *L. plantarum* with 200 ppm of lemon extract; (◇) CO_2 (%) produced by *O. oeni* without lemon extract; (○) CO_2 (%) produced by *O. oeni* with 300 ppm of lemon extract.

lemon extract concentration. The obtained fitting parameters were used only to compare sensitivity to lemon extract of the selected bacteria, grown under optimal conditions. After each fitting the integral curve was calculated and the related area values were used to obtain the GI_{283} data. By fitting these new data, as function of Log of lemon concentrations, two dose-response curves were obtained, one for each bacterium. The two inhibition profiles were reported in Figure 2. Growing and no-growing status were individuated by calculating both NIC and MIC param-

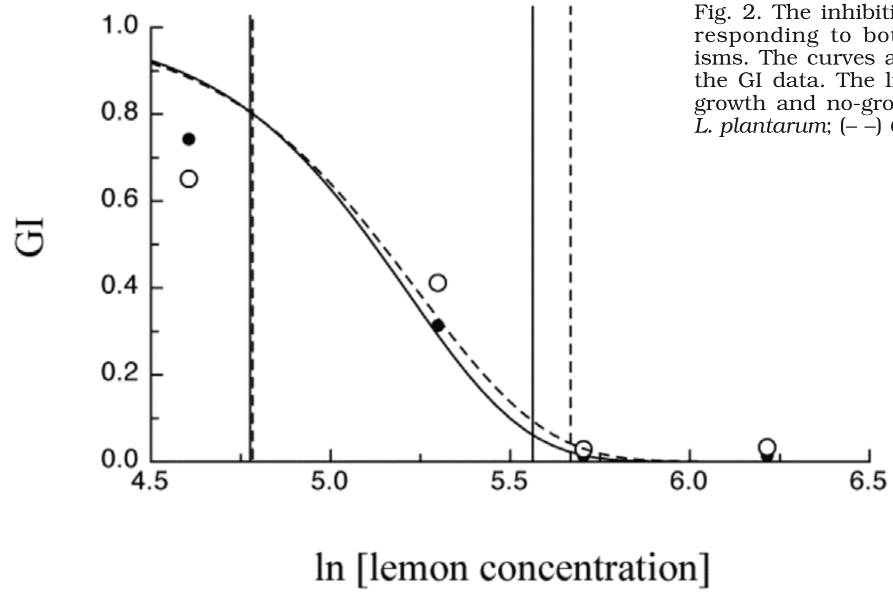

Fig. 2. The inhibition profiles corresponding to both microorganisms. The curves are the fitting to the GI data. The lines delimit the growth and no-growth regions. (–) *L. plantarum*; (– –) *O. oeni*.

eters. For the sake of clarity, each dose-response curve has been subdivided in three primary regions: the first region, lying below the NIC value, which defines a range of lemon extract concentration not significantly affecting microbial activity, the intermediate region bounded by NIC and MIC values, and a third region above the MIC that defines no-growth region. As can be inferred from the figure the susceptibility of both malolactic bacteria to lemon extract was non-linearly related to the active compound concentration and as a consequence, the microbial activity was not detectable within the third region of the inhibition profile. Results on the antimicrobial effects of the investigated natural extract are discussed in terms of NIC and MIC, as expression, respectively, of the sensitivity and the resistance to the active agent. Both microorganisms showed a NIC value of about 112 ppm. On the contrary, the MIC value for *O. oeni* was 273.46 while for *L. plantarum* was equal to 258.17. This finding demonstrated that lower MIC level do not necessarily correspond to lower NIC level. Thus, a new parameter [MIC-NIC] was used to valuate the different microbial susceptibility to lemon extract. As can be inferred from the two new calculated values, *L. plantarum,* with 145.6 ppm, was more susceptible to lemon extract than *O. oeni* with a value equal to 161.5 ppm.

On the basis of the obtained results, the lemon extract represents a promising antimicrobial agent to prevent malolactic fermentation. Because type of growth medium, incubation temperature, and strain variability could affect sensitivity to the active compound, it would be of interest to study and prove the antimicrobial effectiveness of the extract in wine, by simulating the real process conditions.

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EVALUATION OF INTOLERANCE TO FOOD PRESERVATIVES BY NON-INVASIVE BIOPHYSICAL TECHNOLOGIES

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ABSTRACT

Food preservation represents one of the main interests of food science, and various synthetic and natural compounds are available nowadays for food industry. A high concern exists among consumers towards the toxicity of food additives and preservatives, in particular, however the role that some of these compounds can have in causing food intolerance to consumers should be underlined.

In this work we evaluated the feasibility of using an unconventional diagnostic procedure based on bioelectrical skin responses referred to as Electro-Acupuncture Diagnostics according to Voll (EAV) based on the variations of conductivity or cutaneous electric potential in correspondance with the points of acupuncture meridians and due to the interaction between the electromagnetic waves, spontaneously emitted by certain substances, and the human organism. This technique could provide a fast, easy, non invasive and sensitive evaluation of the irritating potential of food preservatives. Four of the most commonly used preservatives in food industry (EDTA, lactoferrine, lisozyme and nisin) were assessed on 20 healthy volunteers. This preliminar study pointed out that the tested preservatives can cause intolerance in sensitive individuals, but further studies are needed to consider the effect of concentration of the pure substances, and of food containing such preservatives on determining intolerance.

Key words: intolerance disease, food preservatives, non-invasive analytical techniques, EAV

INTRODUCTION

Food preservation represents one of the main interests of food science, and various synthetic and natural compounds are available nowadays for food industry. A high concern exists among consumers towards the toxicity of food additives and preservatives, most of which are obtained through chemical synthesis. Therefore, much interest has been focused in the last years on natural preservatives, such as lysozyme, lactoferrin and nisin (often coupled with EDTA to enhance its antimicrobial effect), used with the aim of extending the shelf life of various products, which are thought to be healthier: however the role that some of these compounds can have in causing food intolerance to consumers has not been studied yet.The last decades have been characterized by an exponential progress of knowledges in the scientific field, which have undisclosed the extremely complex nature of the living systems. Such advances allow to understand that at the basis of the cellular transmission there is not only a biochemical message, but also an electromagnetic one. Many unconventional diagnostic procedures based on bioelectrical skin responses are widely used for allergic diseases. The perturbation of the skin electrical response is evoked by a process of bioresonance (Puzzo *et al.*, 2001). According to Voll (1975) the electromagnetic frequencies typical of test substances can be sent to the patient via cables and their effects on the organism can be determined by means of Electro-Acupuncture Diagnostics according to Voll (EAV). Previous studies showed that the EAV acupuncture technique, based on bioelectrical skin responses, and in particular on the variations of conductivity or cutaneous electric potential (in correspondance with the points of acupuncture meridians) due to the interaction between the electromagnetic waves, spontaneously emitted by certain substances, and the human organism, could be regarded as a useful test to evaluate food allergy (Puzzo *et al.*, 2001, Tsuei *et al.*, 1999). Since this diagnostic technique is fast, easy to perform and non-invasive, it could provide an alternative method to patch test and prick test to evaluate the allergenic potential of several substances. Previous studies have pointed out a correspondance >76% between EAV and the prick test (Figure 2), moreover, a further validation of data was obtained by the comparison between diagnostic data obtained with EAV and with Patch test (Figure 3) in the essay of the intolerance to some basic components used in the preparation of cosmetic products. The aim of the present study was to evaluate the feasibility of using Electro-Acupuncture Diagnostics according to Voll (EAV) to test intolerance to some natural food preservatives.

MATERIALS & METHODS

EDTA, lysozyme and nisin were purchased from Sigma-Aldrich (Milan, Italy), lactoferrin was kindly supplied by Glanbia Nutritionals (Monroe, WI, USA).

Subjects

EAV tests were performed on 20 healthy volunteers of both sexes in the age range 18-60. The participants did not suffer from any ailment and were not on any medication at the time of the study. The volunteers were fully informed of the nature of the study and the procedure involved. They were rested for 15 min prior to the tests and room conditions were set at 22 ± 2°C and 40% relative humidity.

EAV tests

EAV bioresonance tests were performed using a Bicom 2000 (Brugermann GmbH, Germany, agent for Italy Medisoft Italia, Milan) equipped with an electrodermal screening device (EDS) (Figure 1). This instrument is basically a galvanometer that measures current variations of acupuncture points and has been used to indicate the energetic state of meridians for the diagnosis and the treatment of body disorders. The device Bicom measures the skin conductivity at acupuncture points via two electrodes. One electrode is a brass cylinder with a large surface and has to be kept by the subject in his hand; the other electrode (stylus) has a small contact area and delivers a direct current (approximately 1 V), by applying a slight pressure at chosen skin meridian points. The body impedance between the skin point and the hand electrode is then measured: the value is shown on an analogical device with an arbitrary scale reading from 0 to 100 units. The operator was an experienced acupuncturist physician. As there is a great variability of skin conductivity among individuals due to a number of factors (skin thickness, humidity, blood flux), a baseline level of resistance was determined for each subject by placing a brass electrode in each hand. The subject was considered “in balance” if the instrument readings were at approximately 80% of the full scale. Subjects with readings below 70% and above 90% were disqualified from the study. Then, the level of conductivity of the terminal point of the meridian selected was measured in the absence of the test compounds, and the value was taken as the reference baseline value. A vial containing the preservative to be tested (Figure 3) was put in a metal vial-holder electrically connected to the hand electrode via Bicom device. Skin conductivity was then measured for each

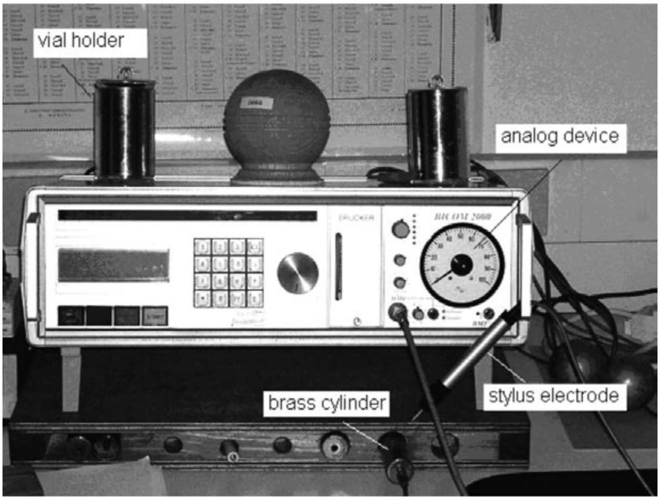

Fig. 1. The Bicom device.

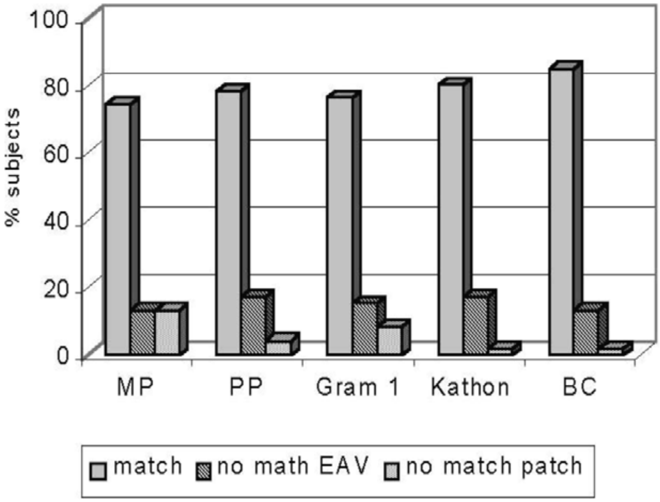

Fig. 2. Validation of EAV test against Patch test. Percentages of subjects showing the same results using patch and EAV tests (match) or a positive response only to patch test (no match patch) or a positive response only to EAV test (no match EAV) for each cosmetic preservative assayed.

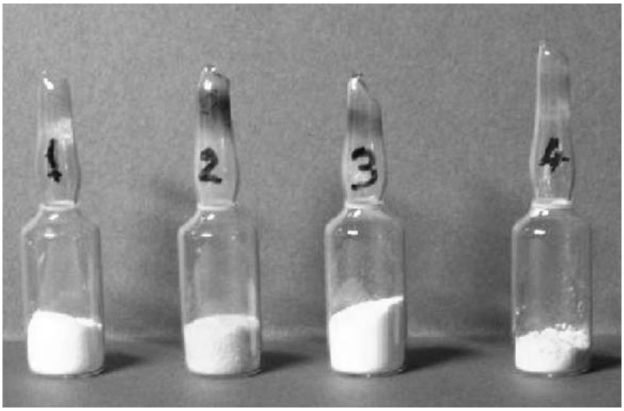

Fig. 3. Vials containing the substances to be tested.

of the substances placed in the vial-holder. A positive reaction to the compound being tested was recorded as a decrease from the baseline value. The same concentrations of preservatives used for the patch test were analysed.

RESULTS AND DISCUSSION

Validation of the method

The validation of the EAV method against the usual diagnostic method gave a statistical correspondance of 75 % between the evaluation with the bioresonance test and data obtained with the prick test (considering positive and negative results). Moreover, in 80% of the cases positive results obtained with the prick test were confirmed by the EAV test.

The comparison between the individual reactions observed using the patch test and the EAV technique showed that at least 70% of the responses matched for all the preservatives tested (**Figure 2**). Similar findings have been reported by Tsuei at al. (1999) comparing the EAV technique results with those obtained by routinely laboratory tests for the diagnosis of food allergy.

EAV test with food additives

This preliminar study pointed out that the tested preservatives can cause intolerance in sensitive individuals. The test pointed out an incidence of 47% for EDTA, and 41% for lysozyme, nisine and lactoferrin. Such high percentages are probably due to the fact that the substances were tested as received (powder). It is likely that the use of diluted samples would determine lower percentages. Future studies will consider the effect of dilution on determining intolerance in tested subjects. Moreover, it will be interesting to test the same substances at the concentration of use in foods, and to test foods containing such additives. The determination of a threshold or a minimum concentration for the onset of intolerance could help food scientists and the food industry to consider the combinate use of different preservatives at lower concentrations instead of fewer or single additives at potentially harmful concentrations.

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EFFECT OF TWO DIFFERENT HUMECTANT INGREDIENTS ON QUALITY OF “AMARETTI” COOKIES

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ABSTRACT

“Amaretti”, typical Sardinian cookies, have a shelf-life strictly limited by a qualitative decay due to severe hardening of the internal almond paste. In this work, the “Amaretti” were manufactured with two different recipes in addition to the original one, adding glucose syrup and bamboo fibre. To evaluate the effect of these ingredients on the quality of the cookies, a_w and moisture content (wet basis) were measured, sorption isotherms were determined and texture and colour evolution were monitored. The new ingredients act in a different way on the “Amaretti” texture and water distribution; although glucose syrup lowers the initial a_w of the “Amaretti” and then reduces the a_w gradient between crust and paste, it leads to significant modifications that affect the typicality of this regional product. In fact, it promotes both the sugar crystallization after about 21 days and the water redistribution between the components of the matrix, resulting in a softening of the crust, well perceptible after 3-4 days of storage. The textural analysis showed that the crust lost its crispness, due to water migration from the paste to the crust (due to the a_w gradient) and to the amorphous hygroscopic sugars . Finally, the high amount of sugars caused a more intense Maillard reaction, as seen by the colour analysis. The addition of the fibre led to an absolutely positive global effect, promoting the water retention into the matrix and maintaining the textural difference between crust and paste. The small difference in colour can be easily solved by making a small change in the time-temperature conditions during baking.

Key words: hardening, humectant, texture, cookies, shelf-life

INTRODUCTION

In this work, we considered Sardinian “Amaretti”, regional cookies that are well-known in Italy for their typical characteristics (soft internal almond-paste and an external hard and dry crust). They are characterised by a moisture content (wet basis) of ~13-14% for the internal paste and of ~ 5-6% for the external crust; the a_w values are ~ 0.74 and ~ 0.40, respectively.

After a few days (four to ten days), a qualitative decay begins due to a severe hardening of the internal almond paste that strictly limits the shelf-life of these cookies. Such a short shelf-life does not permit their marketing in countries far from their traditional manufacturing site. As reported in previous works (Piga *et al.,* 2005; Labuza *et al.,* 1998), this hardening could be due both to the redistribution of water (that leads to the sugar crystallization) and loss of water into the surrounding environment. So, one of the most important tasks is to reduce the a_w gradient between the different components (crust and paste) by decreasing the rate of hardening. The purpose of this research was to test the effects of two different water binding ingredients on the control of moisture loss: glucose syrup and bamboo fibre.

MATERIALS AND METHODS

The manufacturing of the “Amaretti”. “Amaretti” cookies were made in the Department pilot plan, using traditional ingredients in the following amounts: sweet almonds (650 g), sucrose (650 g), egg white (255 g), bitter almond aroma (5 ml). To test the effects of the different humectant agents selected, the “Amaretti” were also produced adding 30 g (~ 2% $^w/_w$) of glucose syrup (D. E.= 47) and 15 g (~ 0,95 % $^w/_w$) of bamboo fibre before starting the mixing. From now on, the three different typologies of “Amaretti” will be indicated as follows: T= traditional; S= syrup; F= fibre.

Storage of the “Amaretti”. For each trial the “Amaretti” were divided in three lots: one for the sorption isotherms determination, one for the textural analysis and the third for the a_w, moisture content (wet basis) and colour evaluation. Cookies were then stored under controlled T-RH conditions (25 °C and 40% RH) for ten days and analysed after 1, 2, 4, 6, and 10 days.

Water activity and moisture content determination. Water activity was measured on three “Amaretti” at a time by an electronic hygrometer previously calibrated with a standard of LiCl of known activity. Gravimetric analysis was performed in triplicate to determine water content (% H_2O on wet basis) on the whole cookie and on the internal paste using an oven at 130 °C for 90 min.

Sorption isotherms. Sorption isotherms determination was performed according to the procedures reported in the COST project - European Cooperation in the Field of Scientific and Technical Research, and the principles described by Labuza (Labuza *et al.,* 2000). Whole samples in triplicate were placed in sealed glass jars containing saturated salt slurries in the a_w range of 0.11 to 0.81 and were stored in an oven at 25 °C.

Texture analysis. Hardness was evaluated in the freshly baked “Amaretti” and after 2, 4, 6 and 10 days using a food texture analyser. Textural determination was made on 15 “Amaretti” per each time of storage by using a 4 mm diameter cylinder probe for a puncturing test (Bourne, 1979).

Colour analysis. The colour of the "Amaretti" was measured using a reflection colorimeter (MINOLTA Chroma Meter mod. CR 210, Osaka, Japan). Cookies were placed on a white standard plate (L*=100) and the CIE L*a*b* coordinates were measured, using D65 illuminant/10° observer (Schoefs, 2002).

Table 1. Moisture content (%) and aw values of the three different recipes of freshly baked "Amaretti".

Moisture content	T		S		F	
	Paste	Paste + crust	Paste	Paste + crust	Paste	Paste + crust
(%)	12.82 (±0.36)	7.56 (±0.09)	15.61 (±0.386)	8.81 (±0.45)	12.90 (±0.44)	8.22 (±0.62)
aw	0.74 (±0.01)	0,57 (±0.01)	0.72 (±0.01)	0.55 (±0.01)	0.70 (±0.01)	0,54 (±0.01)

Sensory evaluation. A sensory evaluation was carried out to check the texture evolution during the storage time and the difference between recipes. In particular, a descriptive test was used in which nine trained panellists described the evolution of the cookies through four parameters that cannot be described by instrumental analysis: resilience, external crispness, internal stickiness and fibre perception. The panellists attributed a value of between 1 (minimum) and 9 (maximum) for each of these parameters.

Statistical analysis. The data were subjected to a one-way analysis of variance (ANOVA). The data were grouped by lots (the three different recipes: T, S and F) to assess the differences for each recipe. The mean values, when appropriate, were separated by LSD's multiple range test at P<0,01.

RESULTS AND CONCLUSION

Moisture content and water activity changes. As far as a_w and moisture content values of the internal paste (Tab. 1) are concerned, the syrup led to a higher level of moisture content in comparison with T and F, whereas both the syrup and the fibre reduced the a_w value of the internal paste. With regard to the evolution of the above-mentioned parameters (Fig. 1), at the last day of storage the loss of moisture from the internal paste was particularly evident in T (61.2% fall), less in S and F (49.3% and 49.7%, respectively). The same behaviour was observed for the a_w values. In fact, all three types of "Amaretti" underwent a progressive decrease in a_w, but it was severer in T than in the other two.

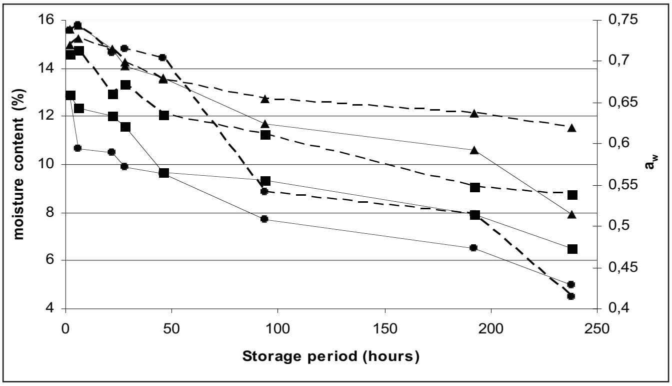

Fig. 1. Change of moisture content (%) and a_w in the three different recipes of "Amaretti".
Data are the means of three determinations.
Legend: - - - - a_w; —— moisture content (%); ● T ; ▲ S ; ■ F.

Sorption isotherms. Fig. 2

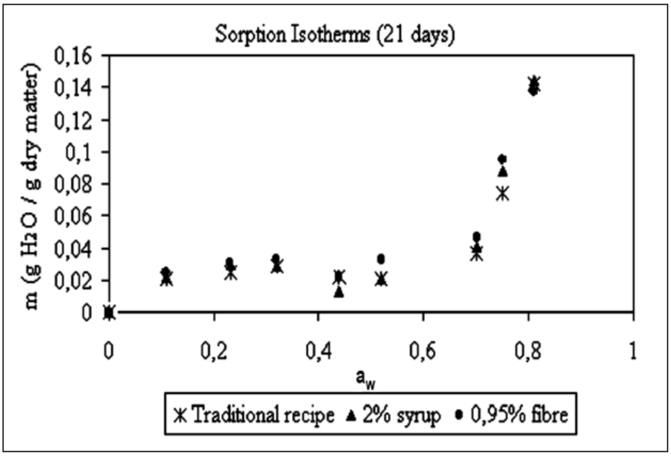

Fig. 2. Experimental sorption isotherm curves for the three different recipes.

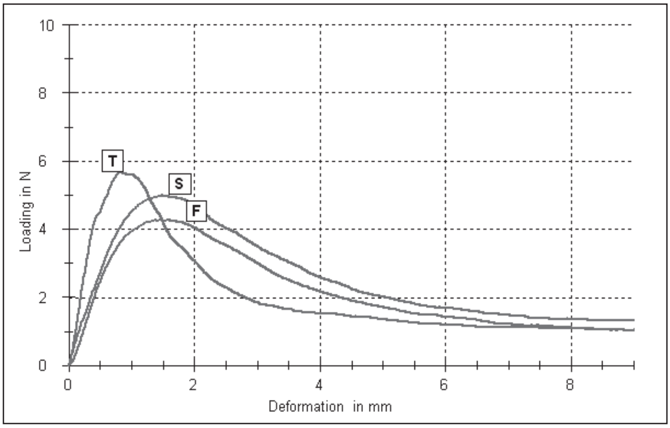

Fig. 3. Three mean curves of freshly baked "Amaretti".

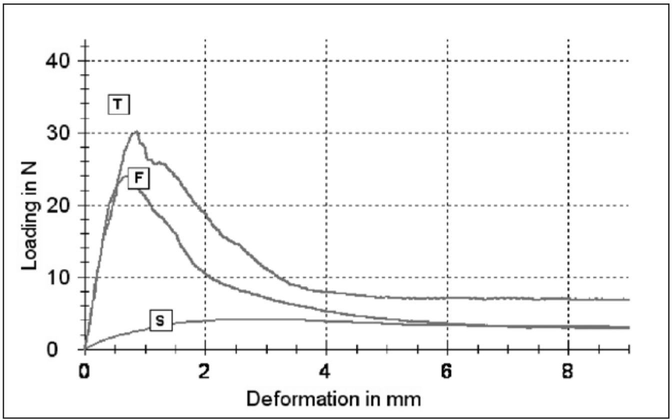

Fig. 4. Three mean curves of "Amaretti" after 2 days of storage.

shows the sorption isotherm curves of the three different recipes of freshly baked "Amaretti" after an equilibrium time of 3 weeks at 25°C. For the first three a_w values there is no statistically significant difference. At 0.44 a_w the difference is between S on the one hand and T and F on the other. At an ERH of 52% a significant difference is observed between S and T on the one hand and F on the other. At an ERH of 70% the difference is between S-T and F, whereas at a_w of 0.75 there is a difference between T on the one hand and S and F on the other. At the higher a_w value there is no statistically significant difference between lots, i.e. the "Amaretti" very easily take up moisture from the surrounding air (Labuza *et al.*, 1979).

Texture evolution. The profile of the three mean curves of freshly baked "Amaretti" is similar (Fig. 3), especially in the final tract: the internal paste had the same consistency, due to the same amount of moisture. The global hardness is also almost the same (see the area under the curves and the corresponding W_{tot} values in Tab. 2).

After 2 days of storage (Fig. 4), the curve relative to S exhibits a shape flatter than before; moreover, the initial peak due to the penetration of the crust is now absent. Finally, the crust lost its crispness as can be seen from the maximum force and Young's modulus values (Tab. 2). The F curve is similar to that of

Table 2. Parameters of texture analysis of freshly baked "Amaretti" and after storage. Legend: x= mean; s= st. dev.

Day of analysis	T						S						F					
	E_{mod} (Nmm^{-1})		F_{max} (N)		W_{tot} (Nmm)		E_{mod} (Nmm^{-1})		F_{max} (N)		W_{tot} (Nmm)		E_{mod} (Nmm^{-1})		F_{max} (N)		W_{tot} (Nmm)	
	x	s	x	s	x	s	x	s	x	s	x	s	x	s	x	s	x	s
0	21.54	6.35	6.01	0.70	17.18	2.88	7.85	1.15	5.18	0.35	21.60	3.34	8.98	2.89	4.49	0.28	18.41	2.60
2	97.96	46.18	42.90	11.07	94.74	19.13	4.94	2.01	4.20	0.52	26.32	3.49	122.74	21.10	25.46	2.34	62.19	10.87
4	202.83	151.61	80.19	10.76	445.07	91.01	8.42	2.34	5.22	1.14	32.75	5.08	33.22	8.72	25.12	3.32	132.05	22.34
6	283.10	160.14	121.01	20.06	666.48	133.00	33.22	6.65	22.29	2.60	98.05	14.80	226.28	28.83	86.16	6.03	286.58	47.91
10	368.47	207.33	139.89	28.88	586.01	162.29	90.32	51.75	39.82	11.10	131.86	36.03	136.45	76.73	100.27	8.33	499.09	68.09

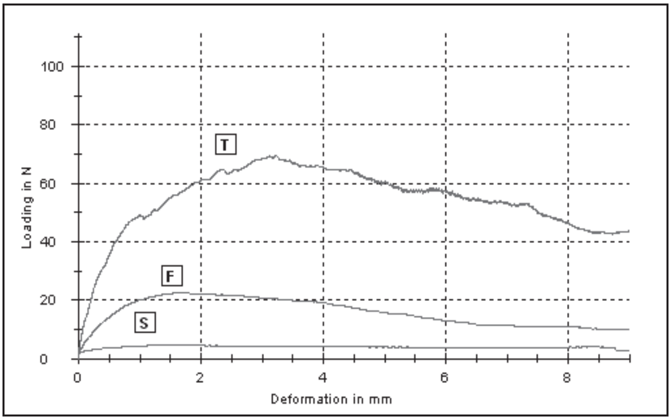

Fig. 5. Three mean curves of "Amaretti" after 4 days of storage.

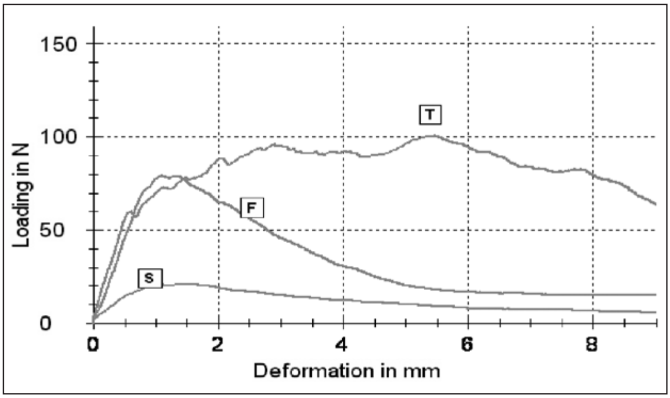

Fig. 6. Three mean curves of "Amaretti" after 6 days of storage.

S in the final part, but very different in the first; in fact, the F "Amaretti" had a hard and crumbly crust still differentiated from the internal soft paste. The T cookies had a crust as stiff as F (similar Young's modulus), but harder (higher maximum force). Moreover, the descending tract of the T curve is slightly shifted over the other two, indicating that, already after two days, the internal paste of T lost a little more amount of moisture. The above trend becomes more evident after 4 days (Fig. 5).

In the sixth day of analysis (Fig. 6) the hardening began to affect S too, when the moisture loss exceeded the moisture redistribution between components. The original difference in texture between paste and crust was tested in F, where the effect of the fibre was well appreciable. However, these "Amaretti" are harder than in the previous analysis as we can deduce observing the value of the area under

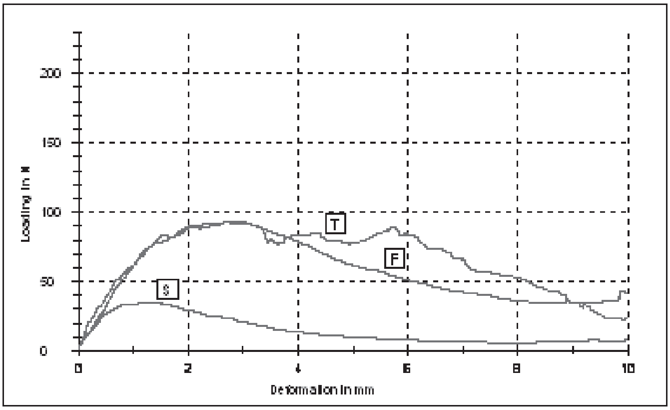

Fig. 7a. Three mean curves of "Amaretti" after 10 days of storage.

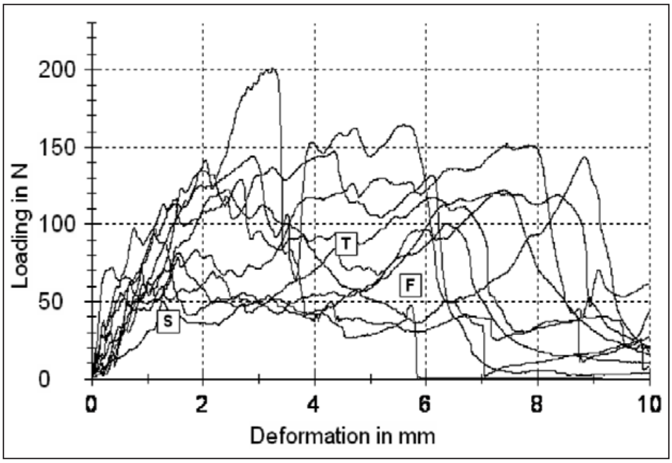

Fig. 7b. Curves of traditional "Amaretti" after 10 days of storage; the "breakage-zones" are well evident.

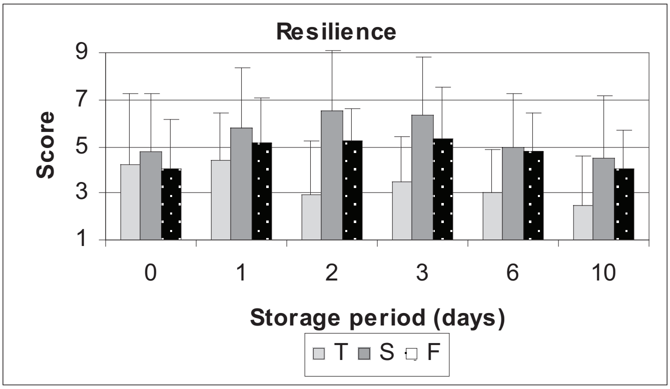

Fig. 8. Sensorial analysis: resilience evolution during 10 days of storage.

the curve (Tab. 2). In the last day (Fig. 7a and 7b), only T "Amaretti" broke into a number of pieces during the test; in fact, in the other two, water is still present into the matrix. The different texture between paste and crust is still appreciable both in S and in F, even though the global hardness is increased in F (value of maximum force under the curve), whereas S exhibited a less hard and stiff crust, as can be seen from the maximum force and Young's modulus values (Tab. 2).

Colour analysis. The results reported in Tab. 3 show the highest value of *a* parameter and the lowest *L* value for S "Amaretti", that indicate the hue of redness/brownness and a global greater darkness of these cookies, respectively. With regard to the F lot, it exhibited the highest *L* value and the lowest *a* and *b* values, due to the effect of the fibre in the lowering of the a_w value of the matrix and, as a consequence, in the slowing down of the non-enzymatic browning reaction.

Sensory evaluation. With regard to resilience (Fig. 8), the panellists confirmed that the "Amaretti" with glucose syrup underwent a water redistribution between components (paste and crust, in this case) that led to a soggy and elastic crust up to the sixth day of storage. In F, the perceived low resilience of the crust can be attributed more to a less baked crust (due to the presence of fibre) than to water redistribution. In T, a low resilience was perceived

Table 3. CIE Lab parameters for the three different recipes. The standard deviations are reported in brackets.

CIE Lab parameters	T	S	F
L	+73.72 (±1.30)	+53.08 (±2.32)	+78.35 (±0.07)
a	+5.05 (±0.13)	+15.54 (±0.50)	+2.79 (±0.01)
b	+27.28 (±0.58)	+26.85 (±2.29)	+23.49 (±0.03)

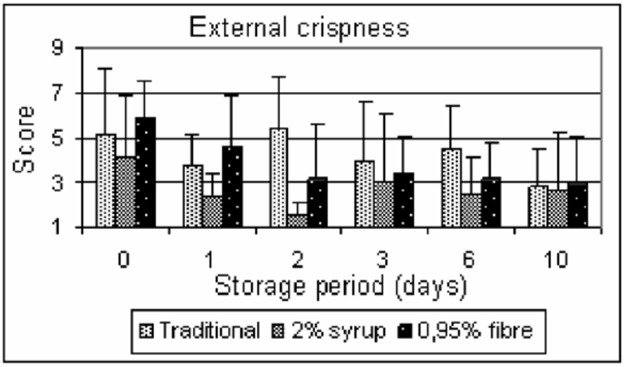

Fig. 9. Sensorial analysis: external crispness evolution during 10 days of storage.

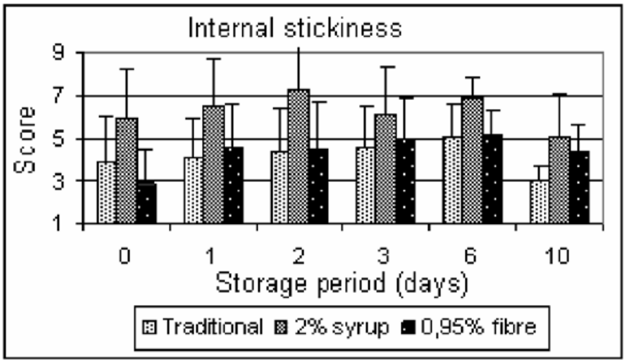

Fig. 10. Sensorial analysis: internal stickiness evolution during 10 days of storage.

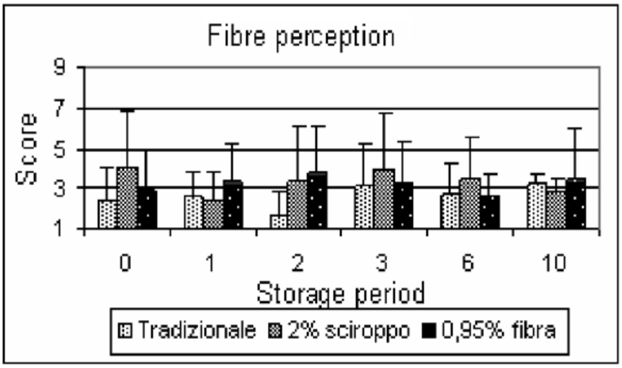

Fig. 11. Sensorial analysis: fibre perception evolution during 10 days of storage.

in the first few days, that disappears after three days due to a quick hardening of the crust. As regards the external crispness (Fig.9), the lowest scores are for S. When the crust began to pick up water, it became less crisp (particularly evident in the third day of analysis), leading to a quality decay of the “Amaretto”, in which crispness or crunchy texture is an essential characteristic influencing consumer acceptance. This parameter was perceived longer in F and T. With regard to internal stickiness (Fig.10), it is interesting to note that the panellists perceived S as sticky, more than the other two lots. Finally, with regard to fibre perception (Fig.11), the panellists were not able to distinguish the presence of fibre among the different lots of “Amaretti”. This aspect is very important because the addition of the fibre did not lead to a change in the original characteristics of the internal paste of the cookies.

In this work, two different ingredients of the traditional recipe of “Amaretti” cookies with known humectant properties were tested, but our study showed that they act differently on the particular product taken into consideration. Although the glucose syrup lowered the initial a_w of the “Amaretti” and reduced the a_w gradient between paste and crust, this led to such a severe modification of the original properties that it affected the typicality of this regional product.

So, only fibre could be added to the traditional recipe, thanks to its capacity of holding water, limiting the crystallization of sugars and maintaining the textural difference between crust and paste. The addition of the humectant ingredients could represent a suitable tool for controlling moisture loss (and extend the shelf-life) in high and intermediate-moisture foods. However, in order to gain better results, it would be preferable to consider it not as a separate means, but combined to others such as suitable packaging solution.

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SESSION III

Shelf-life testing

Chairmen:

Dario Dainelli, Cryovac Sealed-Air, Passirana di Rho, Italy
Nico de Kruijf, TNO, Nutrition and Food Research, Zeist, The Netherlands

INNOVATIVE AND INTEGRATIVE APPROACHES APPLIED TO SHELF-LIFE TESTING AND IMPROVEMENT OF PACKAGED FOODS

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Key words: quality tracers, transfer, reactions, integrative approach

THE EFFECT OF LIGHT OF DIFFERENT WAVELENGTH ON PHOTO-OXIDATION IN ICE CREAM

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Keywords: light, photo-oxidation, ice cream, fluorescence spectroscopy

The use of transparent plastic boxes is becoming more and more common also in the ice cream industry. The purpose of this investigation was to reveal the sensitivity/stability of vanilla ice cream towards light of different wavelengths and to elucidate whether packages with different colour could protect the ice cream against light and still be transparent. Ice cream manufacturing consists of incorporation of gas phase into an emulsion pre-mix before freezing, which represent more than 50% (v/v) air, and is therefore very sensitive to light. Vanilla ice cream was commercially packed in specially made 2 L injection moulded PP tubs and injection-moulded black PP Snap-On lids. During exposure by flat-emitting HMI studio lamps, the black lids were replaced by studio-filters with well-defined transmission-curves. All filters were balanced with neutral (grey) filters and transparent PP-lids to transmit the same amount of light energy (W/m^2) in the 330-800 nm region. Except for one of the clear lid samples, all filters were supplemented with an UV filter. After 24 hours of light exposure the quality was assessed by fluorescence spectroscopy, by measurement of colour, and by a trained sensory panel.

The fluorescence spectra showed two peaks in the 410-500 nm and 500-600 nm region. The peak at 530 nm was the highest, and was due to the riboflavin in the cream. It is generally known that riboflavin in dairy products is degraded when exposed to light and it absorbs light in the UV region and up to about 500 nm. There was a clear decrease of the riboflavin peak for violet, blue and white light with and without UV filter. Red, orange and yellow light had little or no influence on riboflavin. There was an increase in fluorescence intensity in the 410-500 nm region for all colours, but most for blue and green filters. Peaks in this region could be oxidation products from lipid and protein oxidation. This region was closely related to oxidised flavour. The colour was best protected by red, orange, and yellow light. Red and orange transparent plastic boxes would be best to protect vanilla ice cream from photo-oxidation.

STABILITY OF PIGMENTS IN PISTACHIO KERNELS

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ABSTRACT

Often the use of suitable packaging is very important to decrease the degradation of food products. The aim of this study was to test stability of pigments (chl *a*, chl *b* and lutein) in pistachio kernels stored for 14 months, in the dark, at three different temperatures: 10, 25 and 37°C. The samples were packaged using two films (nylon and EVOH), with and without oxygen scavengers; packages were hermetically closed. Moreover, per each temperature, some samples were packaged in bags without a hermetic seal, as reference. After 14 months, at the end of the storage period, pigments underwent a degradation that was influenced by storage time and temperatures. Lower concentrations were observed at 37°C with a degradation of about: 62% for chl *a*, 44% for chl *b*, 57% for lutein. At 10 and 25°C, the samples didn't show any differences statistically significant and the degradation of pigments were: 46% for chl *a*, 33% for chl *b*, 37% for lutein. Among samples, during storage no difference was observed, due to the packaging films or the presence of oxygen scavengers. At 25°C, after 11 months, samples packaged in bags not closed hermetically, showed a presence of moulds and bugs (*Plodia interpunctella - Hubner*). For kinetic study, rate of degradation of the three pigments fitted a pseudo-zero-order kinetic and it was observed an activation energy of: 11.7 KJ/mol, 12.1 KJ/mol and 18.2 KJ/mol, respectively for chl *a*, chl *b* and lutein.

Key words: pistachio, pigments, storage, oxygen scavengers, kinetic

INTRODUCTION

The pistachio kernels are very appreciated fruits in all the world. They have got a high content of oil (about 57 %), in particular they are a good source of unsaturated fatty acids (oleic, linoleic and linolenic fatty acids). Moreover they have got a high content of *potassium* (1025 mg/100g) and *phosphorus* (490 mg/100g), vitamins and antioxidant substances (Maskan and Karatas, 1999; USDA, 2005). Lutein, chlorophylls *a* and *b* are the most important pigments which characterize the pistachio colour (Bellomo and Fallico, in press)

It is common knowledge that oxygen, light and variable temperatures can worsen the appreciable quality characteristics like taste, flavour, colour and nutritional components. The use of suitable packaging and adequate storage conditions are very important to preserve organoleptic and nutritional characteristics of this product. That is very important also to protect the kernels from mould and insects like *Plodia interpunctella – Hubner.*

Up to now, few works were undertaken on the storage of pistachio kernels and they regards the stability of colour parameters and flavour (Andreini et *al.* 2000) and the stability of oil characteristics Maskan and Karatas (1999). No studies were carried out on storage stability of their pigments. Concerning this, it is interesting to remember the studies of Endo et *al.* (1985) which demonstrated the antioxidant effects of chlorophylls and pheophytins on the autoxidation of oils in the dark. As concerns lutein, some authors observed a pro-oxidant activity under light and dark, but it also inhibits lipid oxidation in presence of tocopherol (Subagio and Morita, 2001; Haila *et al.,* 1996).

In this research it was observed as high gas-barrier films (Nylon and EVOH), with/without oxygen scavengers and with/without hermetic seal, at three different temperatures (10, 25 and 37°C), influence stability of pigments in pistachio kernels stored in the dark. The concentrations of pigments (chlorophylls *a-b* and lutein) were analysed during a storage period of 426 days.

MATERIALS AND METHODS

Pistachio kernels were coming from Bronte (Italy), they were picked in 2003 year.

Packaging - For packaging were used two plastic films: Nylon and EVOH (ethylene vinyl alcohol) in a packaging machine DELTA 30; every sample had 150 g of pistachio kernels. In half of packages oxygen scavengers were inserted. The samples, besides, were stored, at three different temperatures: 10, 25 and 37°C. Per each temperature some samples were stored in bags without hermetic seal, as reference.

Chlorophylls and carotenoids extraction and analysis - About 20 g of pistachio kernels were ground for 30s to obtain a homogeneous product. Extraction was carried out by Fuke *et al.* (1985) method with some modification. All operations were done under weak light. Before HPLC analysis, samples were filtered through 0.45 μm filters. The analyses were carried out by HPLC, equipped with a 20 μl loop; detector was a PDA. Chlorophylls and carotenoids pigments were separated on a C18 reverse phase column (MERK LiChrosphere 100 RP-18, LiChrocart 250 x 4 mm, 5 μm) using Pilar Cano (1991) method. The elution was recorded at 300-685 nm, detection and quantification wavelengths for pigments were as follows: Lutein 448 nm, Chlorophyll (chl) *b* 463 nm, Chlorophyll (chl) *a* 430 nm. Chlorophylls and carotenoid pigments identification and quantification were carried out comparing their retention time, absorption spectra and peak areas to pigment standards: *Chl a*, *Chl b,* and *Lutein* (Extrasynthese P > 95%). All results were expressed on dry matter basis.

Statistical elaboration - The statistical elaboration of the results was carried out using the MSTAT WIN 10 program. The program performed the analysis of variance (one-way ANOVA) and the separated means by Tukey test.

RESULTS AND DISCUSSION

The HPLC profile of the extract of pistachio kernels showed the presence of three principal pigments: chl *a*, chl *b* and lutein. At the beginning concentration of pigments were: chl *a* (38.7 ± 2.1 mg/Kg), chl *b* (32.8 ± 2.0 mg/Kg) and lutein (25.8 ± 0.9 mg/Kg); ratio (R) between chl *a* and chl *b* was 1.2. The concentrations observed in pistachio kernels at the beginning were very different and lower than values observed in a previous work (Bellomo and Fallico, in press) and in pistachio picked in 2005 year (data not shown).

As regards storage stability of pigments, statistic treatment of data showed no significant difference among the samples stored with nylon or EVOH, with/without oxygen scavengers and with/without hermetic seal. So, per each temperature, statistics analyses were performed considering all trials as one sample.

The analysed data showed that time and temperatures influenced pigments stability during the storage period. At the end of storage, samples showed the lower concentrations of all the pigments at 37°C; at 10 and 25°C the samples had a statistically similar trend. Independently from the storage temperature, the three pigments highlighted a decreasing trend of the concentration during the whole period (Figures 1, 2 and 3).

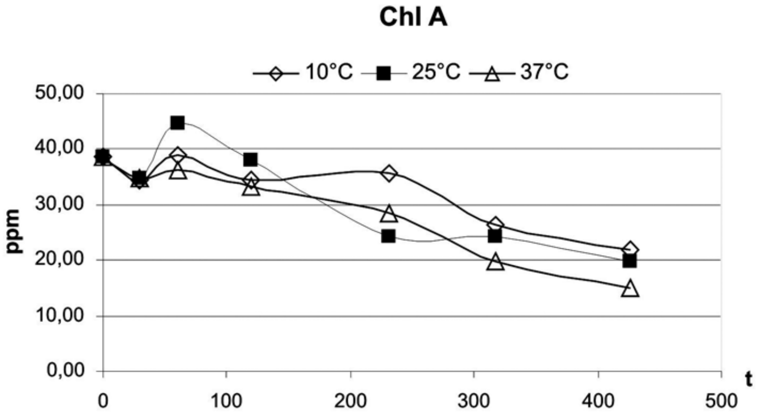

Fig. 1. Trend of Chl *a* concentration in pistachio kernels during storage t=days; ppm=mg/Kg on dry matter, Chl=Chlorophyll.

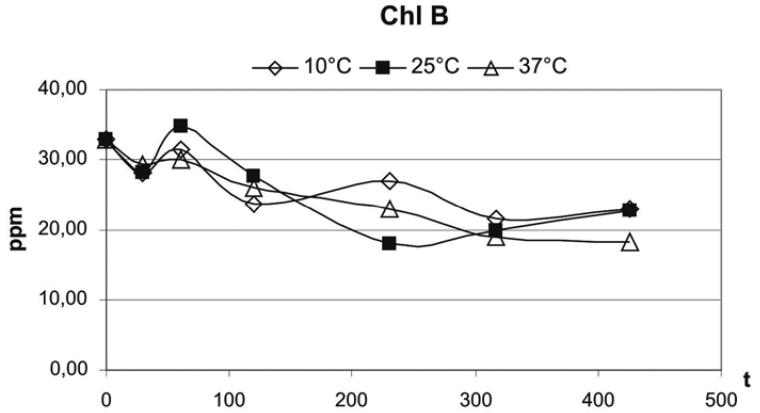

Fig. 2. Trend of Chl *b* concentration in pistachio kernels during storage t=days; ppm=mg/Kg on dry matter, Chl=Chlorophyll.

The degradation rate of pigments was

determined by plotting concentrations of each pigment versus storage time. The linear relationship, which was the best fit for the data, showed zero-order reaction kinetics for all the pigments. From table 1, that shows the degradation rate constants (*k*) of pigments, the higher the temperature the higher degradation rates can be seen. This is in agreement with finding of Pandrangi & Laborde (2004) in fresh spinach stored at three different temperatures. They observed that chlorophylls and carotenoids decreased with increased storage time and degradation was more rapid at higher temperatures.

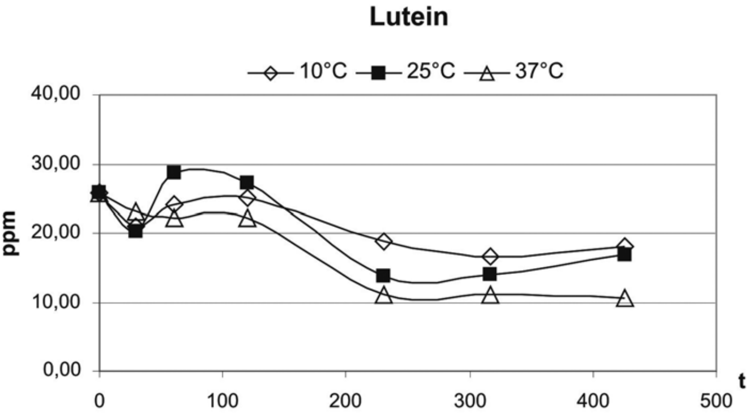

Fig. 3. Trend of Lutein concentration in pistachio kernels during storage t=days; ppm=mg/Kg on dry matter, Chl=Chlorophyll.

Table 1. Rate constants for degradation of pigments and Activation energies.

	K (mg * Kg^{-1} . days^{-1})						Ea	
	10°C	**dv st**	**25°C**	**d st**	**37°C**	**dv st**	**KJ/mol**	**Kcal/mol**
Chl a	0,035	±0,004	0,042	±0,003	0,054	±0,006	11,71	2,80
Chl b	0,023	±0,003	0,029	±0,006	0,036	±0,005	12,11	2,90
Lutein	0,021	±0,002	0,030	±0,002	0,040	±0,005	18,16	4,34

K: rate constant; **Ea:** Activation energy; **d st:** Standard Deviation; **Chl:** Chlorophyll.

Ratio values between rate constants both at 37/25°C and 25/10°C were similar for all the pigments (1.2-1.3, table 2). The only exception was for lutein whose *k* ratio 25/10°C was 1.5.

In pistachio kernels, the most important degradations were highlighted after 8 months. At the end of the storage period the studied pigments degraded at about 46 % chl *a*, 33 % chl *b* and 37 % lutein, both at 10 and 25°C. This degradation was lower than at 37°C: 62 % chl *a*, 44 % chl *b* and 57 % lutein (Figures 1, 2 and 3). From these, it can be seen that only lutein degraded more rapidly than other pigments. In fact, it reached the lowest concentration after 8 months (on the 231st day) at the three storage temperatures (Figure 3). After that, at the end of storage the lutein concentration was the same but chl *a* was more degraded. It was also confirmed by ratio chl *a*/lutein values of pigment concentrations (data not shown). The chl *a* also degraded more rapidly than chl *b*; its degrada-

Table 2. Ratio between rate constants.

K ratio			
Temperatures °C		**37/25**	**25/10**
Chl a		1,3	1,2
Chl b		1,2	1,3
Lutein		1,3	1,5
Pigments	**Chl a/b**	**Chl a/Lute**	**Chl b/Lute**
10°C	1,5	1,7	1,1
25°C	1,5	1,4	1,0
37°C	1,5	1,3	0,9

K: RATE CONSTANT; **CHL**: CHLOROPHYLL; **LUTE**: LUTEIN.

tion was 1.5 faster than chl *b* (Table 2). That is in agreement with other studies (Schwartz and Von Elbe, 1983; Schwartz and Lorenzo, 1991). At 37°C, among the pigments, only chl *b* was less degradable.

As concerns the use of oxygen scavengers, this study highlighted that there weren't differences between samples stored with or without them. Also Schwartz and Lorenzo (1991), in a study about spinach puree (processed and packaged), observed that during storage the oxygen permeability didn't appear to contribute to chlorophyll degradation.

As regards activation energy (Ea), very low values were observed (chl *a*: 2.8 Kcal/mol, chl *b*: 2.9 Kcal/mol and lutein: 4.3 Kcal/mol, table 1) in respect to other studies (Schwartz and Von Elbe, 1983; Schwartz and Lorenzo, 1991; Steet and Tong, 1996; Henry *et al.,* 1998; Martins and Silva, 2002). These authors showed Ea values ranged between: 11.6 and 25 Kcal/mol for chl *a*; 11.1 and 22.8 Kcal/mol for chl *b*; 24.9 Kcal/mol for lutein, respectively. That can be due to many reasons: characteristics of foods (such as water activity, perishability, enzymatic content, etc.), storage temperatures, processing, etc. Moreover, when the rate of a reaction is diffusion-controlled, activation energies are low and they range from 0 to 8 Kcal/mol (Robertson, 1993). Concerning this, pistachio kernels have got a low content of water (at about 5 %), a a_w within a range of 0.65-0.75 and a content of oil at about 57 %. Such a composition indicates that inside the kernels reaction rate could be diffusion controlled; this could explain the low observed Ea values. The presence of antioxidant substances (tocopherols, phosphorus, etc.) could protect the product during storage. In fact, even if it was observed a decreasing trend of various pigment concentration and low activation energy values, after 14 months at 10 and 25°C the remaining % of pigments were: 54% for chl *a*, 70% for chl *b* and 68% for lutein.

CONCLUSION

After 14 months, the studied pigments showed a good stability when storage was made at 10 and 25°C. As concerns the use of oxygen scavengers and plastic films at high gas barrier, it was observed that they didn't influence the stability of the pigments. It means that a cheaper packaging can be enough to store the pistachio kernels. Storage at low temperatures, such as 10°C, was the most important parameter. This temperature guarantees a good quality of pistachios both for pigments stability and the absence of mould and insects.

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SHELF-LIFE MONITORING
OF BROCCOLI
PACKED WITH DIFFERENT FILMS

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ABSTRACT

The shelf-life of broccoli (cv. Marathon) stored at 6°C and 95% R.H. under different packaging conditions, was studied up to 35 days, using physical, chemical and microbiological indexes (weight loss, sulforaphane, vitamin C and chlorophyll content, total mesophilic and psychrophilic bacteria, yeasts, moulds) to assess the product quality throughout the storage time. Five different polyolefinic films were utilized in the study, three perforated, but characterized by a different percentage of perforated area (OPTI 330 A, OPTI 330 C, OPTI 330 E), and two anti-fog non-perforated (MRX, AFG-E 60). The experimental results indicated that the use of packaging could extend the shelf-life of broccoli, with an allowable storing time of about 5 weeks, without any major loss of nutrients and modification of color. In particular, among the different perforated films tested, the OPTI 330 A, characterized by a considerable lower percentage of holes, gave the worst results, in comparison with all the other films. The best results were obtained using the AFG-E 60 film, while no appreciable differences could be pointed out when OPTI 330 C, OPTI 330 E and MRX films were utilized.

Key words: broccoli; chilling; packaging; shelf-life; vegetables

INTRODUCTION

Broccolis, like other cruciferous vegetables, are rich in health-promoting compounds, such as glucosinolates and vitamin C, which due to their antioxidant and free-radical scavenging properties have gained much attention in recent years, because of their marked role in the prevention of cardiovascular diseases and cancer. However, while a regular daily intake of these compounds can exert a positive action, their level in the vegetables can be very variable according to the cultivar, ripening stage and cultivation practices, which as well as postharvest handling, storage conditions, processing and packaging techniques, can affect the final content of the nutrients in broccoli (Galgano *et al.*, 2001; Howard *et al.*, 1999).

Broccoli is a very perishable product, having a rather high respiratory activity, being extremely sensitive to ethylene and losing water readily. Perceivable major alterations are represented by surface dehydration, with weight loss, flavor and color changes, but there can also be an important reduction in the amounts of nutrients. However, from the point of view of the consumers or for the shop management the loss of green color, with floret yellowing due to chlorophyll degradation, is considered one of the major limiting factors in broccoli shelf-life (Jacobsson *et al.*, 2004; Serrano *et al.*, 2006). The shelf-life of broccoli can span over a few days or a few weeks, depending on the storage conditions, such as temperature, relative humidity, light and composition of the surrounding atmosphere and various packaging solutions have been studied in an effort to preserve broccoli during storage and transport (Forney and Rij, 1991; Howard *et al.*, 1997; Jacobsson *et al.*, 2004; Rangkadilok *et al.*, 2002; Serrano *et al.*, 2006).

Polymeric films with different permeabilities to oxygen, carbon dioxide, ethylene and water vapor can act as a barrier to dirt and insects, reduce the dehydration and the loss of sensory quality of the vegetables; in addition, the combination packaging-refrigeration is possible. The choice of the film is also a key factor in order to obtain optimum modification of the atmosphere in the packaging system and avoid extremely low levels of O_2 and/or high levels of CO_2, that could induce anaerobic metabolism with possibility of off-flavor generation, caused by the low gas permeability of the films. Although broccoli are often sold to consumers packaged in polymeric films or in vented plastic bags, a beneficial atmosphere may not always be generated, resulting in a poor final vegetable quality (Jacobsson *et al.*, 2004).

In this research the effect of different packaging films on the shelf-life of broccoli was investigated during 35 days of chilling storage, taking into account not only easily perceivable characteristics, or alterations that can be of interest for the consumer or for the shop management, but also the preservation of acceptable levels of nutrients that play a key role for human health.

MATERIALS AND METHODS

Broccolis (*Brassica oleracea* L. var. *italica*, cv. Marathon) were provided by the Cooperativa Ortitalia in Lavello (Potenza, Italy), and immediately after harvesting the vegetables were washed in potable water, trimmed (stem length 7-8 cm), packed in wooden boxes and transported to the laboratory within two hours. Several packaging methods were tested, using various polyolefinic films, perforated (OPTI 330 A, OPTI 330 C, OPTI 330 E; Cryovac, Italy) and anti-fog non-perforated (MRX, Cryovac; AFG-E 60, Clysar, France) (Table 1). For each experimental thesis two broccolis of similar size (total weight 300-350 g) were packed using a polystyrene tray (19.3 cm x 11.5 cm x 9.5 cm) inserted in a bag (35 cm x 30 cm) made of the various films. When testing the AFG-E 60, the broccolis were packed without a tray and tightly wrapped in the film. The samples were stored at 6 °C and 95% R.H. using a climatic chamber (Mod. KBP 6395 F, Termarks, Sweden). The tests lasted up to 35 days and the broccolis were sampled every 7 days for analyses. Unpacked broccolis stored under the same conditions were utilized as a control.

Table 1. Principal technical characteristics of packaging films.

Characteristics	OPTI 330 A	OPTI 330 C	OPTI 330 E	MRX	AFG-E 60
Thickness (µm)	15	15	15	15	15
WVTR (g/m^2 24 h)$^{(1)}$ (38°C, 100% R.H.)	10	10	10	10	10
PO2 (g/m^2 24 h atm)$^{(1)}$ (23°C, 50% R.H.)	3500	3500	3500	10000	14000
PCO2 (g/m^2 24h atm)$^{(1)}$ (23°C, 0% R.H.)	17700	17700	17700	41000	36000
Holes diameter (mm)	0.5	1.0	0.5	--	--
Holes/cm^2	1.08	10.23	31	--	--
Percentage of perforated area	0.21	8.03	6.08	--	--

(1) Reported values are for the plain film.

The broccoli moisture content was determined according to official methods (AOAC, 1990), while the vitamin C and sulforaphane content were assessed by HPLC or GC-MS according to Galgano *et al.* (2002) and Chiang *et al.* (1998), respectively. The total chlorophyll content was determined spectrophotometrically, according to Inskeep and Bloom (1985), while broccoli weight loss was determined gravimetrically and expressed as a percentage of the original weight. The vegetables were also monitored for their microbiological quality, assessing the levels of mesophilic and psychrophilic bacteria, yeasts and moulds, at each sampling according to Brackett (1989).

RESULTS AND DISCUSSION

The shelf-life study was carried out up to 35 days. However, due to an excessive development of moulds, the control sample was considered only up to 28 days. One of the main problems during the storage of broccoli is the high weight loss, which may have a negative influence on their marketability. From this point of view all the films allowed a reduced dehydration of the vegetables in comparison with the unpacked samples, with final weight losses after 35 days between 1.5 and 6%. The lowest and the highest values were recorded when using the AFG-E 60 (1.52%) and the OPTI 330 C (5.70%) films, respectively, while the control showed a weight loss of about 10% after 28 days of storage (Fig. 1). Similar results were reported by Jacobsson *et al.* (2004), who used films with similar technical characteristics for the packaging of chilled broccoli.

Broccolis are vegetables rich in health promoting compounds, such as vitamin C and sulforaphane, and also in this case packaging contributed to minimize the loss of these nutrients; in fact, vitamin C content, initially 7.82 mg/g dry weight (d.w.), decreased sharply in the control throughout the whole period of study, with a final loss of more than 35% after 4 weeks. Similar losses were reported by Serrano *et al.* (2006) for unpacked broccoli (cv. Marathon), but stored at 1°C. Packing the vegetables resulted in a higher retention of vitamin C, and after 35 days the losses were substantially limited to no more than 20%, with the AFG-E 60 film giving the best retention results, with negligible vitamin losses. Only when using the OPTI 330 A, characterized by the lowest percentage of perforated area, the losses were slightly higher reaching about 26% of the initial content of vitamin C (Fig. 2).

Broccoli showed a quite limited initial sulforaphane content of 1.13 mg/g d.w., in comparison with the levels found in the literature (1.9 - 3.7 mg/g d.w.) (Chaing *et al.*, 1998). However, this could be explained by taking into account the differences in varieties, cultivation practices, season, post-harvest handling and storage conditions. During storage, the concentration of sulforaphane appeared to fluctuate or increased slightly, remaining substantially constant in all packed samples, and being higher than the concentration observed in the control, in which the moiety showed a decrease of 12% after 7 days, and a final loss of 18% after 28 days of chilling storage (Fig.

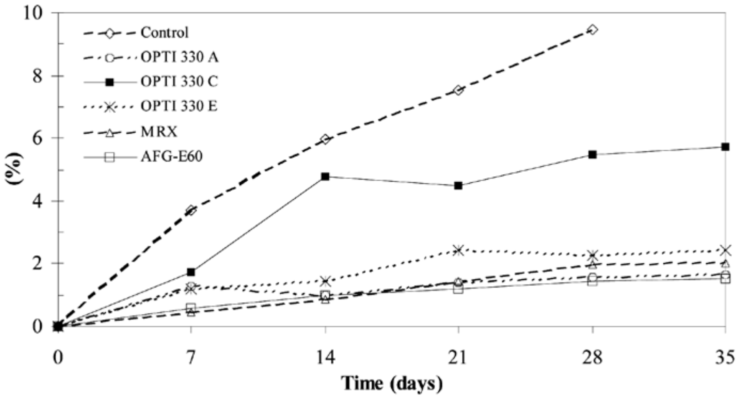

Fig. 1. Percentage of weight loss in broccoli during storage at 6°C and 95% R.H.

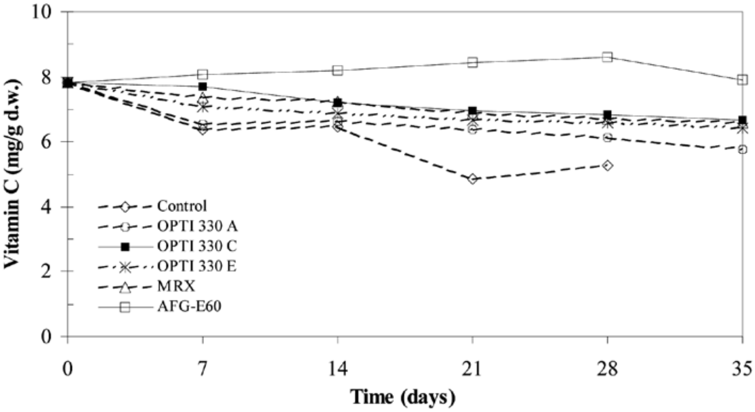

Fig. 2. Vitamin C content in broccoli during storage at 6°C and 95% R.H.

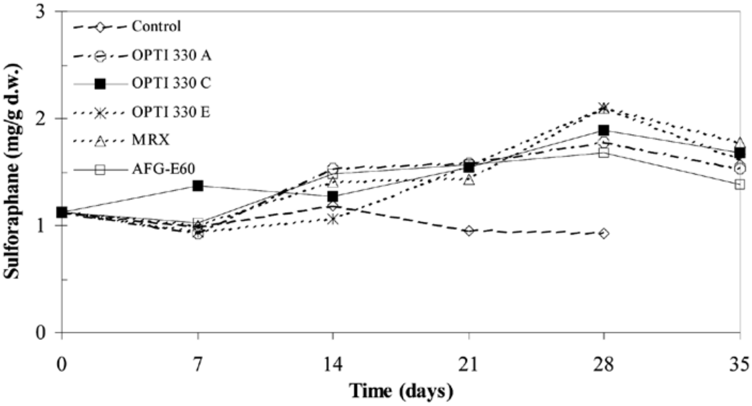

Fig. 3. Sulforaphane content in broccoli during storage at 6°C and 95% R.H.

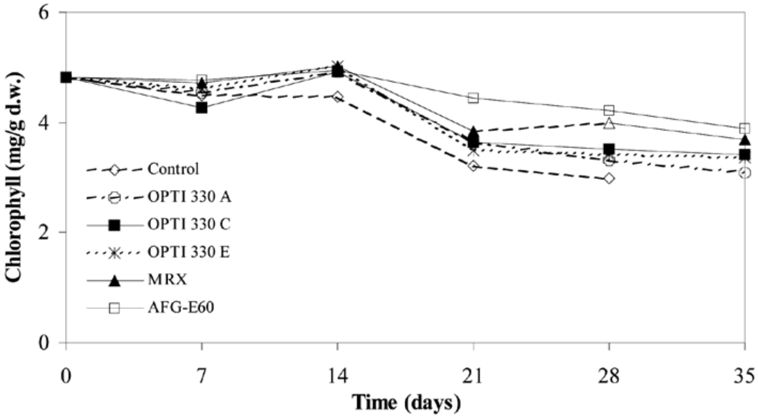

Fig. 4. Chlorophyll content in broccoli during storage at 6°C and 95% R.H.

3). A similar behavior was observed by Rangkadilok *et al.* (2002), who studied the glucoraphanin content, precursor of sulforaphane, in broccoli stored under chilling conditions in a controlled atmosphere and modified atmosphere packaging, but using films different from those utilized in the present study. However, in the literature contradictory results are also reported about the changes in concentration of this moiety during broccoli storage (Hansen *et al.*, 1995). Also for sulforaphane the scientific results are not

Table 2. Microbial counts in unpacked and packed broccoli.

			Polyolefinic film				
Microorganisms (log cfu/g)	Time (days)	Control	OPTI 330 A	OPTI 330 C	OPTI 330 E	MRX	AFG-E 60
Mesophilics	0	4.23	4.23	4.23	4.23	4.23	4.23
	7	4.45	4.18	4.30	4.38	4.15	4.22
	14	4.62	4.47	4.32	4.60	4.20	4.43
	21	5.78	5.29	4.82	5.53	5.18	5.40
	28	5.94	5.48	5.08	5.71	5.00	5.39
	35	--	6.18	5.45	5.63	5.66	5.00
Psychrophilics	0	2.00	2.00	2.00	2.00	2.00	2.00
	7	4.36	3.30	3.60	3.15	3.90	3.85
	14	4.48	4.16	3.78	4.15	3.70	4.20
	21	5.60	5.30	5.56	5.16	4.71	5.30
	28	5.67	5.70	5.69	5.64	5.67	5.62
	35	--	5.81	5.67	5.68	5.38	5.15
Yeasts	0	2.00	2.00	2.00	2.00	2.00	2.00
	7	4.79	3.51	3.15	3.36	3.18	4.40
	14	4.81	3.81	3.31	3.16	3.35	3.81
	21	5.10	3.90	4.41	3.95	3.00	4.83
	28	5.60	4.26	4.22	3.90	3.36	4.85
	35	--	5.54	4.48	4.00	3.78	4.62
Moulds	0	2.00	2.00	2.00	2.00	2.00	2.00
	7	2.00	2.00	2.00	2.00	2.00	2.00
	14	2.68	2.36	2.00	2.48	2.18	2.00
	21	3.74	2.72	2.54	2.65	2.00	3.30
	28	4.52	2.98	3.60	3.00	2.00	3.65
	35	--	3.00	4.18	3.89	2.00	4.31

always in agreement, and contrary to the data of the present study, Howard *et al.* (1997) have reported a sulforaphane decrease of about 39% after only 7 days of storage in broccoli packaged in perforated polyethylene bags (2.5 microholes/cm^2) and stored at 4°C.

Another important factor in quality deterioration of stored broccoli is tissue yellowing, due to chlorophyll degradation. Chlorophyll content, initially 4.82 mg/g d.w., remained relatively constant up to 14 days in the whole thesis, but then began to decrease throughout the whole subsequent period (Fig. 4). After 35 days the chlorophyll loss was higher for the perforated films, particularly for OPTI 330 A (36%), than for those non-perforated, AFG-E 60 (19%) and MRX (23%), thus leading the non-perforated films to a higher retention of the green color in broccoli, as also reported by Serrano *et al.* (2006). Conversely, in the control the chlorophyll loss increased more sharply, reaching the value of 34% after only 21 days of storage.

As far as the microbiological development is concerned, the use of polymeric films limited the microbial growth, as also reported by Brackett (1989), while unpacked broccoli resulted as no more marketable after 28 days, because of a strong mould presence (Table 2). No great differences in mesophilic and psychrophilic counts were recorded among the different films, and after 35 days of storage all the packed samples were still acceptable from a microbiological point of view, the total mesophilic count being lower than 6 log cfu/g. Furthermore, throughout the whole storage period, packaging resulted also in a higher inhibition of the yeast and mould development, with the MRX film giving the best results, and similar observations have been reported by Mohd Som *et al.* (1994). Only with the OPTI 330 A the mesophilic count (6.18 log cfu/g) was slightly higher than that normally found in fresh vegetables, ranging from 4 to 6 log cfu/g (De Felip, 1991). Moreover, the OPTI 330 A film was the only one that allowed a yeast population higher than 5 log cfu/g.

CONCLUSION

In conclusion, the experimental results indicate that packaging could extend the shelf-life of broccoli, with an allowable storage time of about 5 weeks, without any major loss of nutrients and modification of color. In particular, among the different films tested, OPTI 330 A gave slightly worse results than all the other films, but allowing in any case a still marketable product after 35 days of storage. The best results were obtained using the AFG-E 60 film, while no appreciable differences could be pointed out among OPTI 330 C, OPTI 330 E and MRX films. However, it should be noted that the cost of these films is significantly different, with the price of MRX and AFG-E 60 being two or three times higher, respectively, in comparison with the price of the films of the OPTI family.

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SURFACE ANALYSIS BY ATOMIC FORCE MICROSCOPY OF BACTERIOCIN ACTIVATED PLASTIC FILMS FOR FOOD PACKAGING

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Antimicrobial agents can be used to give antimicrobial properties to polymeric materials used to envelope foods for packaging purposes. Different kinds of antimicrobial packaging are described in the current literature and the bacteriocin coating of polyethylene surfaces is reported as an effective method to confer antimicrobial properties to food packages. However, no information is reported about the interactions, between bacteriocins and the surface of plastic films, which are implicated in the adhesion and release mechanisms of antimicrobials. In this study we exploited an Atomic Force Microscopy (AFM) analysis of active and non active surfaces of plastic films. The analysis was carried out by using an easyScan 2 (Nanosurf AG, Switzerland) followed by post-processing of images by the software Scanning Probe Image Processor (SPIP, Image Metrology). In particular, the surface images were obtained by a dynamic AFM analysis and roughness parameters of 3D images were investigated by SPIP. Different polyethylene films were involved in this research and different bacteriocins were used for their activation. Significant differences were found between the bacteriocin activated and control films and the activated surfaces showed lower values of average roughness and higher values of summit density indicating that an homogeneous distribution of the bacteriocin preparation was obtained following the coating procedure. Information on the interactions between plastic film and antimicrobial preparations could dramatically improve the production and implementation of bacteriocin activated food packages.

Key words: active packaging, bacteriocin, Atomic Force Microscopy

NEAR INFRARED SPECTROSCOPY AS A TOOL TO STUDY THE SHELF LIFE OF FOODS

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Key words: NIR, shelf life, cheese, fish

Classical studies on shelf life of foods are based on the parameterisation of the time dependence of some microbiological, chemical and physico-chemical indices. These tests are usually time consuming, and require high manual ability and qualified personnel.

Recently, NIR spectroscopy has been used more and more in the agricultural and food industries. This analytical technique is relatively low-cost and it can be applied in both fundamental research and in factory for on-line control, by using optic probe. NIR technique is easy to use, fast and non destructive method, and in recent years it is becoming a valid tool, alternative to classic wet chemical methods. As known, NIR spectra contain some information related to macro composition of food (water, proteins, lipids an carbohydrates) and to its chemical or structural modifications.

The aim of this research was to investigate the feasibility of applying Near Infrared Spectroscopy (NIR) to study the shelf life of selected foods.

As first study, the shelf life of three different types of dairy products was studied: Crescenza cheese, stored at two different temperatures (8° and 15°C) and analysed at fixed times; Italian Butter, having its shelf life influenced by oxidation reactions, stored at 10°C up to 47 days of storage and Ricotta samples, stored at three different temperatures (3°, 10° and 20°C) up to 48 days of storage. FT-NIR spectral data were collected in the whole NIR range (from 12000 cm^{-1} to 4000 cm^{-1}) by using a FT-NIR spectrometer equipped with a fibre optic probe working in diffuse reflectance (MPA, Bruker Optics). Measurements were carried out in different replicates directly on the surface of the sample. Results shows that NIR spectroscopy reveal the water and fat modifications occurring during the storage.

FT-NIR spectroscopy was also applied to discriminate fresh olive oils from olive oils stored in different conditions: under light for one year and under dark for one and two years. The results showed how this method was able to define olive oils freshness and olive oils oxidation and appeared to be able to describe the different storage conditions.

The freshness of farm raised European sea bass (*Dicentrarchus labrax*) was studied by applying both chemical methods and FT-NIR spectroscopy, using a fibre optic probe operating in diffuse reflectance directly on the fish surface. The results showed the ability of FT-NIR spectroscopy to monitor the shelf life of this types of fishes stored at three different temperatures, underling a critical day of shelf life for each storage temperature. Results are in agreement with those obtained by classical techniques, allowing to define also a Q_{10} value in order to describe the time-temperature relationship.

Overall, the results obtained in these case studies, show that FT-NIR spectroscopy, being a non destructive methods, could be implemented together with conventional techniques to study the shelf life of foods and to monitor the quality of the products during storage.

RANCIDITY EVALUATION AND SHELF-LIFE MONITORING OF VIRGIN OLIVE OIL BY ELECTRONIC-NOSE

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ABSTRACT

This study was carried out to evaluate the applicability of Electronic Nose (EN) for the rapid and objective evaluation of rancidity in virgin olive oils and to monitor the shelf-life of bottled virgin olive oils. An EN equipped with an array of 10 electronic chemical sensors with partial specificity and an appropriate pattern-recognition system was tested. The results obtained by the EN were compared with those obtained by the sensory assessment and those obtained by the GC/MS analysis of volatile markers of oxidation.

Analytical parameters of EN were optimized: volume of the flask and sample amount, time and temperature of analysis, data collection interval. Principal component analysis (PCA) was applied to a first data sets evaluation. Usefulness and limits in applying the EN for the screening among different quality oils (extra virgin, virgin and lampante) and to evaluate the rancidity development defect in bottled virgin olive oil during 12 month storage are discussed.

Key words: rancidity, Electronic Nose, virgin olive oil, sensory quality

INTRODUCTION

The main alteration occurring during the shelf-life of bottled virgin olive oils is oxidation of polyunsatured fatty acids. Temperature and light promote the reaction between the oxygen dissolved in the oil matrix and unsaturated fatty acids with the formation of hydroperoxides. The homolytic cleavage of hydroperoxides, leading to the production of several undesirable volatile compounds, accounts for the rancid off flavour appearance and the loss of nutritional and sensory quality of the oils (Frankel, 1998; Guillen and Ruiz, 2005). The definition of sensory characteristics is a legal requirement in order to classify virgin olive oils in different categories (extra virgin, virgin, lampante) and the official evaluation of the rancid off flavour is achieved by sensory analysis (Panel test). Following the International Olive Oil Council (IOOC, Madrid, Spain) methodology (EC Regulation n. 2568/91; EC Regulation 796/02) a trained panel of 10 – 12 assessors taste the oil samples and, using an appropriate profile sheet, detect and quantify positive and negative attributes. So the appearance of a sensory defect during extra virgin olive oil storage means the end of its shelf life. A virgin olive oil is considered rancid when the median of the rancid perception measured by panel test is different from zero (EC Regulation 796/02). The effectiveness of sensory analysis must be checked by the robust coefficient of variation (CVr%), a statistical index informing about the homogeneity of the judges. Sensory evaluations, however, are often difficult for a routine control because they need a lot of qualified human resources and they are time consuming. On the other hand, different analytical techniques have been employed in the last few years to study olive oil "aroma" (Guth and Grosh, 1991; Morales *et al.,* 1994; Aparicio *et al.,* 1996; Angerosa *et al.,* 1999; Morales *et al.,* 2005). The volatile compounds responsible of rancidity can be analysed by static head-space (SHS), dynamic head-space (DHS) or solid phase micro-extraction (SPME) and quantified and monitored by gas chromatography (GC) or GC coupled with mass spectrometry (GC/MS). These techniques, however, are still very slow and quite expensive. Moreover, the detection of single compounds is not always related to the human perception even thought Odour Activity Value (ratio of the concentration to the odor threshold) is considered(Guth and Grosh, 1991; Guth and Grosh, 1993). In the recent years, the requirement of low cost, simple handling and objective instrumental methods for the analyses of volatile compounds brought the application of Artificial Olfactive System (AOS) or "Electronic Nose"(EN) based on organic, not specific sensors of olive oil evaluation (Gardner and Barlett, 1999).

The use of EN for the screening of virgin olive oils, infact, could represent a valid and direct method to evaluate oil rancidity and monitor its shelf-life. EN is normally equipped with low specificity sensors and with an appropriate data treatment software to characterize and recognize simple and complex odours (Gardner and Barlett, 1994; Deinsingh *et al.,* 2004). This technique is clearly different from the others used in quali-quantitative analyses (HPLC, GC, GC-MS) because it does not identify the single compounds making up a flavour and its relative amount, but it offers a global analysis, operating in a way similar to human nose (Camurati *et al.,* 2003). From a chemical mixture low selectivity sensors give a characteristic pattern that is classified on the basis of the information acquired during a training phase. The main advantages of the AOS are the immediacy of the response and the absence of sample pre-treatments. Therefore, there are several applications in food field. In the industrial production and packaging of olive oils the sensory evaluation is a critical point. A great number of oil samples must be tasted each day to assess its quality level and to standardize the final flavour of the product. This represent a considerable task for the internal organization (Camurati *et al.,* 2003). Therefore, an instrumental screening of oils samples made in order to classify them in a faster and cheaper way could be of great interest. The aim of the present study was to develop and verify a method for a sensory quality evaluation of the virgin olive oils by EN. In particular, the attention was focused on the possibility of discriminating "rancid olive oils" from extra virgin olive oils and monitor the rancidity development during its shelf life.

MATERIALS AND METHODS

Oils. Sixty four (64) olive oil samples were studied. Thirty two rancid olive oil samples were purchased by the ASA (Naples, Italy) and CRIOL (Montesarchio, BN, Italy) from their storage collection (Table 1). Thirty two monovarietal extra virgin olive oil samples, eight of which (from 23f to 40f) were certified by a PDO (Protect Denomination of Origin), were produced during 2004/05 and 2005/06 seasons in different geographical areas of Campania Region (Table 2). Among these, eight samples (from 1f to 8f) were stored for 18 months in optimal storage conditions. A "rancid training set" was built by dilution of rancid standard provided by IOOC following the procedure reported in EC Regulation n. 2568/91 (Annex 12). A "fruity training set" was obtained by continuous dilutions (v/v 1:1) of a 23f oil sample (Table 2) with a fresh, odourless, refined olive oil for 4 times.

SPME-HRGC-MS. The volatile compounds were extracted and concentrated by SPME and analyzed by a GC/MS system. Extraction and concentration of volatiles was carried out with a 50/30 µm DVB-CAR-PDMS fiber (Supelco, Bellefonte, USA). Olive oil samples (3 ml) were equilibrated for 20 minutes in a 20 ml pierceable vial at 40°C and for 10 minutes in the presence of the SPME fibre in the oil headspace (Vichi *et al.*, 2003). After the equilibration time the fibre was placed in the injection port of the GC system where it was desorbed in splittless mode for 10 minutes at 230°C. The desorbed compounds were separated on a Shimadzu gas chromatograph (QP5050A) with mass spectrometer detector (E.I. 70eV) (Shimadzu, Milan, Italy) equipped with a 60 m x 0,32 mm i.d., film thickness 0,50 µm, Supelcowax™ 10 (Supelco, Bellefonte, USA). Conditions used for GC analysis were: initial temperature, 40°C for 4 minutes, increased to 240°C at a rate of 3,5 ml/min, held for 3 minutes. The operating MS conditions were: temperature interface 250°C, temperature ion source 200°C. The mass range varied from 30 to 250 amu, the solvent delay was 5 min and scan speed 0,4 scan/s.

Table 1. List of the rancid defected oil samples: identification label, median of rancid defect and median for fruity are reported for each sample.

Samples	Median of defect	Median for fruity	Samples	Median of defect	Median for fruity
1r	5,8	0,5	***15r***	8,0	0,0
2r	2,5	1,0	***16r***	0,7	3,9
3r	1,1	1,2	***17r***	0,8	3,4
4r	0,4	3,5	***18r***	3,5	1,0
5r	3,0	2,0	***19r***	1,0	1,8
6r	0,6	3,8	***20r***	2,5	1,4
7r	0,3	4,8	***21r***	4,0	0,0
8r	2,5	0,9	***22r***	3,6	0,5
9r	0,5	3,6	***23r***	4,6	0,1
10r	0,4	3,8	***24r***	1,2	2,3
11r	2,3	1,1	***25r***	1,1	2,8
12r	1,8	1,9	***26r***	0,4	3,5
13r	2,2	1,0	***27r***	0,6	3,1
14r	0,6	3,9	***28r***	4,5	0,4

Identification of the peaks was obtained on comparison of their mass spectra of the NIST library. Quantification was carried out using isobutyl acetate as Internal Standard (Morales and Aparicio, 1993). All samples were analysed in duplicate.

Sensory Analysis. 64 olive oil samples were evaluated by a 10 member trained panel according to the IOOC panel test (EC Regulation n. 2568/91). The "fruity" and "rancid" sensory attributes were quantified by calculating the median of intensities perceived by assessors.

Electronic nose. An Electronic Nose (Airsense Analytics, Germany) PEN2 with 10 MOS (Metal Oxide Semiconductor) sensors was used. The sensors are n-type semiconductors (stannum dioxide) which are reactive to oxidant compounds (particularly to aldehydes, ketones, alcohols, fatty acids and fatty esters). The operating conditions were: the baseline was acquired in a filtered dry air in a continuous flow of 600 ml/min for 300 seconds; aliquots of 1 ml of each olive oil samples have been introduced in 20 ml vials with a pierceable Silicon/Teflon disks in the cap and incubated at 30°C for 20 min before injection. After a headspace generation, the volatile compounds have been directly transferred by the carrier gas at a constant flow rate (400 ml/min) into the sensors chamber and the sensor responses acquired for 100 sec. Then, sensors have been exposed to air in order to keep the gas sensor signal back to the baseline. Sensor responses towards olive oils samples were analysed by the software Winmuster vers.1.6 (Airsense Analytics, Germany).

RESULTS AND DISCUSSION

In the first stage of the work the EN parameters were evaluated in order to analyse virgin olive oil. Volume of the flask (20 ml, 10 ml), sample amount (3 ml, 2 ml, 1 ml), time and temperature of analysis, data collection interval were tried (Guadamarra *et al.*, 2000) (data not shown). A "rancid training set" was prepared by progressive dilution of the IOOC rancid standard sample with a fresh, odourless, refined olive oil (v/v 2:1). The 12 training samples obtained were analyzed by the EN. Figure 1 shows the distribution of the samples on the plane obtained from two Principal Component arising from the PCA carried out on data acquired by the electronic sensors. EN is able to distinguish oil samples characterized by different amount of standard rancid oil. Sensibility appears higher for strongly and medium rancid samples (from 1 to 8) than for those presenting low levels of rancid defect (9-12). This results agree with those found during the intensity rating test carried out by the assessors. (Figure 2). The EN appear an effective analytical instrument in the analysis of extra virgin olive oils. Further improvement can represent a useful task to the quality control in order to make easier the assessors' work. The intensity of the rancid perception evaluated by a trained panel in a positioning test has been compared with the EN response showing a good correlation ($R^2 = 0.64$)(Figure 2). The differences between contiguous samples are less evident when the standard concentration is under the panel's mean threshold. The pattern shown before (Figure 1) is referred to the ability of the instrument to discriminate the intensities of the rancid defect added to a refined olive oil. Tests actually in progress on others "off flavours training sets" (fusty and winey-vinegary) doesn't give the same linearity (data not shown). Things can be different with a real oil sample which is characterized by a complex sensory profile. The contemporary presence of more attributes (positive and negative) could make difficult the EN analyses. Therefore, in order to confirm the instrument ability in separating the different intensities of extra virgin

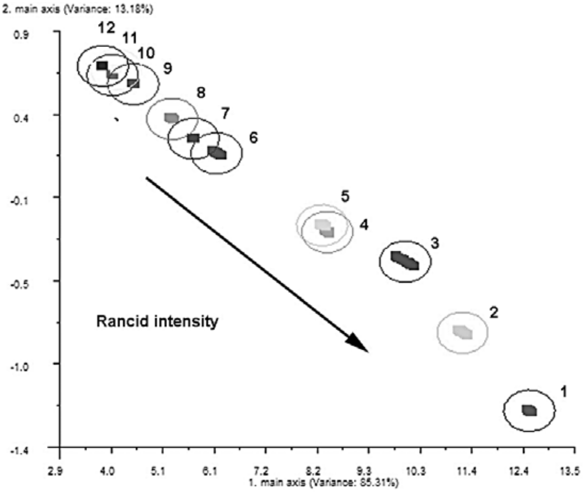

Fig. 1. PCA resulting on Electronic Nose (EN) data related to “rancid training set” carried out directly by the EN software.

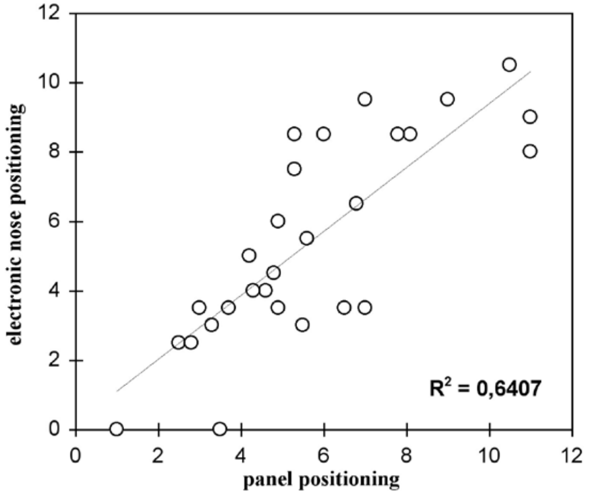

Fig. 2. Comparison between Electronic Nose (EN) and panel responses in a positioning test carried out on virgin olive oils characterized by different rancid intensity attribute.

olive oil fruity attribute, a “fruity scale” was built. The oil sample 23f was chosen on the base of the judgment of a trained panel and of the data obtained by the quali-quantitative analysis of volatile compounds (median for fruity = 7; content of *trans*-2-esenal measured by SPME-GC/MS = 25 ppm). The pattern resulting from the EN analyses of the “fruity scale” is reported in Figure 3. The EN shows a good ability in separating also the “fruity scale” samples. However the bouquet of an extra virgin olive oil is extremely complex, and therefore, the pattern must be considered only indicative of the fruity type used to build the scale.

A useful application of EN in oil industry could be the rapid discrimination of extra virgin olive oils from lampante olive oils. The PCA (Figure 4) elaborated by the instrument software shows that electronic nose can separate extra virgin olive oils (fruity and without off flavours) (Table 2) from rancid oils (Table 1).

For a better understanding of the EN operating system a selection of oil samples were analyzed by conventional instrumental analysis: SPME associated to the GC/MS. The amounts of the marker compounds for fruity and rancid attributes measured by SPME/GC-MS on a set of 12 samples are reported in Table 3. The identification of the peaks relative to “rancid” (octane, butanal, pentanal, hexanal, t-2-pentanal, heptanal, octanale, 2-heptenal, 6-methyl-5-hepten-2-one, nonanal, 2,4-heptadienal, 2-nonenal, 2-decenal, hexanoic acid) and “fruity” (t-2-hexenal, hexyl-acetate, hexanol, c-3-hexenol, t-2-hexenol,) were carried out according to literature (Aparicio R. and Morales M.T., 1994; Angerosa *et al.*, 2004; Morales *et al.*, 2005). The TIC chromatograms of a fruity and a rancid oil analyzed with SPME-GC/MS method are shown in Figure 5. PCA analysis was then performed on data obtained both by SPME-GC/MS and by EN. The results are shown in Figure

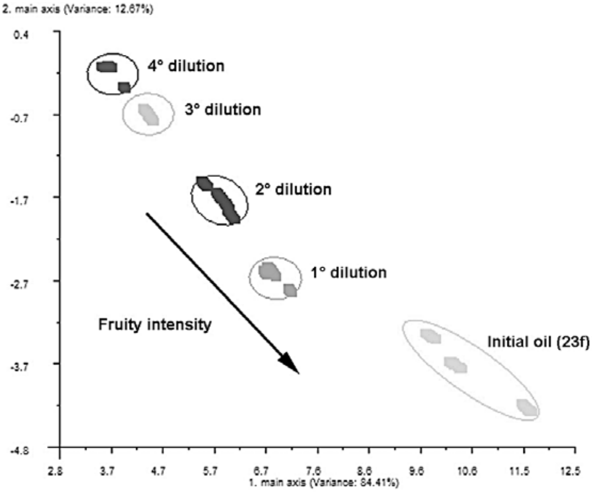

Fig. 3. PCA resulting on Electronic Nose (EN) data related to “fruity scale” carried out directly by the EN software.

6. A separation between “fruity” and “rancid” oils was observed. The loading plot, however, didn’t evidence any correlation among EN data and SPME data. The ability of EN to monitor the evolution of extra virgin olive oils during storage was evaluated by the analysis of 8 oil samples undergone to a shelf life test. Figure 7 shows the PCA carried out on EN sensors responses to the initial oil samples and to the same oils after 12 and 18 months of storage. EN proved to be able to distinguish between “fresh” extra virgin olive oils and “old” ones and between oils stored for different times.

CONCLUSION

The tests carried out in the course of the present work confirmed the advantages supplied by the EN respect to sensory analysis and instrumental traditional techniques: short time analysis; absence of sample pretreatments; response expressed in simple, synthetic easy interpretable way; possibility to monitoring processes on line. The main problems of with EN analysis are essentially connected to the ability to guarantee an adequate sensibility, selectivity and repeatability of the measures. The MOS ensors used and method optimized showed good reliability and stable

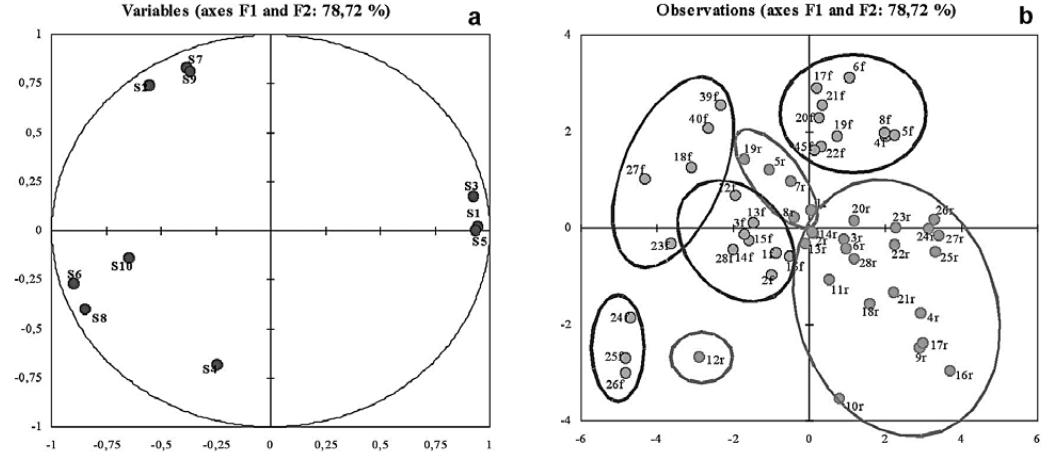

Fig. 4. Loading plot (a) and score plot (b) resulting from the PCA carried out on Electronic Nose (EN) data arising from the analysis of 64 oil samples (f = fruity; r = rancid).

Table 2. Extra virgin olive oil (EVOO) samples used in the study: identification label, cultivar, harvest date, median of rancid defect and median for fruity are reported for each sample.

Samples	Cultivar	Harvest date	Median of defect	Median for fruity	Samples	Cultivar	Harvest date	Median of defect	Median for fruity
1f	Minucciola	2004/05	0,0	5,2	***26f***	DOP Val di Mazara	2005/06	0,0	4,9
2f	Minucciola	2004/05	0,0	4,5	***27f***	DOPMontilblei	2005/06	0,0	5,5
3f	Minucciola	2004/05	0,0	4,0	***28f***	DOPMontilblei	2005/06	0,0	2,9
4f	Minucciola	2004/05	0,0	4,2	***29f***	DOPMonlilblei	2005/06	0,0	5,2
5f	Minucciola	2004/05	0,0	5,0	***30f***	DOPMontilblei	2005/06	0,0	6,0
6f	Minucciola	2004/05	0,0	5,0	***31f***	DOPMontilblei	2005/06	0,0	5,8
7f	Minucciola	2004/05	0,0	5,1	***32f***	DOPMonlilblei	2005/06	0,0	4,9
8f	Minucciola	2004/05	0,0	4,4	***33f***	DOPMontilblei	2005/06	0,0	5,2
9f	Olivone	2005/06	2,0	2,1	***34f***	DOPMontilblei	2005/06	0,0	4,5
10f	Marinese	2005/06	0,0	2,4	***35f***	DOPMontilblei	2005/06	0,0	4,9
11f	Ogliarola	2005/06	0,0	3,6	***36f***	DOP Terre di Siena	2005/06	0,0	4,0
12f	Ravece	2005/06	0,0	5,4	***37f***	DOP Terre di Siena	2005/06	0,0	4,2
13f	Ravece	2005/06	0,0	3,3	***38f***	DOP Terre di Siena	2005/06	0,0	4,1
14f	Ravece	2005/06	0,0	4,4	***39f***	DOP Umbria	2005/06	0,0	4,5
15f	Ravece	2005/06	0,0	3,9	***40f***	DOP Umbria	2005/06	0,0	4,6
16f	Ravece	2005/06	0,0	5,1	***41f***	DOP Valli Trapanesi	2005/06	0,0	5,1
17f	Frantoio	2004/05	0,0	3,8	***42f***	DOP Valli Trapanesi	2005/06	0,0	5,2
18f	Frantoio	2004/05	0,0	3,5	***43f***	DOP Valli Trapanesi	2005/06	0,0	4,9
19f	Frantoio	2004/05	0,0	3,3	***44f***	Ravece	2004/05	0,0	4,5
20f	Leccino	2004/05	0,0	4,5	***45f***	Ravece	2004/05	0,0	5,2
21f	Leccino	2004/05	0,0	3,4	***46f***	Ravece	2004/05	0,0	4,5
22f	Leccino	2004/05	0,0	3,9	***47f***	Ravece	2004/05	0,0	5,7
23f	DOP Chianti Classico	2005/06	0,0	7,0	***48f***	NostralediLauro	2004/05	0,8	1,2
24f	DOP Chianti Classico	2005/06	0,0	4,9	***49f***	NostralediLauro	2004/05	1,3	2,0
25f	DOP Monte Etna	2005/06	0,0	4,5	***50f***	NostralediLauro	2004/05	2,4	2,2

measures for numerous samples analyzed. It was possible to discriminate between oils of different quality (EVOO and lampante) and between different intensity for "fruity" or "rancid" attribute. Furthermore, the EN proved to be an useful tool for the monitoring of oil flavour evolution during storage.

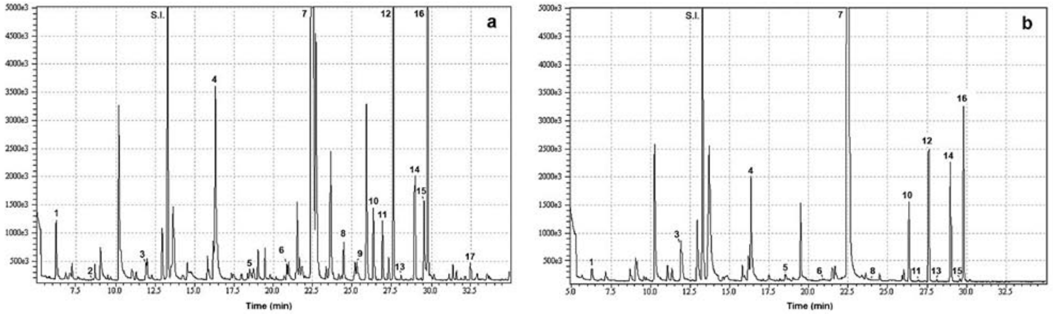

Fig. 5. Score plot (a) and loading plot (b) resulting from the PCA carried out on Electronic Nose (EN) results compared with SPME-GCMS data.

Table 3. Main volatile compounds identified by Solid Phase Micro Extraction (SPME) coupled with gas-cromatography/ mass-spectrometry (GC/MS) in a set of 12 olive oil samples (f = fruity; r = rancid). nd = not detect

Peak	Compounds	1r	2r	3r	4r	5r	6r
1	Octane	4285±50,36	1130 ± 18,55	1179 ± 754,82	224 ± 27,59	448±151,32	235±0,35
2	Butanal	36±0,62	35±2,55	16±12,46	nd	104±24,33	nd
3	Pentanal	965±26,33	472 ± 66,09	689±486,90	145±10,49	1431 ±336,30	93±5,96
4	Hexanal	16016±2607,83	6358±218,71	5530 ± 2604,25	2287 ± 79,96	15238 ± 3800,81	1083±19,97
5	2-pentanal (E)	413±114,25	221 ± 16,39	223±79,29	98 ± 6,15	860±130,45	56±2,14
6	Heptanal	1691 ±327,24	292±13,43	236±50,81	53 ± 3,27	1158 ± 238,76	56 ± 2,09
7	2-hexenal (E)	8000±2458,03	3753 ± 243,60	5417 ± 431,22	19958 ± 1523,09	43566 ± 7235,97	12076 ± 197,59
8	Hexyl acetate	1120 ± 362,03	599 ± 56,19	112±11,49	nd	358±61,82	140±6,80
9	Octanal	2896±955,38	362±30,68	203±5,53	50±5,13	1305±296,14	86±3,71
10	3-hexenil acetate(Z)	3871 '1238,29	1354. 112,79	258.9,35	294 .35,34	1484.300,07	328.3,33
11	2-heptanal (Z)	3386.992,73	864.79,62	566'148,81	62.3,64	1216'188,07	235.13,88
12	6-methyl-5-hepten-2-one	5111±1625,40	955±80,43	499±25,11	32 ± 5,61	1065±218,96	86±6,37
13	Hexanol	381 ±80,87	3404±150,08	379±76,35	489±46,78	484±100,54	1091 ±28,55
14	3-hexenol (Z)	390±86,32	722 ± 35,22	113±15,58	272 ± 26,14	635±128,80	337±4,50
15	Nonanal	7043 ± 2729,68	1943 ± 195,77	561 ±126,54	116 ± 10,58	1109 ± 253,69	346±7,45
16	2-hexenol	1193.380,00	6708.613,62	447.54,23	597.58,79	1019±222,42	1750'45,18
17	2,4-heptadienal	555'190,30	286.25,06	108'40,91	51 '4,35	398.59,12	58±1,14
18	2,4-heptadienal	232±76,70	55±5,58	22±11,23	nd	54±21,14	14 ± 1,77
19	2-nonenal	327±87,70	51 ± 14,42	44±39,38	nd	44±7,41	6±1,03
20	2-decenal	220±70,37	66 ± 10,28	52±28,05	nd	43±9,13	17±2,50
21	Nonanol	47±22,63	35±2,99	5±1,16	nd	8±0,12	21 ±4,58
22	Hexanoic acid	1682 ± 723,67	560 ± 34,09	178±34,57	nd	2255±479,85	11 ± 0,72

Peak	Compounds	nf	24f	39f	40f	2Sf	26f
1	Octane	72 ± 9,87	50±19,27	31 ±4,51	35±5,91	85±10,26	111±15,79
2	Butanal	nd	nd	nd	nd	nd	nd
3	Pentanal	71 '1,89	46.5,10	52.17,82	62.0,93	nd	214.11,89
4	Hexanal	1704±608,37	782±213,56	711±62,31	754±42,96	1919±207,10	1465±764,50
5	2-pentanal (E)	145±52,80	42±10,59	42±1,35	57±8,49	42±2,58	57±4,98
6	Heptanal	17±5,88	4±6,21	10±0,90	8±1,53	14±1,08	18±8,82
7	2-hexenal (E)	60144 ± 3127,60	22687 ± 4121,34	23378±2512,73	29080±3247,81	7865±521,12	4803 ± 6727,21
8	Hexyl acetate	61 '13,99	32.0,46	19.5,36	23±4,45	10.2,88	204'67,57
9	Octanal	nd	nd	5.0,74	5.1,92	3.0,24	nd
10	3-hexenilacetate(Z)	1220±481,57	460 ± 77,83	415±44,02	538±51,35	530±51,05	1693 ± 370,33
11	2-heptanal (Z)	22±9,85	9±0,01	11±5,18	8 ± 2,42	13±2,45	14±2,26
12	6-methyl-5-hepten-2-one	12±16,79	8 ± 2,03	11±0,21	19±13,69	nd	22±7,45
13	Hexanol	944±287,28	613 ± 72,94	898±21,56	789 ± 132,16	2679±354,27	1342 ± 283,60
14	3-hexenol(Z)	979 ± 354,12	554 ± 71,28	756±39,22	456 ± 86,65	5072 ± 350,79	3344±629,21
15	Nonanal	54±20,44	14 ± 0,49	34±3,83	18±7,92	32±8,47	81 ± 46,74
16	2-hexenol	2981 ±1030,93	788±111,00	1186 ± 38,98	1110 ± 202,95	936±259,84	1466'353,98
17	2,4-heptadienal	3±3,70	nd	nd	nd	nd	nd
18	2,4-heptadienal	nd	nd	nd	nd	nd	nd
19	2-nonenal	nd	nd	nd	nd	nd	nd
20	2-decenal	3±4,76	nd	nd	nd	nd	nd
21	Nonanol	nd	nd	nd	nd	nd	nd
22	Hexanoic acid	nd	nd	nd	nd	nd	nd

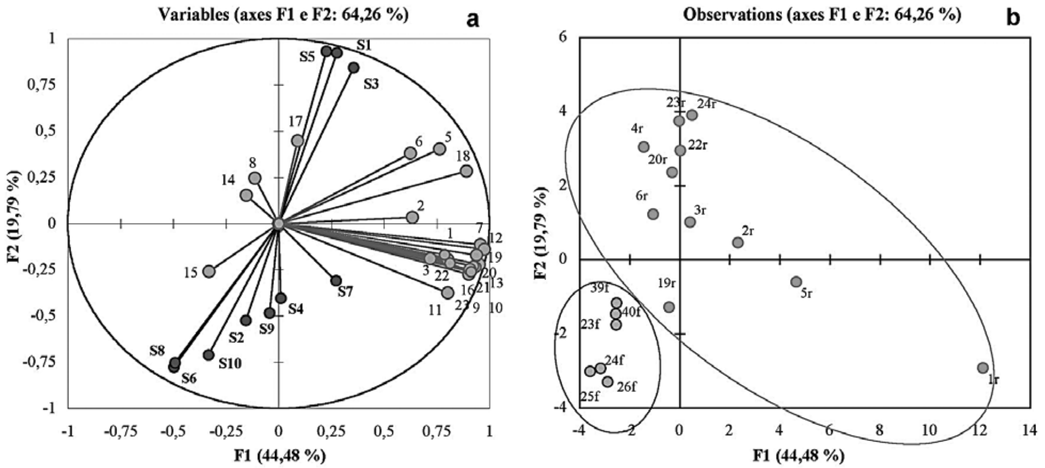

Fig. 6. Total Ionic Current (TIC) chromatogram of a rancid oil (a) and a fruity oil (b) analyzed with SPME-GCMS.
Peaks identification: 1 Octane; 2 Butanal; 3 Pentanal; 4 Hexanal; 5 2-Pentenal, (E); 6 Heptanal; 7 2-Hexenal, (E); 8 Hexyl acetate; 9 Octanal; 10 3-Hexenyl, acetate, (Z); 11 2-Heptanal, (Z); 12 5-Hepten-2-one, 6-methyl; 13 1-Hexanol; 14 3-Hexen-1-ol, (Z); 15 Nonanal; 16 2-Hexen-1-ol, (E); 17 2,4-Heptadienal.

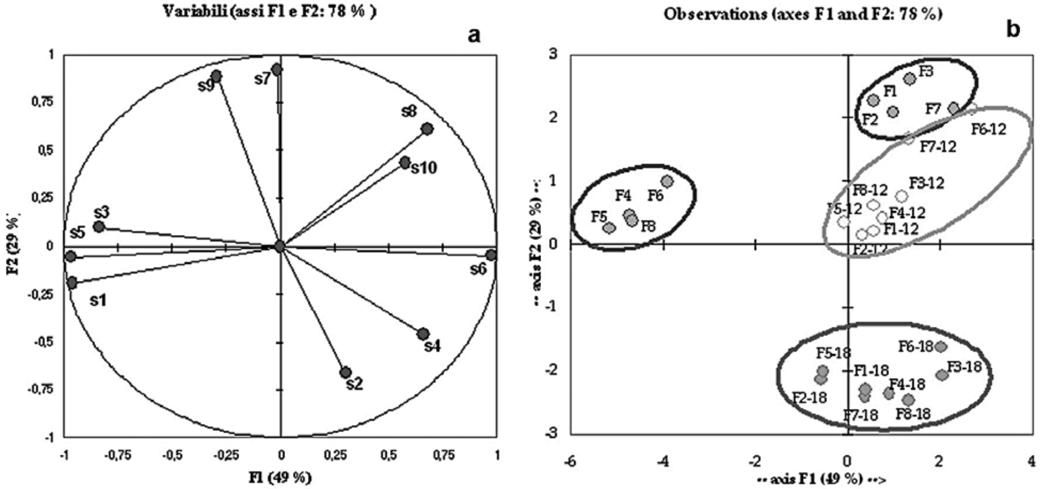

Fig. 7. Loading plot (a) and score plot (b) resulting from a PCA carried out on EN data arising from the analysis of 8 "fresh" extra virgin olive oil samples and of the same samples after 12 and 18 months of storage.

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MEASURING THE QUALITY OF PACKED FOODS

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ABSTRACT

Measuring the quality of food is important for product and process optimization in order to avoid or food degradation as much as possible. This paper provides an overview of advanced analytical approaches in combination with food packaging research techniques to obtain insight in the key processes of food degradation. Also examples are given in which the obtained insight resulted in an appropriate packaging strategy.

Key words: packaging performance, advanced food analysis

INTRODUCTION

Degradation of food products may cause off-flavors or loss of nutritional value resulting in inferior product quality and consumer rejection. The mechanism of degradation and the reaction products highly depend on the type of product. Degradation of nutrients, flavor and additives, are mainly determined by light, pH, oxygen, moisture and other product matrix components.

Optimization of the composition and packaging of food products with respect to shelf-life and sensory quality can be done with classical techniques as high performance liquid chromatography (LC) and gas chromatography (GC) and microbial testing.

Application of these techniques allows e.g. the selection of an appropriate UV barrier in packaging materials to avoid unwanted photo degradation of vitamins.

The combination of analytical techniques and food packaging research techniques such as gas permeability and optical oxygen measurements allow the selection of appropriate measures to avoid food degradation processes like lipid oxidation and growth of aerobic microorganisms.

To study complex research issues such as off-flavor research, optimization and control of products and ageing or alteration of food during storage such as oxidation in complex food stuffs, holistic analytical methods are applied. Holistic analysis is the comparative non-biased determination of nearly all components in a broad range of polarity and molecular weight in a sample. This is in contrast to target analytical techniques as mentioned above which aims at measuring a limited number of known parameters and/or compounds. Such comprehensive techniques are based on GC or LC coupled to mass spectrometry (MS). Next, data pre-processing tools and multivariate data analysis (MVA) are applied to find the relevant correlations between the analytical data with certain properties of interest.

DEGRADATION CAUSED BY LIGHT

It is known that light causes oxidation of lipids and other nutritional compounds in foods.

For this reason potato chips are packed in metal coated barrier packaging and beer in brown bottles. In beer and milk the presence of the vitamin B2 (riboflavin) amongst others is responsible for these light-induced oxidative reactions. Also light can cause the sun struck flavor in milk as a result of degradation of S-containing amino acids.

Under commercial conditions of storage, light in the visible range (blue to green, broad maximum at 430 to 460 nm) is mainly responsible for the photo-oxidation. Commonly used fluorescent lamps in retail displays predominantly emit light of wavelengths above 400 nm.

In some cases it is unknown which part of the spectrum is responsible for degra-

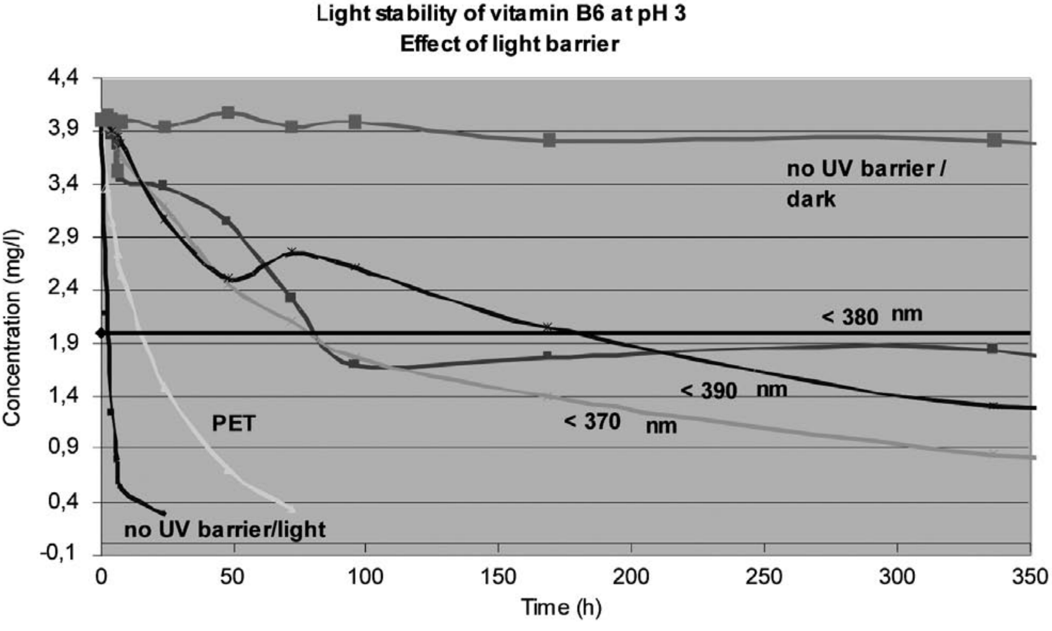


Fig. 1. The effect of different light barriers on stability of the vitamin B6 when exposed to simulated daylight (UV-A, UV-B and the visible part of the spectrum) under controlled temperature, oxygen, pH conditions.

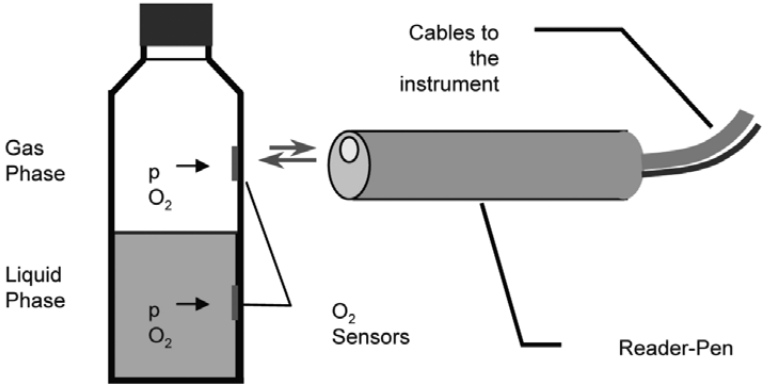


Fig. 2. Optical oxygen measurement with a non-invasive oxygen detector.

dation and research is necessary to allow selection of packaging materials that offer sufficient protection. For instance for food constituents such as several vitamins this is often not the high energy part of the spectrum (UV light). Figure 1 shows the result of a study in which the effect of different light barriers has been investigated at TNO. In this example the stability of the vitamin B6, when exposed to simulated daylight (UV-A, UV-B and the visible part of the spectrum) under controlled temperature, oxygen, pH conditions is demonstrated to vary strongly. The graph shows that glass (no UV barrier) provides no protection at all, while blocking below 270 nm improves the shelf-life strongly. Blocking of higher wavelengths, which is more expensive, does however not significantly improve the shelf-life of this vitamin.

OXYGEN PERMEABILITY OF PACKAGING

The effects of oxygen on the stability of foods can be measured in many ways. Often the methods are destructive and invasive and each measurement requires its own sample. To avoid this TNO developed an non-invasive optical technique that is based on the quenching of fluorescence of a dye in the presence of oxygen (Figure 2). A small oxygen sensitive coated glass disk is mounted onto the inner surface of a packaging. A small external laser diode is used to excite the dots and to measure the resulting fluorescence. The optical oxygen sensing measurement technique is based on the fluorescence quenching of a metal organic fluorescent dye, immobilized in a gas permeable hydrophobic polymer. This effect is proportional to the quencher (oxygen) concentration. The oxygen concentrations can be measured in almost any matrix either liquid or gas. Today this technique is being marketed as the Oxysense™ technique.

By means of this technique, the oxygen concentration can be measured continuously in liquids, gasses and oils without affecting the contents of a packaging.

This way the oxygen concentrations ranging from 20% down to 0.01 mg/l in liquids or 0.02% by volume in gas. The sensor is independent of pH (2-12) and salt and the quenching process does not consume oxygen.

Applications are:

- Oxygen barrier measurements of packaging materials
- Shelf life studies of packed foods and Feed & Cosmetics
- Fat oxidation
- Oxygen consumption by microorganisms
- Oxygen consumption by vegetables

- Effectiveness of closures
- Diffusion coefficients of O_2 in liquid matrices
- Leak detection.

In this way not only the effect of oxygen on the food but also the effect of a packaging on the oxygen concentration can be measured. In other words the oxygen ingress, permeability or effect of an oxygen absorber can be determined.

Fig. 3. Ready-use-tool to measure oxygen permeation and adsorption by scavengers in closed containers.

To measure the contribution of a closure, TNO developed a metal neck system with a precision finish (PCO and crown) that allows fast and sensitive measurement of these effects (Figure 3). Customer bottling conditions (e.g. very low O_2 and CO_2 levels) can be used. If desired, bottle concept evaluation (bottle + closure) is also possible.

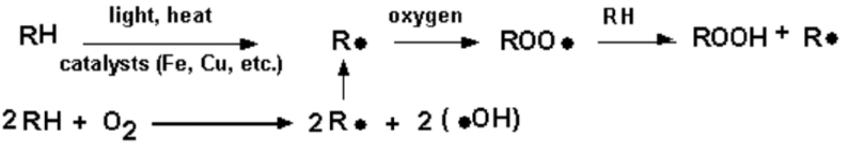
The technique is very fast (in general <600h), and sensitive (low ppb level). It avoids the contribution/influence of the packaging material (barrier, finish defects or irregularities) or withdrawal of samples for analysis as the measurements are noninvasive. The metal necks (see Figure 3) contain oxygen sensors in headspace and liquid area to allow fast and sensitive optical measurements with time via an optical fiber.

The system is very suitable for:

- Determination of the quality of oxygen scavengers in liners/caps
- Oxygen scavenging rate in ppb/h
- Oxygen ingress rate (or permeability) in ppb/h
- Efficiency/capacity of scavenger over shelf life (O_2 half-life values and in ppb/h)
- Theoretical shelf life base on test results and allowed O_2 level
- Permeability of sealing surface
- Leakage rate of finish.

Food oxidation

Oxygen can enter a package via the wall, a seal, the opening between a closure and a finish or through the closure materials. The presence of oxygen is one of the most important causes of chemical food degradation. It is responsible for lipid oxidation in many food products but also for growth of aerobic microorganisms. Lipid oxidation is takes place after a certain lag time during which hydro peroxides and free radicals are being formed from unsaturated fatty acids. In a second phase, formation of volatile carbonyl compounds takes place, and consequently off-flavors are being formed and the products become rancid.



The unsaturated fatty acid (RH) is being transformed into the unstable peroxiradical (ROO•) and subsequently into a more stable hydroperoxide (ROOH).

$$\mathrm{ROOH} \xrightarrow[\text{fission}]{\text{heat}} \mathrm{RO}\bullet + \bullet\mathrm{OH} \longrightarrow \text{Carbonyls}$$

Beer in plastic

Glass bottles cannot be replaced by plastic bottles without looking at the consequences. Plastic is more susceptible for oxygen permeation than the conventional glass bottles. When oxygen enters a bottled beer it causes lipid oxidation resulting in carbonyl formation influencing the flavor of beer. For example, the stale "cardboard" flavor, which may appear upon storage, is mainly due to very small amounts of trans-2-nonenal, formed during malting and wort production. Also less clearly assignable flavor deterioration occurs as a result of oxidation and target analysis alone cannot provide insight in the chemical causes anymore. For this type of complex issues holistic anlytical approaches are required.

To determine the influence of plastic bottles on the taste of beer various packaging concepts (glass, PET, PEN, multilayers) and closures were compared. The aim was to develop a model to predict staling of beer and to allow the optimisation of the packaging material. Various types of bottles were filled with beer and stored for various weeks at various temperatures (8°C, 23°C and 30°C). After 0, 1, 6 and 12 weeks the samples were assessed by an extensive sensory evaluation. The beer was analyzed by GC-MS. The GC-MS fingerprints of the volatile compounds present in the beer and advanced statistical analysis were used to obtain correlations between changes in

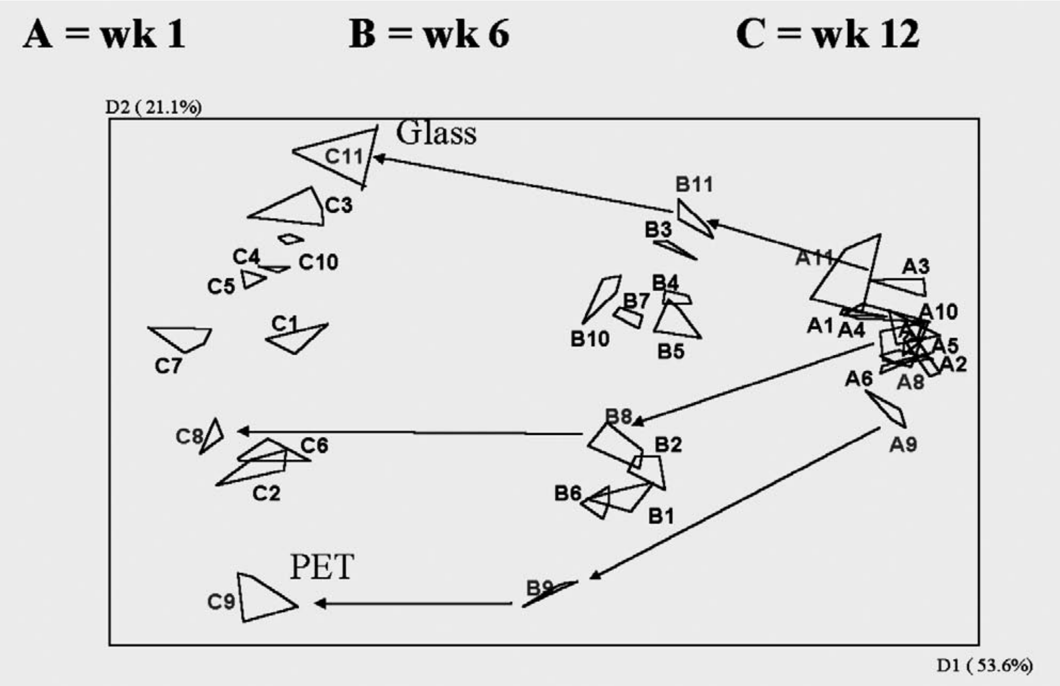


Fig. 4. Discriminant analysis plot of GC-MS profiles of beer stored in various bottles for 1, 6 and 12 weeks. Assignment: A. storage of 1 week; B storage of 6 weeks; C storage of 6 weeks.

sensory quality and changes in the chemical composition. The cluster analysis in Figure 4 gives the difference between beers kept in different bottle concepts. For instance, a clear difference between beer stored in glass (11) and PET (9) can be seen. As was expected, storage in PET resulted in the largest change in odor and taste. This study resulted in a suitable plastic bottle concept (multi-layer bottles, closure with O_2-scavenger) and provided insight in the influence of the packaging on time dependent quality development.

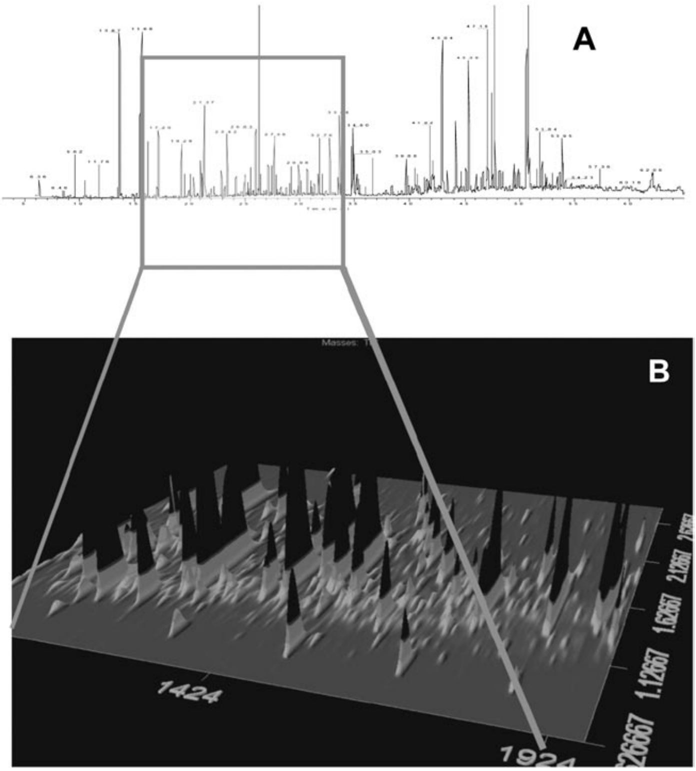


Fig. 5. GCxGC-TOF-MS analysis of a plant extract; A) from this perspective only the first separation (based on boiling point) is visible. B) Helicopter perspective makes second separation (on polarity) visible.

CONCLUSIONS AND FURTHER DIRECTIONS

Depending on the sample complexity and the problem studied target and/or comprehensive analytical techniques are able to obtain insight in relevant key processes. In combination with food packaging research, analytical techniques allow the identification of quality determining markers which than can be applied to select the optimal packaging strategy. However, with the current techniques it is still very difficult to study degradation of complex foods.

In the case of carbonyl patterns in beer, the GC patterns are relatively simple compared to more complex food products.

The next step in measuring the quality of packed complex foods will be the development and application of analytical protocols based on multidimensional LC*LC-MS and GC*GC-MS. The application of these techniques provides more insight into the chemical composition of a food. Currently, TNO is carrying out experiments with a GCxGC equipped with a fast time-of-flight (TOF)-MS detector. In Figure 5A a chromatogram shows a 1-dimensional GC-MS peak pattern of a plant extract. When the multi-dimensional GCxGC pattern of this extract is being plotted (see Figure 5B) it becomes clear that many peaks are co-eluting in the first dimension. These compounds could be separated in the second dimension. In the plot the color of the peaks represents their concentration and the vertical position the elution time.

Multidimensional techniques will provide much more data containing inherently more information. New statistical pattern recognition techniques are being developed at TNO to be able to abstract the useful information from these large datasets.

A HEAD SPACE GAS ANALYZING APPROACH FOR EVALUATING THE SHELF LIFE OF MOZZARELLA CHEESE

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ABSTRACT

A gas evaluating method, based on the analysis of the CO_2 of the head space produced by coliforms, was used to study the shelf life of mozzarella cheese, packaged in dilute salt solution with and without lysozyme and Na_2-EDTA. The data regarding the CO_2 production by coliforms were modeled according to the reparameterized Gompertz equation, in order to calculate the Minimum Detection Time (MDT).

The addition of lysozyme and Na_2-EDTA increased significantly MDT and inhibited the metabolism of coliforms. Moreover, the proposed method showed a good correlation with the standard plate count of coliforms and could be an alternative and simple approach to study the shelf-life of mozzarella-cheese.

Key words: coliforms, lysozyme, minimum detection time, mozzarella cheese

INTRODUCTION

Mozzarella is the most popular member of the “Pasta Filata” cheeses (stretched curds) (Coppola *et al.*, 2001). It is manufactured according to traditional procedures or by using pasteurized milk and commercial starter cultures of lactic acid bacteria (Coppola *et al.*, 2001); moreover, mozzarella cheese is produced by direct acidification with lactic acid, citric acid or glucono-δ-lactone (Parente and Moschetti, 1997).

Within the complex bacterial community of traditional cheeses, lactic acid bacteria are considered to be the dominant microflora, but many indesiderable microorganisms may lead to defects in the appearance, flavour and texture of mozzarella cheese. According to the Italian law (Anonymous, 1997) the coliforms are the test microorganisms for evaluating the shelf-life of mozzarella cheese. The presence of coliforms in cheese is an indication of poor sanitation (Tamime, 2000); they grow rapidly during the first days of storage, producing lactic acid, acetic acid, formic acid, succinic acid, ethanol, 2,3-butylenglycol, H_2 and CO_2 and causing the gassy defect and the swelling of the plastic bags (Tamime, 2000).

The cell load of coliforms of mozzarella cheese is monitored through the pour plate on a selective medium (Violet Red Bile Agar), after 18-24 hr of incubation at 37 °C, but the viable and not cultivable cells cannot be detected.

In the last years many alternative and indirect methods were proposed for evaluating the cell load of different microbial groups in foods or in model systems. A promising technique is the gas-chromatographic approach, because of its simplicity, linearity, high sensitivity and high recovery capacity (Gardini *et al.*, 1997).

On these basis the aim of this work was to study the suitability of a head space gas analyzing approach to evaluate the cell load of total coliforms and to study the shelf-life of mozzarella cheese.

MATERIALS AND METHODS

Samples preparation. Samples of mozzarella cheese of 50 g were purchased from a local market in Foggia (Italy) and packaged with 200 mL of dilute salt solution previously added with a 50 mM phosphate buffer (K_2HPO_4/KH_2PO_4; J.T. Baker, Milan, Italy). This solution was added with lysozyme (0.25 mg*mL^{-1}; Sigma-Aldrich, Milan) and 3.72 (50 mM) or 1.79 g (20 mM) of Na_2-EDTA (J.T. Baker). Samples of mozzarella cheese packaged in dilute salt solution without lysozyme and Na_2-EDTA were used as control. The samples were stored at 4 °C and analyzed after packaging and 1, 3, 6 and 8 days of storage.

Microbiological analyses. 10 g of mozzarella cheese were diluted with 90 mL of a sterile saline solution (0.9% w*v^{-1} NaCl) in Stomacher bag and blended for 1 min with a Stomacher Lab Blender 400 (PBI International, Milan, Italy). Decimal dilutions of cheese homogenates were performed and microbiological counts of total coliforms were carried out on VRBA (Violet Red Bile Agar, Oxoid, Milan), incubated at 37 °C for 18-24 hr. The analyses were performed in duplicate and the data were submitted to one-way analysis of variance (ANOVA) and to Duncan's test, through the software Statistic for Windows (Statsoft, Tulsa, USA).

Evaluation of the metabolic activity of coliforms. The metabolic activity of coliforms was studied by inoculating 1 mL of cheese homogenates in 20 mL vials, containing 10 mL of a synthetic medium (yeast extract 3.0 g*L^{-1}; bacteriological peptone 7.0 g*L^{-1}; NaCl 5.0 g*L^{-1} and biliar salts n. 3 1.5 g*L^{-1}; Oxoid). The vials were ermetically sealed, incubated at 37 °C and analyzed over 72 hr, for monitoring the CO_2 in the head space through a Gas Analyzer Checkmate 9900 O_2/CO_2 (PBI). The data were expressed as v*v^{-1}.

All the analyses were performed in duplicate and the data of each storage time were submitted to one-way ANOVA and modeled according to the Gompertz equation, modified by Zwietering et al. (1990) and reparameterized by Corbo et al. (2006), in order to estimate the Minimum Detection Time (MDT), defined as the time necessary to obtain 3% of CO_2 in the head space (Gardini *et al.*, 1997)

RESULTS AND CONCLUSIONS

Table 1 reports the Gompertz parameters regarding the CO_2 production by coliforms in the head space of sealed vials, at different time of storage of mozzarella cheese at 4 °C.

The R values underlines the high adequacy of the reparameterized Gompertz function to the experimental data. In the control samples the cell load of total coliforms, as determined on VRBA, increased from 3.46 to 5.87 Log CFU*g^{-1} within 8 days (storage time); moreover the increase of this parameter resulted in a light decrease of the lag phase and MDT values of the CO_2 production in the head space. The kinetic parameters A (maximum increase of CO_2) and μ_{max} (rate production) were not affected by the cell load.

The addition of the antimicrobial mixture in the dilute salt solution increased significantly the MDT within 8 days up to 45.87 and 28.19 hr, at 50 and 20 mM of Na_2-EDTA, respectively. On the contrary the cell load of coliforms, as evaluated on VRBA, decreased up to the undetectable level (1 Log CFU*g^{-1}).

Within the experimental time, a strong decrease of the maximum amount of CO_2 produced in the head space was observed; in fact, this parameter attained at 3.40

Table 1. Gompertz parameters regarding the production of CO_2 by coliforms of mozzarella cheese in the head space of sealed vials, incubated at 37 °C.

	A[a]	μ_{max}	λ	MDT	R	Cell load[c]
Control						
0[b]	20.41±0.69	1.21±0.15	9.22±1.98	10.63±1.57	0.999	3.46±0.24[d]
1	20.29±0.83	1.27±0.14	9.44±1.54	10.75±1.21	0.999	3.43±0.19
3	19.37±0.18	1.66±0.07	9.96±0.51	10.98±0.44	0.999	4.07±0.05
6	20.06±0.53	1.32±0.10	9.04±1.00	10.34±0.78	0.999	5.53±0.19
8	20.43±0.57	1.19±0.07	7.29±0.81	8.86±0.55	0.999	5.87±0.08
Na_2-EDTA 50 mM						
0	19.84±0.93	1.55±0.23	11.56±1.54	12.95±1.18	0.994	3.46±0.24
1	20.56±0.99	0.60±0.07	11.92±2.27	14.81±1.52	0.997	3.19±0.21
3	15.52±0.20	0.83±0.16	20.41±0.67	22.83±0.28	0.999	1.22±0.11
6	6.37±0.06	0.51±0.03	19.96±0.32	24.37±0.11	0.999	1.16±0.09
8	3.40±0.12	0.12±0.01	25.20±1.13	45.87±1.13	0.998	-[e]
Na_2-EDTA 20 mM						
0	19.47±0.78	1.85±0.29	11.98±1.38	13.04±1.09	0.996	3.46±0.24
1	19.19±1.60	0.95±0.20	17.41±2.24	19.03±1.89	0.993	3.18±0.25
3	14.64±0.20	0.91±0.04	19.37±0.32	21.68±0.22	0.999	1.93±0.05
6	12.64±0.06	1.47±0.02	22.84±0.05	24.25±0.04	0.999	1.47±0.18
8	10.97±0.05	1.19±0.02	26.32±0.09	28.19±0.05	0.999	-

[a] Gompertz parameters: A, maximum increase of the amount of CO_2 (v*v^{-1}); μ_{max},, maximal CO_2 production rate (CO_2*hr^{-1}); λ, lag phase (hr); MDT (minimum detection time), time (hr) to attain 3% of CO_2 in the head space (Gardini *et al.,* 1997); R, regression coefficient. The parameters are accompanied by the standard error.

[b] Days of storage at 4 °C of mozzarella cheese.

[c] Log CFU*g^{-1}.

[d] Standard deviation.

[e] Cell load < 1 Log CFU*g^{-1}.

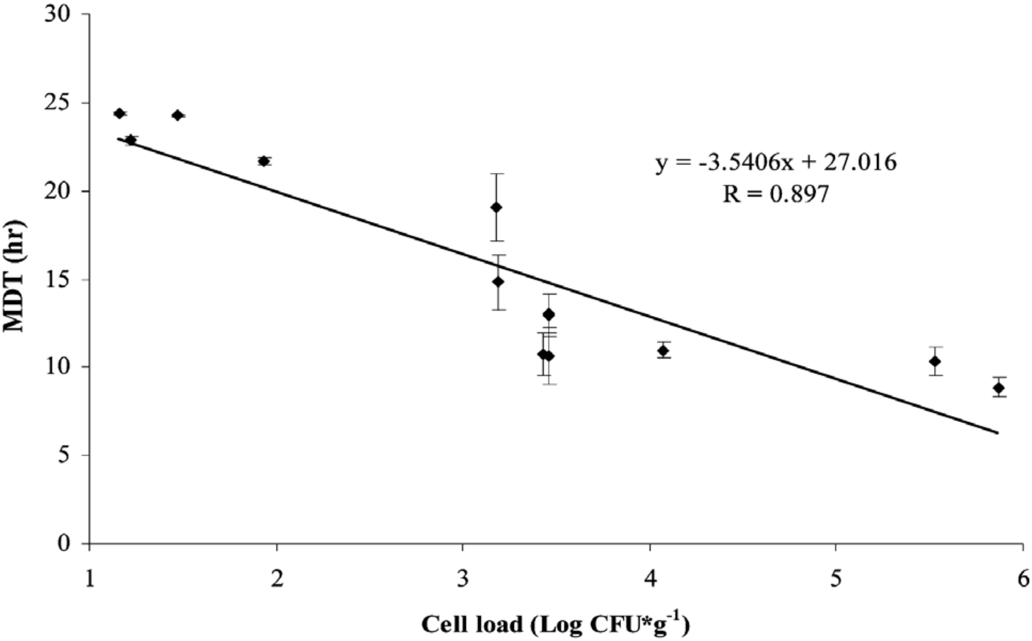

Fig. 1. Linear correlation of Minimum Detection Time ± standard error (MDT) vs cell load of coliforms of mozzarella cheese.

and 10.97% at 50 and 20 mM of Na_2-EDTA, respectively. As in the control sample, the parameter μ_{max} was not influenced by the time or by the antimicrobial mix.

The parameter A was not related linearly to the viable cell load, but it could be considered as an indirect measure of the coliforms metabolism. Moreover it pointed out that lysozyme and Na_2-EDTA stressed the cells within a prolonged storage time; in fact, after 8 days the cell load was at the undetectable level, but there was a residual microbial metabolism, due to a little amount of viable but not cultivable cells, as it could be inferred by the value of the parameter A. On the contrary a good correlation of MDT vs the viable cell load (figure 1) was observed within 1 and 6 Log CFU*g^{-1}, as showed by R value (0.897).

According to the Italian law (Anonymous, 1997), the critical value of coliforms was 5 Log CFU*g^{-1}, corresponding to a 9.3 hr MDT (*break-point*). In our experimental conditions the *break-point* was attained in the control sample within 3rd and 6th day of storage and agreed to the traditional shelf-life (calculated on the basis of the cell load on VRBA), which was estimated in 4.58 days, with a 95% confidence intervals of 3.13-5.88 days (Sinigaglia *et al.*, 2006; unpublished data).

In the samples added with the antimicrobial mix, shelf-life values were > 8 days (running time). These data were confirmed by MDT values, which were always higher than the predicted *break-point*.

This study can be considered as a preliminary step in order to get ready the head space approach for evaluating the shelf-life of mozzarella cheese, but it showed several interesting properties: a) the linear relationship of MDT vs cell load of coliforms of mozzarella cheese; b) the sensitivity for analizyng the physiological state of the cells, in particular to point out the viable but not cultivable cells; c) the suitability for modeling with the Gompertz function; d) the possibility of an indirect evaluation of mozzarella shelf-life with a MDT values *break-point*.

In conclusion the evaluation of the CO_2 in the head space could be regarded as a convenient alternative to the traditional pour plate on VRBA to control the cell load of coliforms and mozzarella cheese shelf-life, even if a better calibration curve has to be defined by analyzing a greater number of samples than that used in this work, in order to calculate a more precise *break-point* for MDT values. Moreover this approach could be convenient for a rapid monitoring of coliform cell load, in order to decide mozzarella balls as safe or not safe for human consumption. The response time is lower than that of the traditional technique, but higher than those observed for some ultrarapid methods, like DEFT and bioluminescence (Gardini *et al.,* 1997). The advantages of the proposed methods, if compared to the ultrarapid techniques, are the simplicity and the low cost, that could allow its diffusion and use in the food industry.

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APPLICATION OF PCR/DGGE AND CLONING ANALYSIS TO STUDY BACTERIAL POPULATION IN MINIMALLY PROCESSED VEGETABLES

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ABSTRACT

In the present study, the microbial ecology of minimally processed vegetables (MPV) packaged under ordinary atmosphere (OA) and modified atmosphere (MA) was studied by culture-independent method. The bacterial DNA was extracted directly from MPV samples and amplification of the 16S rRNA genes was carried out using universal primers. Fingerprints of 1500 bp were obtained by denaturing gradient gel electrophoresis (DGGE). Moreover, clone libraries of PCR fragments were constructed. PCR-DGGE results indicated microbial community structure was similar on both MPV samples packaged under OA and MA and the dominance of plant pathogens. In fact, a total of 8 different 16S rDNA sequences derived from the eubacterial clone library of MPV products, was affiliated to the families *Microbacteriaceae*, *Pseudomonadaceae* and *Enterobacteriaceae.*

Key words: minimally, processed vegetables products, bacterial communities, denaturing gradient gel electrophorens and cloning

INTRODUCTION

Routine methods for detection microrganisms in foods usually use methods which are based on cultivation of bacteria, where only 0.1-3% of the total bacterial population can be grown under laboratory conditions (Amann *et al.*, 1995).

This approach is tedious, time consuming and liable to introduce serious biases to community analyses (Boivin-jahns *et al.*, 1995). Thus, cultivation independent methods based on the 16S rRNA genes are important in the description of microbial communities. In particular, denaturant gradient gel electrophoresis (DGGE) and related temporal gradient gel electrophoresis (TGGE) methods of PCR-amplified rDNA fragment separation have been applied to a variety of environmental studied for analyzing microbial communities (Rantsiou *et al.*, 2005; Handschur *et al.*, 2005) and to profile pathogens directly in food samples (Cocolin *et al.*, 2001). Generally, the principal bacteria present in vegetables are members of *Pseudomonas*, *Enterobacter* or *Erwinia* and lactic acid bacteria (LAB) and to lesser extent species of moulds and yeast (Ahvenainen, 1996). Usually, plant tissues are trimmed, cut and washed during processing for ready to eat salads. The process can alter the integrity of the commodity's tissues and can induce wounding stress (Baur, 2003) changing bacterial communities in the product (Schuenzel and Harrison, 2002). Recently, methods have been developed to increase the shelf-life of vegetables through (Day and Wiktorowicz, 1999; Francis *et al.*, 1999) combining the effect of washing, MA packaging and low temperature microorganisms (Francis *et al.*,1999). However, the occurrence of psychrophilic facultative anaerobic human pathogens can not be excluded since the product is consumed without heating.

In the present study, we describe the application of PCR-DGGE approach combined with the analysis of the clone libraries of the 16S rDNA amplified fragments to study the ecology of MPV products packaged both under ordinary and modified atmosphere, and to monitor the microbial dynamics throughout the storage.

MATERIAL AND METHODS

Salad composition and processing

Minimally processed vegetables used in the present study were taken from the regular production of a commercial company are described in Table 1. Portion of 250 g were packaged in ordinary atmosphere (OA) and modified atmosphere (MA) in polypropylene bags and into a 17 × 21 cm of polyamide-polyethylene film bags (PA/PE), (Technoplast, Reggio Emilia, Italy) respectively. The modified atmosphere packaging was obtain flushed for ca. 5 sec a gas mixture of 90% nitrogen, 6% carbon dioxide, 3 % oxygen (Delta 30, Delta, Brindisi, Italy). All bags were stored at 4°C for 6 days.

Measurements of CO_2, O_2 and pH

The CO_2 and O_2 concentrations in the packages were measured using the gas analysing equipment CheckPoint (PBI Dansensor, Denmark). The pH were measured with pH meter HI 9017 (Hanna Instrument, Italy) according to the manufacturer's recommendations.

Total DNA extraction

Twenty-five gram samples, in triplicates, were homogenized in a stomacher with saline peptone and 4 mL of supernantant were centrifuged (10 min at 8,000 rpm) and the cell *pellets* were resuspended in 1 mL of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0) and recentrifuged. Then, the supernatant was removed and the *pellets* transferred to a sterile screw-cap tube containing 0.3 g of zirconium beads (diameter, 0.1 mm). The samples were treated at 5,000 rpm for 180 sec in a bead beater (Biospec products, Bartlesville, Okla), cooled on ice for 2 min and centrifuged at 13,000 rpm for 5 min. The supernatant fluid was transferred to a sterile tube and stored at -20° C until use.

PCR-DGGE analysis

PCR-DGGE analysis of the 16S rRNA genes was performed as previous reported (Randazzo *et al.*, 2002) PCR amplification was performed in a volume of 50 μL of 20 mM Tris HCl (pH 8.4) containing 50 mM KCl, 3 mM $MgCl_2$, 50 μM each deoxynucleotide triphosphate, 1,25 U of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), 5 pmol each primers and 1 μL of template DNA. The universal PCR primers U968-GC forward and L1401 reverse were used to amplify the V6 to V8 regions of eubacterial 16S rDNA (Nubel *et al.*, 1996). The cycling program was: initial denaturation of DNA for 5 min at 94°C; 35 cycles each consisting of 30s at 94°C, 30s at 56°C and 40s at 68°C; and extension for 7 min at 68°C. PCR products were quantified by electrophoresis on a 1.2% (wt/vol) agarose gel containing ethidium bromide. The DGGE analysis was carried out using the DCode Universal Mutation Detection system (Bio-Rad, Hercules, California) Electrophoresis was performed as previously reported (Randazzo *et al.*, 2002).

Cloning of the 16S rDNA fragment

Three μL of the PCR products were directly ligated into the pGEM-T easy vector (Promega) following the manufaturer's instructions. The appropriate regions of the 16S rDNA in the cell lysates of trasfomants were amplified and their mobilities were compared to the rDNA derived patterns of MPV sample by DGGE (data not shown). The clones that produced a single DGGE amplicon with a melting position identical to one of the dominant bands in the vegetable samples DNA patterns were selected for sequencing analysis. The plasmid insert from the corresponding clones was sequenced from Biodiversity srl Company (Brescia, Italy).

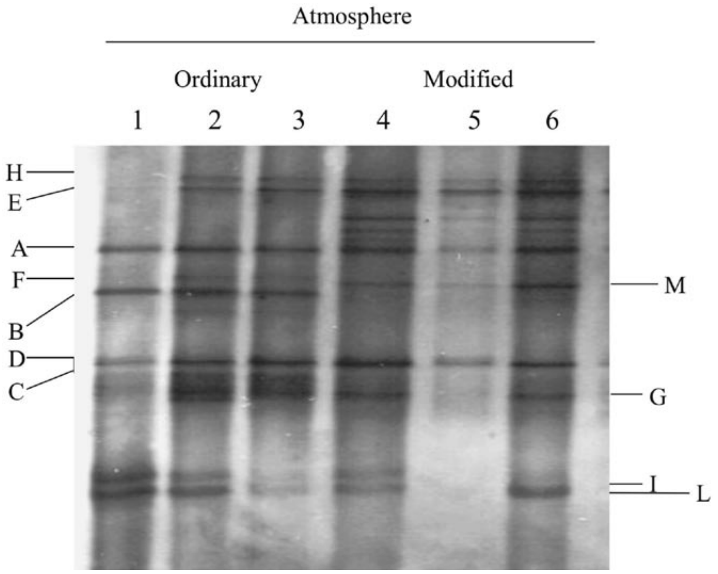

Fig. 1. DGGE of PCR products of the V6 to V8 regions of 16S rDNA that represent the biodiversity in minimally processed vegetables samples packaged both in ordinary and modified atmosphere. Lanes 1, 2 and 3 MPV packaged in ordinary atmosphere at t_0, t_3 and t_6 respectively, lanes 4, 5, and 6 MPV packaged in modified atmosphere at t_0, t_3 and t_6 respectively. The position of band discussed in the text are indicated by letter correspond to species bacteria: A, *Erwinia persicinus*; B, *Pantoae agglomerans*; C, *Pseudomonas rodhesiae*; D, *Acinetobacter* spp. E, *Enterobacter aerogenes*; F, *Pseudomonas siringae*; G, *Pseudomonas putida*; and M, *Pantoae agglomerans*. The identification of the bands is discussed in the text.

RESULTS AND CONCLUSIONS

Measurements of CO_2, O_2 and pH

The concentration of CO_2 and O_2, in the salad packaged under ordinary atmosphere did not change throughout storage, while, as expected, in samples packaged under modified atmosphere the CO_2 concentration increased from 9 % to 19% and the O_2 decreased rapidly during the storage. The pH values, both in ordinary and modified atmosphere, were similar from the day of packaging to the end of storage (data not shown).

PCR-DGGE analysis

DGGE profiles obtained from ordinary and modified atmosphere samples at the same day of packaging showed detectable differences (Fig. 1, lanes 1 and 4) suggesting a variability of the microbial community composition in raw vegetables. To identify the origin of the bands in the DGGE patterns, the migration of the 16S rDNA amplicons of related clones, isolated from the MPV samples packaged both in ordinary and modified atmosphere, were compared with the 16S rDNA DGGE profiles. Samples packaged in OA, ranging from the same day of production to the end of storage, showed similar patterns, with a high number of bands. In particular, the dominant amplicons A, B, and C and the weak band D, that corresponded to *Erwinia persicinus*, *Pantoae agglomerans*, *Pseudomonas rodhesiae* and *Acinetobacter* spp species, respectively, were stably present in the DGGE gel throughout the storage at 4°C (Fig. 1, lanes 1, 2, 3). In contrast, the amplicons E, F and G were determined to be *Enterobacter aerogenes, Pseudomonas siringae* and *Pseudomonas putida*, respectively, and the amplicons H, unidentified by using clone libraries, were not present in the MPV sample at 0 days of storage but appeared after 3 days of storage and remained dominant bands until the end of storage (Fig. 1, lanes 2 and 3). Moreover, DGGE profiles of MPV samples packaged under OA showed two not identified, intense bands I and L. Most of the dominant amplicons appeared in the MPV samples packed under MA showed the same position of those generated from samples packaged under OA, strongly suggesting similarity in the microbial composition during storage. Only the band M, not visible in samples packaged under OA (Fig. 1 lanes 4 to 6), was cloned, sequenced and by comparison with the EMLB database identified as *Pantoa agglomerans.*

The results obtained combining PCR-DGGE analysis of the 16S rDNA fragments and the construction of the clone libraries highlighted a complex bacterial communities and the dominance of plant pathogens in both (OA) and (MA) MPV samples. This evidence allows us to carefully conclude these bacteria could have been selected during the washing step used in the manufacturing of the salads, since plant-pathogenic bacteria are tightly associated with plant matrices.

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PHYSICAL-CHEMICAL CHARACTERISTICS OF FROZEN GROUND BEEF AND AGED BEEF FROM *BOS INDICUS* STEERS SUPPLEMENTED WITH α-TOCOPHEROL ACETATE

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ABSTRACT

The color and lipid stability are important factors, among others, on the quality and acceptability of meat and meat products. The objective of this work was to evaluate the effects of diet supplementation with vitamin E of Nellore steers, on physical and chemical characteristics of frozen stored ground or aged *Quadriceps femoris* (QF) and *Longissimus dorsi* (LD) muscles. These muscles were obtained of 24 animals, with 30 months of age and 279 kg of mean live weight, confined for 98 days. Daily, half of animals received 1000 mg of α-tocopherol acetate (VITE) added to 100 g of corn meal. Other half received 100 g of corn meal without the antioxidant. The animals were slaughter when the fat thickness measured between the 12th and 13th ribs reached 6 mm. Twenty four hours after slaughter, 7 samples of QF were grounded, frozen at -25°C and stored for 0, 1, 2, 3, 4, 5 and 6 months at -18°C. Moreover, 4 samples with 2.5 cm in thickness were cut from the LD, individually vacuum packed and kept between 0 and 1°C for aging for 1, 7, 14 and 21 days. All samples were analyzed to determination of the pH, color, and water holding capacity. Thus, the VITE supplementation improved only the water loss characteristics of frozen ground QF and did not present positive effect over the physical-chemical characteristics of the LD aged for up to 21 days.

Key words: antioxidant, color, stability, pH, Nellore

INTRODUCTION

The color and the lipid stability are limiting factors, among others, on the quality and the acceptability of meat and of meat products. In red meats, such as beef, a bright red color is perceived by consumers as being indicative of freshness, while consumers discriminate against meat which has turned brown in color. The rate of discoloration of meat is believed to be related to the effectiveness of oxidative processes and enzymic reducing systems in controlling metamyoglobin levels in beef (Faustman and others, 1989). Therefore, the muscle stability depends mainly on the balance between the anti-oxidant agents, such as α-tocopherol, and pro-oxidants, including the polyunsaturated fatty acids, and the free iron within the muscle (Mitsumoto and others, 1998).

In the case of refrigerated vacuum packed meat and frozen ground beef, the loss of quality of the product may occur caused also by color changes (Dufrasne and others, 2000). The refrigerated meat and frozen ground beef decolorizing is consequence of myoglobin alterations, commonly due to some lipid oxidation reactions. Therefore, meat flavor and shelf-life are mostly limited by the occurrence of lipid oxidation and surface decolorizing. In this particular case, some technologies has been tested recently, aiming to guarantee the quality of bovine meat during storage, standing out the use of vitamin E in animal feeding prior to slaughtering (Lynch and others, 1999; Dufrasne and others, 2000). Besides retarding lipid oxidation, this antioxidant may diminish myoglobin oxidation.

The objective of this work was to evaluate the effects of diet supplementation with vitamin E (as synthetic α-tocopherol) of Nellore steers, on physical and chemical characteristics of frozen stored ground *Quadriceps femoris* (QF) or aged *Longissimus dorsi* (LD) muscles.

MATERIALS AND METHODS

The studied muscles were obtained of 24 animals, with 30 months of age and 279 kg of mean live weight, confined in Pirassununga, São Paulo state, Brazil, for 98 days with high proportion of concentrate in diet. Daily, half the animals received 1000 mg α-tocopherol acetate added to 100 g of corn meal, the other half received 100 g of corn meal without any additives. The animals were slaughtered when the thickness of under-skin fat, measured between the 12th and 13th ribs with and ultrasound equipment, reached 6 mm.

Twenty four hours after slaughtering, 7 samples of *Quadriceps femoris* (QF) were grounded, frozen at -25°C and stored for 6 months at -18°C. Moreover, 4 samples with 2.5 cm were cutted from the *Longissimus dorsi* (LD), individually vacuum packed and kept between 0 and 1°C, for 21 days, and finally frozen at -25°C until characterizations. There were realized pH analyses, with a digital phmeter (model HI8314, Hanna Instruments), water holding capacity (WHC) calculated as [WHC=We/(Wb+We) x100] were Wb is the weight of the beef and We the weight of the exudate. The color of the meat was determined in triplicates using a Hunter-Lab portable colorimeter (mod. MiniScan XE), using the L*, a*, b* CIElab system scale.

Vitamin E (VITE) and cholesterol analyzes were also realized in the first day of aging for 12 samples of LD. The equipment used for the vitamin E and cholesterol determination was a high performance liquid chromatograph (HP series 050), with simultaneous detection at several wave lengths. The systems were controlled by the software *CHEMSTATION-HP* (Katsanidis and others, 1999). The statistical analyses were done by procedure of software SAS®.

RESULTS AND DISCUSSIONS

No significant differences were observed for the pH and LD values between the VITE and the Control group along the aging time (Figure 1). Also, there was not significant difference between time and treatment for the WHC (Figure 2). However, Mitsumoto and others (1998) studied the vacuum packed LD, kept refrigerated at 1°C for 6 days, and observed that the meat of the animals supplemented with 5000 mg of vitamin E significantly lost less water by exudation than the control samples, maintaining the integrity of the membranes and keeping the sarcoplasmatic components, resulting in less exudation loss. Besides, Jensen and others (1997) did not find significant difference of WHC in the LD of pork supplemented with 100, 200 and 700 mg of α-tocoferyl acetate, 48 after slaughtering, with values of about 3.7, 4.0 and 4.4, respectively.

The L* and a* color parameters had linear effects ($p<0.01$) (Figures 3 and 4), and the b* parameter had cubic effect ($p<0.01$) among the days of aging, for the muscle (LD) but were not affected by the treatments (Figure 5). A possible explanation for the high L* values could be the presence of liquid over the meat surfaces, causing higher moisture and higher values for the 21st day of aging. In general, a* did not vary with the time, almost for all aging periods, therefore, presumably there was not any protecting effect of the membranes due to vitamin E supplementation. However, the loss of color pigment did not occur in the studied times, moreover, in spite of the vacuum package, the color of the meat was recovered after the packaging removal due to the my-

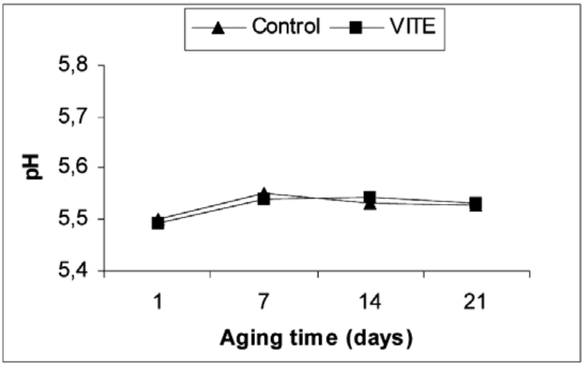

Fig. 1. *Longissimus dorsi* muscle pH variation with aging time.

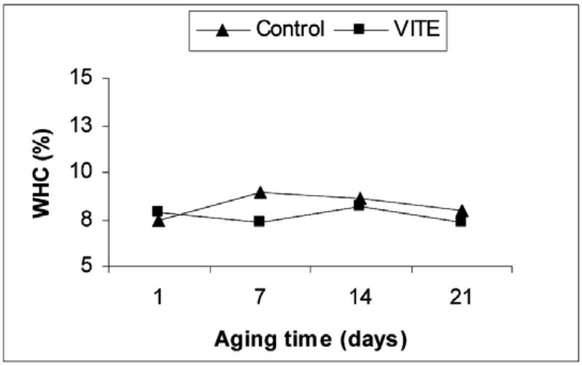

Fig. 2. *Longissimus dorsi* muscle Water holding capacity (WHC) variation with aging time.

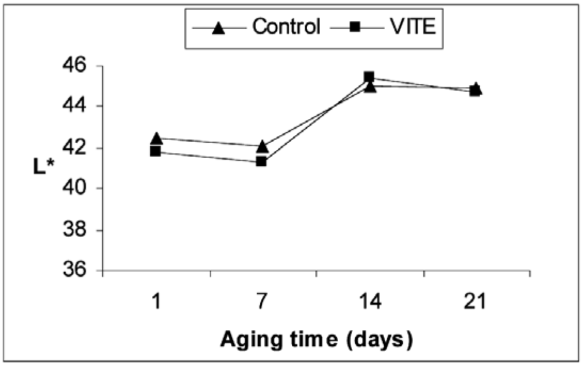

Fig. 3. *Longissimus dorsi* muscle (L*) variation with aging time.

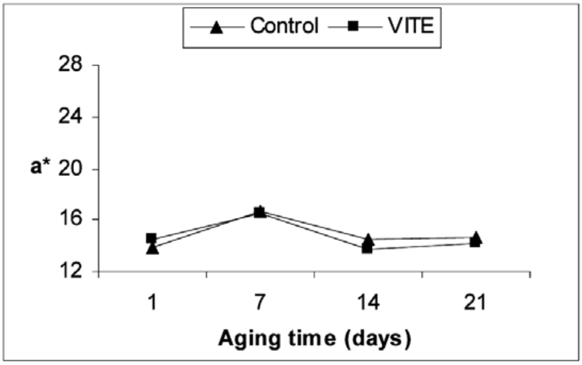

Fig. 4. *Longissimus dorsi* muscle (a*) variation with aging time.

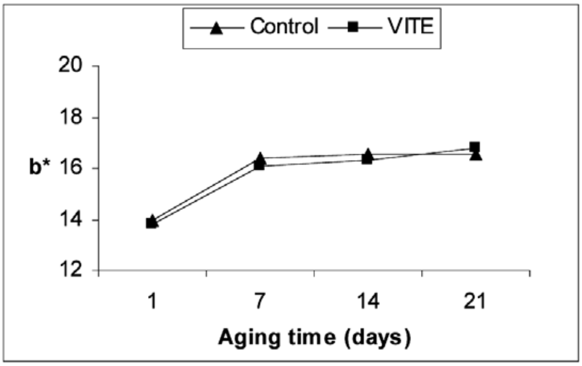

Fig. 5. *Longissimus dorsi* muscle (b*) variation with aging time.

oglobin oxygenation (Mitsumoto and others, 1998). Therefore, the decolorizing rate looks to be related to the efficiency of the oxidative and enzymatic processes, decreasing the meat control systems of the metamyoglobin levels.

Correlating the results from the supplemented animals with the control group, in general a significant difference was not observed between the treatments. However, the vitamin E concentration of the LD, at day 1 of aging, presented significant interaction ($p<0.01$) between the treatments and levels of concentrate, with mean values of 1.7 mg/kg and 4.7 mg/kg, for the control group and VITE, respectively. Dufrasne and others (2000) also found a significant increase of the α-tocopherol concentration in the *Longissimus thoracis* muscle of bovines, stored at 4°C for up to 14 days, with controlled illumination, with values of 1.9 mg/kg of VITE in the meat of studied animals and 0.9 mg/kg in the meat of the control group. These results indicated, that there were possible effects on the lipid oxidation with low α-tocopherol concentration in the aged meat for up to 14 days. However, studies of vitamin E supplementation related to levels of concentrate in bovine feed were not found.

The cholesterol concentration in the LD of VITE supplemented steers and in the control group, were 51.0 mg/100g and 52.5 mg/100 g, respectively, without significant difference. Also, the cholesterol values presented a linear effect ($p<0.01$) with frozen time, but was not affected by treatments.

During the frozen time of the ground beef, the WHC was affected by the treatments ($p<0.01$), with less water lost on animals treated with VITE (Table 1). This result suggests that the VITE supplementation maintained the cell membrane integrity and the lipid protection against oxidation. It was observed a quadratic effect ($p<0.01$) with frozen time.

The pH presented a linear effect ($p<0.01$) with frozen time, but was not affected

Table 1. Means values of water holding capacity (WHC), pH, L*, a* e b* in ground M. *Quadríceps femoris*, during frozen storage for control and vitamin E supplemented groups.

	PHYSICAL-CHEMICAL CHARACTERISTICS					
Treatments	Month	WHC	pH	L*	a*	b*
Control	0	4.45	5.67	44.81	16.36	16.22
	1	7.93	5.68	44.56	15.40	16.28
	2	9.00	5.66	44.71	15.77	16.58
	3	10.14	5.68	41.61	16.71	16.96
	4	8.32	5.67	42.17	14.87	16.07
	5	12.15	5.72	40.79	16.27	16.61
	6	8.44	5.69	42.07	14.45	15.93
Vitamin E	0	4.44	5.70	44.38	16.07	15.96
	1	7.33	5.72	43.77	14.85	15.89
	2	8.19	5.68	44.00	14.93	15.85
	3	7.93	5.71	40.63	16.66	16.51
	4	8.92	5.70	41.81	15.04	15.96
	5	11.22	5.74	40.10	15.86	16.16
	6	9.87	5.70	41.24	15.24	15.90

by treatments. The colour characteristics were not affected by treatments. However L* had cubic effect ($p<0.01$), a* linear ($p<0.05$) and b* quadratic effect ($p<0.01$) with frozen time. Lynch and others (1999) reported that the dietary supplementation of cattle with vitamin E increased tissue α-tocopherol levels in M. *Longissimus dorsi*, M. *Gluteus medius* and M. *Psoas major* and appeared to be an effective means for improving the color and oxidative stability of fresh, frozen and vacuum-packaged beef cuts. Also, they concluded that the differences in lipid stability of different muscles could explain intermuscular variability in meat colour. The lipid stability of the different muscles generally reflects the different color stability of the muscles. In this way, the behavior association of L* and a* seems to indicate losses on the color of the meat. It suggests that vitamin E did not protect the membranes in the ground beef, against the results expected. The VITE supplementation improved only the water holding capacity (WHC) of QF, of frozen ground beef of cattle.

CONCLUSION

In general, it was observed that in spite of the vitamin E high concentration in the supplemented feed, it was not confirmed the protecting effect of this vitamin related to color of meat. The results indicated that at the concentration level of acetate α-tocopherol of the present study, vitamin E supplementation of Nellore bovine did not have influence on the physical and chemical characteristics of meat aged for 21 days and frozen for 6 months.

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PHYSICO-CHEMICAL, SENSORY AND MICROBIOLOGICAL EVALUATIONS OF MINIMALLY PROCESSED BLOOD ORANGES USING A PILOT-PLANT

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ABSTRACT

The possibility of providing an immediately utilizable new technology for citrus factories, has prompted our interest into the study and layout of a pilot-plant for fresh-cut blood orange productions. Fruits of Tarocco 'TDV', Moro 'NL 58-8D-1' and Sanguinello 'NL 49-5-5' cultivars were processed and packaged with three films of different permeability to oxygen in order to evaluate the efficiency of our pilot-plant at producing fresh-cut blood oranges. No important physico-chemical changes were observed in orange slices during 15 storage days. Only a decrease in vitamin C content was noted in almost all the examined cultivars. Regarding the sensory evaluation, Tarocco 'TDV' was the most suitable cultivar to be processed since the best sensory values were observed during storage. Microbiological results showed that fruits, packaged with the three films, had, up to the 6th storage day, lower values than those established by microbiological criteria for minimally processed fruits and vegetables.

Key words: pilot-plant, fresh-cut blood oranges, shelf-life, packaging

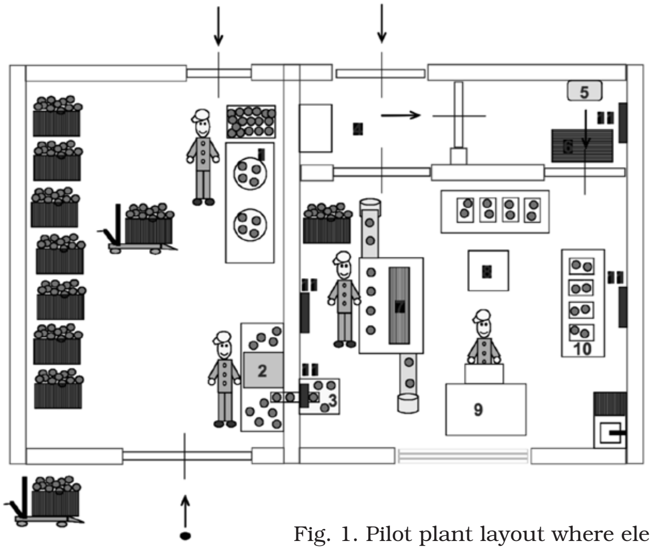
INTRODUCTION

The objective of studies on minimally processed fruits and vegetables is to obtain ready to eat fresh products and extend the shelf life to make transport and retail feasible. Minimal processing removes the natural protection of fruits and vegetables, making them susceptible to desiccation and wilting, and exposes internal tissue to microbes and potentially deleterious endogenous enzymes (Garcia and Barrett, 2002). Therefore, the goal of most processes is to stabilize the product by physical and chemical treatments. In addition, hygienic condition of product and processing environment have great importance, since it aids to prevent potential contaminations. Oranges, being non-climateric fruits, and owing to their morphological and physiological characteristics, present a good potentiality for preparation as minimally processed products (Pretel *et al.*, 1998). Several studies on fresh-cut oranges evaluated only blond varieties, such as autochthon Portugal oranges (Rocha *et al.*, 1995), Salustiana (Pretel *et al.*, 1996; 1998), Hamlin and Valencia (Pao and Petracek, 1997), and Shamouti (Del Caro *et al.*, 2004). Recently, researches are focused on the possibility to produce minimally processed blood oranges, with the aim of finding a new market segment for these fruits (Caggia *et al.*, 2004; Rapisarda *et al.*, 2006). The objective of this work was to plan a layout and realize a pilot-plant for fresh-cut blood orange productions, in order to provide an immediately utilizable new technology for citrus factories and study the physicochemical, microbiological and sensory evaluation of minimally processed blood oranges packaged with three films having a different permeability to gases and stored for 14 days at 4°C.

MATERIALS AND METHODS

Pilot plant description

Two adjoining rooms were equipped with a pilot plant for fresh-cut orange production. The planning required a preliminary definition of "elementary activities" (EA) (Figure 1), "functional spaces" (FS), necessary for the various activities, and their



1) Fruits washing and disinfection;
2) Air-drying fruit system;
3) Steel tunnel for orange entrance in The processing area;
4) Operators wearing;
5) Disinfecting solution dispenser for hands;
6) Rot soaked in sanitized solution;
7) Orange peeling machine;
8) Commercial slicing machine;
9) Heat-sealing vaccum/gas machine;
10) Boxes for sliced fruits;
11) UV Lamps.

Fig. 1. Pilot plant layout where elementary activities (EA) were located.

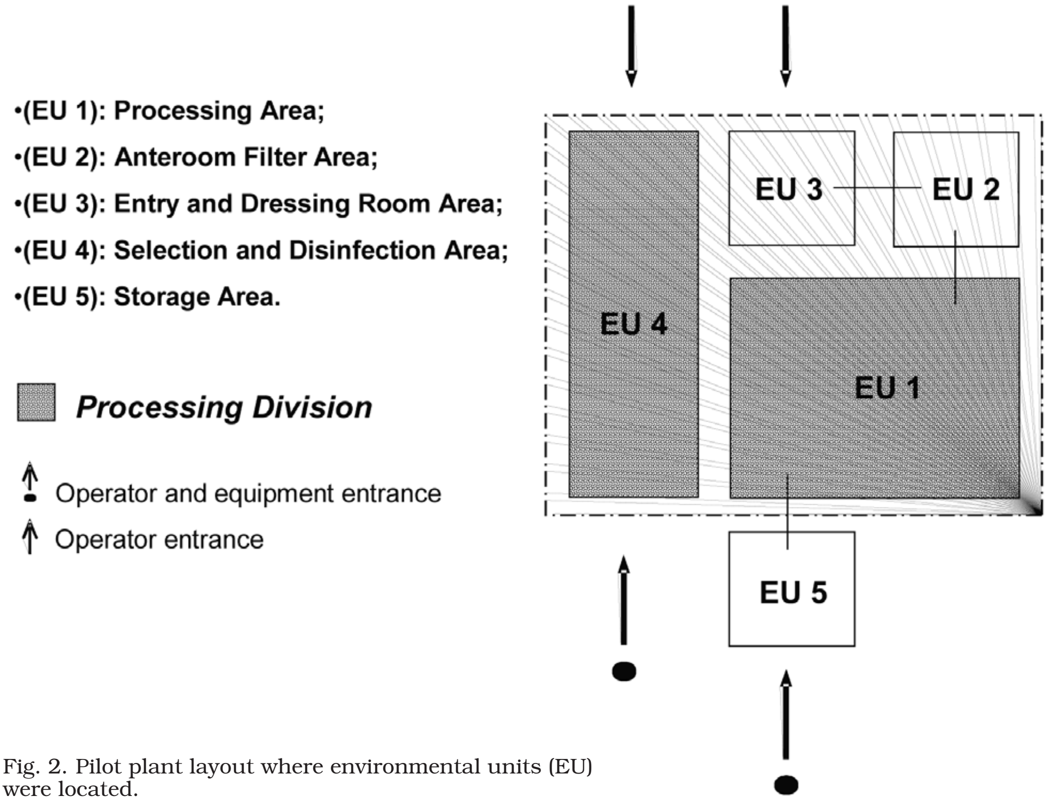


Fig. 2. Pilot plant layout where environmental units (EU) were located.

grouping in "environmental units" (EU). In particular, a total of 5 EU (processing area – EU1, anteroom filter area - EU2, entry and dressing-room area – EU3, selection and disinfection fruits area – EU4, storage area – EU5) were located (Figure 2). The EU4 selection and disinfection area was equipped for fruit washing and disinfection. The entry and dressing-room area (EU3) was used for operators equipment (wearing overalls, caps, gloves, masks and rubber boots) in order to reduce microbial contamination. Moreover, before the operators entered the processing area (EU1), they passed through the anteroom filter area and washed their hands with a disinfected solution and their boots with a rot soaked in a sanitized solution. In the processing area, UV lamps and air-conditioner systems (room temperature 18 ± 1°C) were installed. This area was equipped with a machine suitable for peeling orange fruits (PND mod. BABY, Salerno, Italy), a heat-sealing vacuum/gas machine (TecnoFoodpack mod. Easy Pack, Pavia, Italy) and two commercial slicing machines (model ALA 430, Treviso-Italy). The oranges were transferred from the selection and disinfection fruits area into the processing area through a sanitized tunnel, equipped with UV lamps.

Sample preparation and analyses

Fruits of blood orange cultivars Tarocco 'TDV', Moro 'NL 58-8D-1' and Sanguinello 'NL 49-5-5' were harvested at maturity in the experimental field of the CRA-Istituto

Table 1. Technical characteristics of films[a] used for packaging the different Tarocco clone slices.

Property	**PLASTIC FILM**		
	Film A	**Film B**	**Film C**
Name	NPL Mailar Triplex®	NPL Mailar Monostrato®	NPL Accoppiato (PET12+PP50)®
Stratigraphy	Oriented Nylon (OPA) 17 g/m^2	Polyester (PET) 41.7 g/m^2	Polyester (PET) 16.8 g/m^2
	Adhesive 2 g/m^2	- -	Adhesive 2 g/m^2
	Polyester (PET) 42 g/m^2	- -	Polypropylene (PP) 45.5 g/m^2
Permeability to oxygen	35 cc/m^2/24h	56 cc/m^2/24h	110 cc/m^2/24h
Permeability to water vapour	15 g/m^2/24h	13 g/m^2/24h	45 g/m^2/24h
Permeability to CO_2	-	217 cc/m^2/24h	-
Sealing point	140/220°C	140/220°C	135/150°C

[a] Supplied by TecnoFood-pack (Parma, Italy).

Sperimentale per l'Agrumicoltura (Acireale, Italy). Then oranges were sanitized by immersion for 2 min. in 200 ppm Cl_2 solution, rinsed with distilled water and air-dried. From the EU4, fruits were transferred to the EU1 and mechanically peeled and sliced along the equatorial axis. Fruit slices were packaged, under normal air, with films of different permeability (Table 1) using CPET (crystal polyethylene teraftalate) boxes, if they were packaged with film A and B, and PP (polypropylene) boxes, if they were packaged with film C. Thickness of each orange slice was about 1 cm. Each box, containing four orange slices (about 180 g), was stored at 4±1°C, for about 14 days. During the storage period, groups of five boxes for each orange cultivar were sampled every 3-4 days to determine the physicochemical [head-space gases (O_2 and CO_2), firmness, color, pH, total acidity (TA), total soluble solid (TSS), vitamin C, anthocyanins, glucose, fructose, sucrose]. Statistical elaboration of the physicochemical results was carried out with the STATISTICA 6.0 program for Windows. The physicochemical parameters evolution was evaluated by one way variance analysis (ANOVA) to determine statistical differences during the storage days, and means were separated by Tukey's test at 5 or 1% levels of significance. To simplify the sensory data interpretation, a new parameter, named *sensory acceptability* (SA), was elaborated:

$$SA = \left(\frac{F_n + Fr_n + A_n + S_n + Ac_n + J_n - O_n}{F_0 + Fr_0 + A_0 + S_0 + Ac_0 + J_0 - O_0} \right) \text{ X } 100$$

with F (firmness), Fr (freshness), A (aroma), S (sweetness), Ac (acidity), J (juiciness), O (off-flavor), *n* (storage days: 0, 3, 5, 7, 10, 12), *0* (descriptor values of product at processing day). Sensory acceptability was submitted to one way variance analysis (ANOVA) to verify significant differences among packaging films, during storage days, and the means separation by Tukey's test.

Microbiological analyses [mesophilic viable counts (PCA), yeast and mould (SAB),

Table 2. Change in physicochemical parameters of fresh-cut blood oranges packaged with three films of different permeability to oxygen.

	FILM A							FILM B							FILM C						
Storage days	**0**	**3**	**5**	**7**	**10**	**12**	**14**	**0**	**3**	**5**	**7**	**10**	**12**	**14**	**0**	**3**	**5**	**7**	**10**	**12**	**14**
											MORO										
Vitamin C	41,62 AB	40,75 AB	42,44 AB	43,70 A	38,08 ABC	36,71 BC	31,82 C	47,16 a	45,45 ab	44,21 ab	43,33 ab	42,76 ab	40,38 b	41,79 ab	52,62 A	46,33 AB	44,44 AB	40,09 B	41,34 B	39,15 B	37,59 B
Anthocyanins	179,27 B	190,10 AB	185,50 AB	175,66 B	180,53 AB	212,17 AB	232,51 A	147,52	156,60	164,58	159,98 NS	161,82	156,62	146,36	139,47	140,05	140,22	144,97 NS	144,43	137,57	140,18
a*	14,54	15,32	15,58	14,18 NS	14,16	14,31	16,37	10,95	11,20	11,00	10,72 NS	10,51	10,78	11,92	13,51	13,31	13,20	11,67 NS	11,65	11,15	13,70
											SANGUINELLO										
Vitamin C	57,70 A	58,45 A	55,95 AB	60,14 A	54,14 AB	48,14 AB	45,27 B	55,02	48,42	51,42	51,27 NS	50,11	48,95	51,40	61,81 A	46,91 B	43,97 BC	41,34 BC	41,46 BC	41,58 BC	37,07 C
Anthocyanins	119,14	117,58	119,11	118,37 NS	125,14	124,70	121,36	129,85	129,80	131,40	127,76 NS	132,19	128,61	125,76	133,99	135,98	131,73	135,72 NS	136,26	132,79	133,15
a*	12,88	12,87	12,46	12,68 NS	12,74	12,81	11,63	11,06	10,85	10,49	10,91 NS	11,08	11,25	10,30	7,21	7,24	7,09	7,37 NS	7,06	6,74	6,74
											TAROCCO										
Vitamin C	58,40 A	53,64 AB	53,35 AB	46,41 AB	48,36 AB	48,64 AB	44,44 B	63,68 ab	66,10 a	63,02 ab	60,53 ab	55,70 ab	52,43 b	51,83 b	54,16 a	52,57 ab	38,96 bc	41,36 abc	53,56 a	45,00 abc	38,29 c
Anthocyanins	61,65	47,76	55,49	47,47 NS	53,50	47,13	35,83	65,29	70,04	77,37	77,06 NS	63,94	62,09	56,62	64,67	57,47	55,90	64,42 NS	63,66	46,61	59,85
a*	12,22	13,42	12,46	12,68 NS	13,31	12,71	12,95	11,48 AB	10,85 AB	10,49 B	10,91 AB	11,98 AB	11,75 AB	12,12 A	10,95 A	7,24 BC	7,09 C	10,12 A	9,71 A	9,46 AB	9,60 A

significant differences p≤0.01 - capital letter; p≤0.05 - small letter; n.s. - not significant.

acetic acid bacteria (GYCA), acidophilic bacteria (OSA)] were carried out on groups of three boxes.

RESULTS AND CONCLUSIONS

In Table 2, only vitamin C and color parameter (anthocyanins and a*) data are reported, since no important changes in the other physicochemical parameters were observed during storage. With exception of Sanguinello packaged with film B, a decrease in vitamin C content was observed in fresh-cut oranges of the three cultivars packaged with the different permeability films and stored at 4±1°C for 14 days. This decrease, however, did not lead to an excessive reduction in the antioxidant defenses of the fresh-cut oranges, since the level of this component in the slices, at the end of storage, was about 31-52 mg/100 mL. The substances responsible for the typical color of blood orange flesh are anthocyanins (Rapisarda *et al.*, 2001). These pigments, in fresh-cut fruits, may be degraded by light, oxygen or endogenous enzymes (Dorantes-Alvares and Chiralt, 2000); however, the high acidity of fresh-cut oranges and the persistence of high vitamin C levels during storage, contributed to the inhibition of enzymatic browning processes of the slices and, thus, to the preserving of the slice color. In fact, no significant variations in the anthocyanin content were observed in the three examined cultivar fruits, with the exception of Moro packaged with film A, where an increase of about 23% was observed. This increase, is

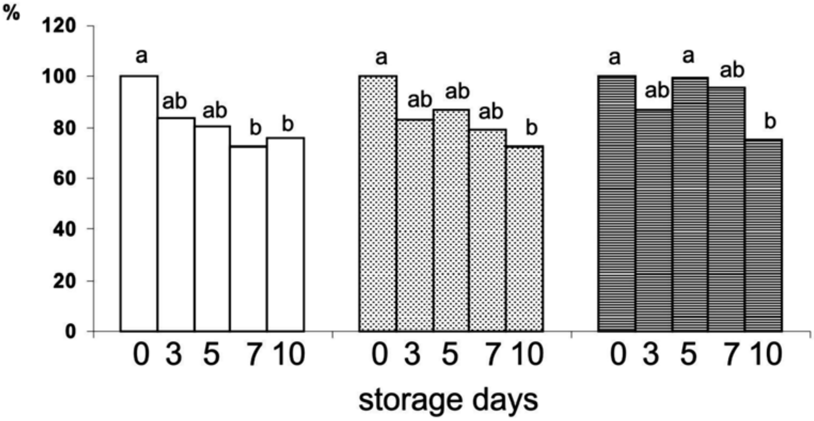


Fig. 3. Change of sensory acceptability (SA) parameter of Moro fresh-cut fruits packaged with three different permeability films.

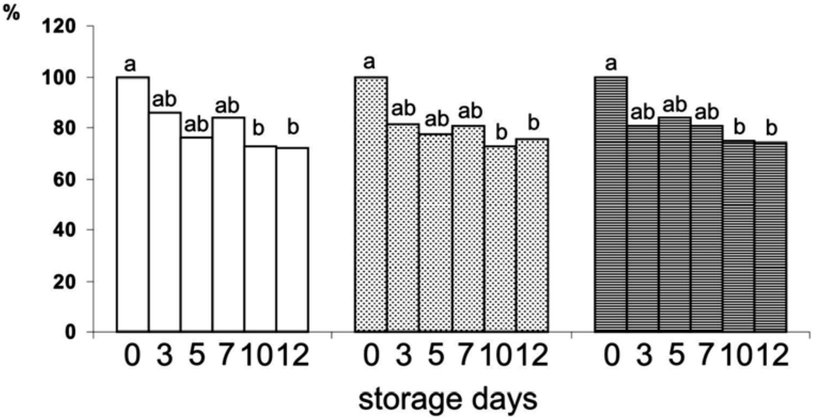


Fig. 4. Change of sensory acceptability (SA) parameter of Sanguinello fresh-cut fruits packaged with three different permeability films.

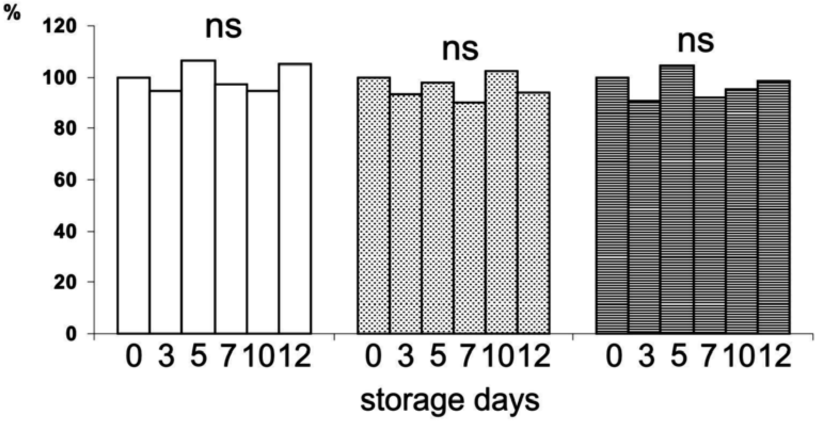


Fig. 5. Change of sensory acceptability (SA) parameter of Tarocco fresh-cut fruits packaged with three different permeability films.

Table 3. Microbiological evolution during storage of fresh-cut blood oranges packaged with the three different permeability films (log cfu/g).

storage days	Film A				Film B				Film C			
	OSA	PCA	SAB	GYCA	OSA	PCA	SAB	GYCA	OSA	PCA	SAB	GYCA
						MORO						
0	2,30	1,57	2,17	2,15	1,34	1,48	1,02	1,15	1,10	1,25	1,30	1,15
3	1,30	1,40	1,50	1,60	1,55	1,70	1,80	2,00	2,30	2,10	2,70	2,55
6	4,04	4,58	4,01	4,81	3,81	4,95	4,05	3,41	7,12	6,80	8,30	7,70
9	7,80	8,15	8,47	8,20	2,00	8,95	2,40	2,90	4,80	5,00	5,60	4,90
12	8,00	8,64	9,43	8,58	7,48	6,84	8,30	8,54	9,00	7,90	9,50	7,00
						SANGUINELLO						
0	<1	<1	<1	<1	<1	<1	<1	<1	<1	1,69	1,79	1,30
3	4,05	2,72	1,56	2,37	0,96	0,96	<1	2,65	<1	1,52	2,00	<1
6	7,53	1,20	7,17	2,76	7,59	5,74	7,55	3,78	1,75	7,61	5,19	2,46
9	5,04	5,85	4,96	5,54	3,83	2,96	<1	3,16	1,21	3,44	3,00	3,26
12	<1	3,96	4,96	5,46	3,96	3,96	<1	5,19	1,45	4,26	3,96	5,19
						TAROCCO						
0	1,20	1,50	1,80	1,00	1,00	1,50	1,30	1,10	<1	<1	<1	<1
3	3,08	2,80	2,50	2,75	2,75	1,53	4,00	3,20	4,20	3,30	5,30	4,50
6	4,25	3,75	4,90	4,00	5,40	5,85	6,00	5,10	8,15	5,60	8,56	7,19
9	4,00	2,80	3,50	2,00	1,00	2,49	3,23	2,05	5,10	5,10	4,26	6,10
12	6,75	6,48	6,37	6,38	6,87	6,79	6,09	4,84	6,99	5,58	6,64	6,07

probably due to the biosynthesis of these pigments induced by low temperature storage (Rapisarda *et al.*, 2001). Moreover, significant changes of the red color index a* for Tarocco 'TDV' packaged with B and C films were observed. No correlation between this color index and anthocyanin content in the slices ($R^2 = 0.08$ for film B, $R^2 = 0.07$ for film C) was observed, due to the high variability in the distribution of the pigments in the juice sacs. Therefore, the choice of the best cultivar and film suitable for the production of minimally processed blood oranges was assigned to sensory and microbiological analyses. Figures 3, 4 and 5 show that the best sensory results were obtained with Tarocco fruits packaged with the three films, since no statistical difference among the storage days was observed. Regarding cv. Sanguinello, a SA decreasing of about 25-28% was observed in products packaged with the three films. A similar behavior was observed in cv. Moro, even if sensory analyses of products packaged with the three films were stopped at the 10th day of storage, since slices were not pleasant to sight. Regarding microbiological analysis results, mould and yeast were the most worrying microrganisms. In fact, orange sample contamination was high after the 6th storage day, but, with regard to the other examined microorganisms, yeast exceeded the CNERNA-CNRS (1996) values (Table 3). Among the cultivars, the worst results were obtained with cv. Moro; in fact, the number of most detected microbial groups, expressed as log CFU/g, was higher than 10^8 especially between the 9th and 12th storage days.

With respect to the packaging films, no important differences in sensory and microbiological values were observed among the different orange cultivars studied.

ACKNOWLEDGEMENT

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VIRGIN OLIVE OIL SHELF LIFE: STUDY OF OIL STABILITY AT ROOM TEMPERATURE AND UNDER ACCELERATED STORAGE CONDITIONS

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ABSTRACT

Virgin olive oil stability is very high in comparison with other vegetable oils. Accelerated shelf-life tests (ASLT) using temperatures around 100 °C are generally employed to estimate the induction period of the oxidation reaction in a short period of time, but no satisfactory correlation has been found with the VOO shelf-life at room temperature. The main goal of this research is, therefore, to study the chemical stability and the autoxidation process of VOO in the attempt to estimate the shelf-life of this product. A good correlation between the time required to reach EU Regulation upper limits for PV, K_{232} and K_{270}, and the temperatures of the assay, up to 60°C, by a potential equation in all samples studied was found. Moreover, a similar slope was obtained; this makes feasible to establish an ASLT method at a temperature below 60°C to estimate shelf-life at room temperature.

Key words: accelerated storage conditions, antioxidants, oxidation, shelf life, virgin olive oil

INTRODUCTION

Virgin olive oil (VOO) is an appreciated product nowadays due to its peculiar sensory characteristics and composition. Its triacylglycerides are mainly composed by oleic acid which confers VOO higher resistance to oxidation than other vegetable oils richer in polyunsaturated fatty acids. It is also a source of minor compounds nutritionally valuable such as α-tocopherol (Vit. E), β-carotene (precursor of vit. A), and phenolic compounds with antioxidant activity, which can play an important role in the prevention of certain diseases such as cardiovascular disease and cancer (Visioli et al, 1998; Simopoulos, 2001).

Although lipid oxidation occurs fairly slowly in VOO at room temperature (Gómez-Alonso, 2005) it is the main cause of olive oil sensory and nutritional quality deterioration and its reaction rate determines the shelf-life of this product. Accelerated shelf-life tests (ASLT) using temperatures around 100 °C are generally employed to estimate the induction period of the oxidation reaction in a short period of time (Abaza, L., 2006; Mateos, R., 2005; Frega, 1999), but no satisfactory correlation has been found with VOO shelf-life at room temperature (Kaya, 1993; Gómez-Alonso, 2005; Mancebo-Campos, 2005). As a consequence, oxidative stability is not recognized as a VOO legal quality index.

The main goal of this research is, therefore, to study the chemical stability and the autoxidation process of VOO in the attempt to estimate the shelf-life of this product.

MATERIALS AND METHODS

Olive oil samples. Seven industrial extra virgin olive oil samples (I-VII) of *Cornicabra* variety, which mainly differed in the phenolic compounds and α-tocopherol content, were employed.

Oxidation studies. Aliquots of 40 ml of each EVOO were stored in darkness at 25, 40, 50 and 60 °C in 25 ml open amber glass bottles. One bottle was taken from the incubator for analysis at scheduled times. All experiments were carried out in duplicate. The induction period was also determined by the Rancimat 679 apparatus, using an oil sample of 3.5 g, with a temperature of 100 °C and an air flow of 10 l/h.

Analytical determinations. *Peroxide value* (PV, meq O_2/kg), *specific extinction coefficients* K_{232} and K_{270} and *fatty acid composition* were measured following the European Regulation EEC 2568/91 (and following amendments) and the *α-tocopherol content* by the AOCS official method Ce 8-89. *Phenolic compounds* were extracted by SPE using a diol cartridge and analyzed by HPLC using a Spherisorb S3 ODS2 column (250 x 4.6 i.d. mm, 5 mm particle size). The mobile phase used during the elution gradient was a mixture of water/acetic acid, methanol and acetonitrile. Syringic acid was added as internal standard.

Statistical analysis was performed using SPSS 11.0 statistical software.

RESULTS AND DISCUSSION

The shelf-life of extra virgin olive oil (EVOO) is very long, if storage conditions are adequate for avoiding lipid oxidation. In the present research, with the aim of studying the effect of temperature on oxidation rate and mechanism and the antioxidants role, the autoxidation process was accelerated by exposing oil to oxygen using open bottles, but in darkness to avoid photooxidation effects.

Figure 1 shows the increment of the peroxide value (PV) and K_{232} with time at room temperature (25°C) in the seven EVOOs studied, which mainly differed in the content of phenols (1,08–3,88 mmol/kg) and α-tocopherol (0,33-0,55 mmol/kg). The

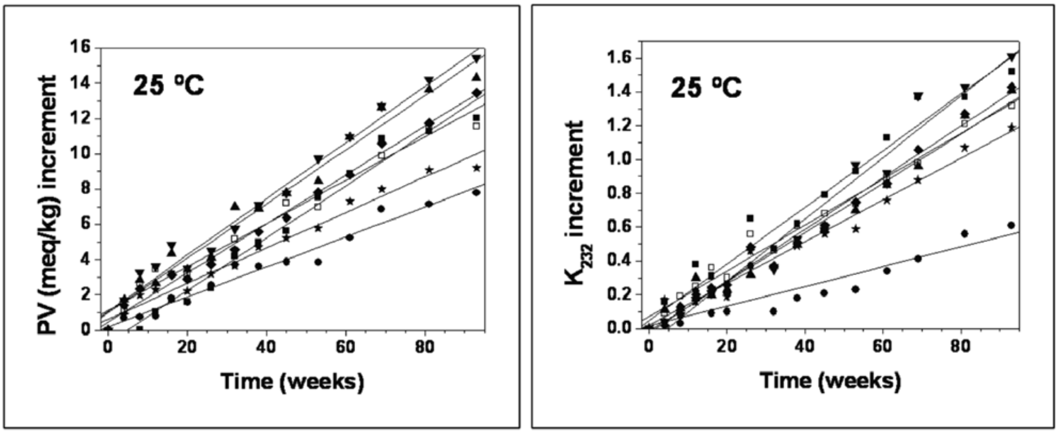

Fig. 1. Evolution of PV and K_{232} of extra virgin olive oil samples at 25°C.
Samples: ■, I; ●, II; ▲, III; ▼, IV; ◆, V; □, VI; ★, VII.

Table 1. Kinetic parameters of PV and K_{232} evolution at 25°C.

	Peroxide value		K_{232}	
Sample	$k / 10^{-10}$ (mol L^{-1} s^{-1})	r^2	$k / 10^{-10}$ (mol L^{-1} s^{-1})	r^2
I	1.19	0.981	0.25	0.964
II	0.65	0.949	0.09	0.809
III	1.21	0.965	0.22	0.978
IV	1.21	0.965	0.27	0.966
V	1.03	0.991	0.23	0.986
VI	0.97	0.969	0.20	0.982
VII	0.80	0.985	0.18	0.976

evolution of these two primary oxidation indexes apparently followed a pseudo-zero-order kinetic ($C = kt + C_0$), whose reaction rate constants are reported in Table 1. All samples presented similar oxidation rates in spite of their different antioxidants content, with the exception of sample II whose lowest oxidation rate could be only explained by its highest chlorophylls content, since no correlation was found between the initial antioxidants content and the oxidation rate at 25°C (Gómez-Alonso, 2005). However, there is no doubt about the great effect

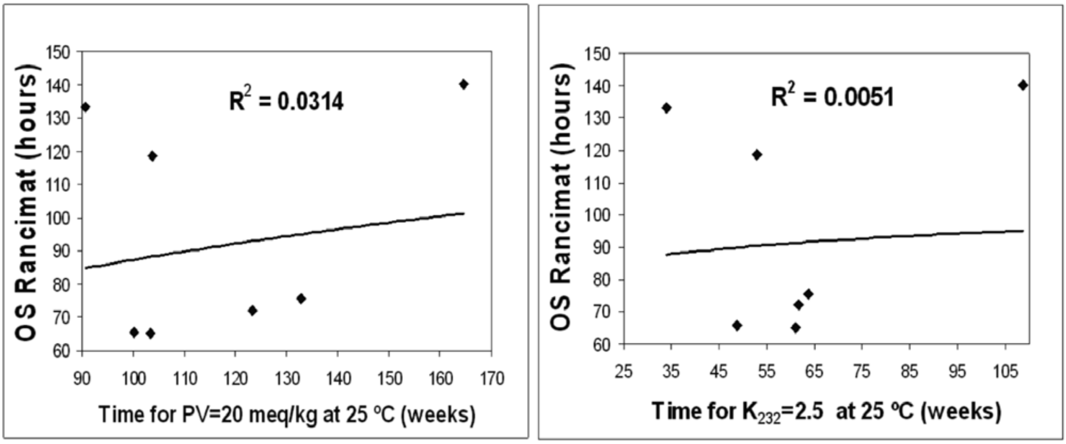

Fig. 2. Correlation between Rancimat oxidative stability and EVOO shelf-life at 25°C.

of these compounds on oil stability since oxidation rate in OO triacylglycerides is much higher (Gómez-Alonso, 2004).

K_{232} was the first quality index to reach the upper limit established by EU Regulations (2.5 for EVOO). In addition, its lowest within-sample variability, highest linearity and best correlation with the loss of polyunsaturated fatty acids ($r^2 = 0.924$, $p < 0.001$) led us to use it as the best parameter to monitor the early stages of lipid oxidation and for shelf-life prediction by means of accelerated methods.

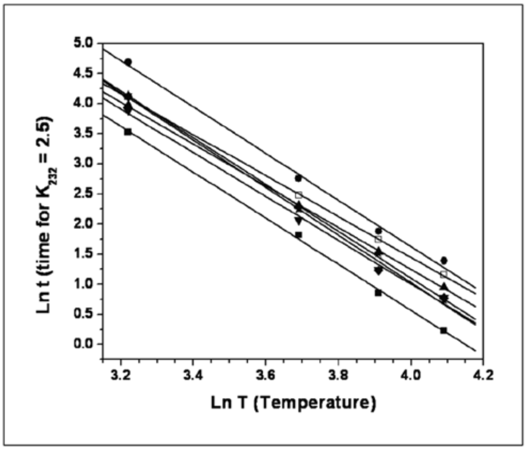

Fig. 3. Relationship between time required to reach K_{232} upper limit and storage temperature.
Samples: ■, I; ●, II; ▲, III; ▼, IV; ◆, V; □, VI; ★, VII.

Figure 2 shows the lack of correlation found between Rancimat induction period and shelf-life at room temperature and under the conditions studied, expressed as the time needed to reach the PV or K_{232} legal limits. This is easily explicable by the fact that at this high temperature the oxidation proceeds drastically and hydroperoxides rapidly decompose to secondary oxidation products. The difference in the oxidation mechanism and therefore in the chemical parameters that affects oxidation rate at different temperatures explains that the good relationship found between phenolic compounds and oxidative stability at 100°C (Abaza, 2006; Salvador, 1999) is not found at lower temperatures, particularly at 25°C.

On the contrary, it was found that the time required to reach Regulation limits for K_{232}, but also for PV and K_{270}, satisfactorily correlates with temperature, up to 60°C, by a potential equation of ($t = a\, T^{b}$) in all samples studied (Figure 3). Moreover, a similar slope was obtained in practically all the samples, which ranged between -3.39 and -3.93 ($R^2 > 0.995$) for the parameter K_{232}. This makes feasible to estab-

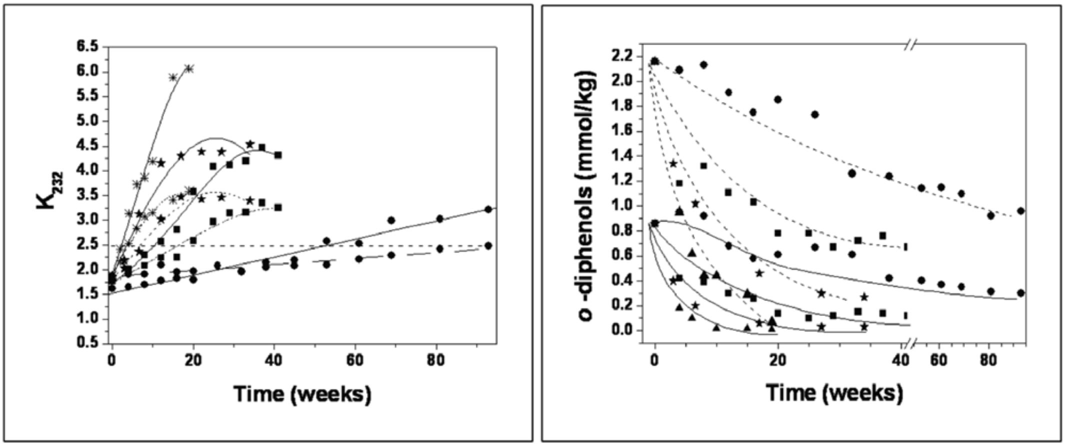

Fig. 4. Evolution of K_{232} and o-diphenols in two EVOO samples at different temperatures.
Temperatures: ●, 25°C; ■, 40°C; ★, 50°C; ▲, 60°C.

lish an accelerated shelf-life testing method (ASLT) at temperature below 60°C to estimate shelf-life at room temperature.

The relationship between the initial composition of EVOO and oil stability, in search of a model to predict shelf-life, is a matter currently under study, and a preliminary relationship involving the initial contents of (i) chlorophyll, (ii) free acidity and (iii) linolenic acid content was found. Moreover, the relationship between the behaviour of individual phenolic compounds, as well as other minor components, and the oxidation reaction at different temperatures (Figure 4) may help to understand the role of these components in EVOO stability through the market period.

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DIFFERENTIAL RESPONSES OF ANTIOXIDANTS TO SALT STRESS IN BERRIES OF WILD TYPE AND NON-RIPENING TOMATO MUTANT

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ABSTRACT

The goal of the present research is to evaluate the possibility of using diluted sea water as alternative irrigation water for tomato (*Lycopersicon esculentum* Mill.) plants, with the purpose to combine economic needs and environmental sustainability with merceological traits and quality of the fruit (antioxidant contents). To reach this goal we have compared the antioxidant power of non-ripening tomato, a genotype with long shelf-life, with that of the wild one. In the berries, picked at well ripe stage, antioxidant activity of both lipophilic and hydrophilic extract, flavonoids and tocopherols have been evaluated.

Key words: antioxidant power, flavonoids, mutant, sea water, tocopherol, tomato

INTRODUCTION

The shelf-life of many leafy and root products has been extended successfully, but for fruit that continue to ripen after harvest the results are far from being satisfactory. Tomato fruits are climacteric, in that the beginning of ripening is associated with a transient burst in the respiration rate and ethylene evolution. Ethylene is known to play a pivotal role in ripening and post-harvest physiology of tomatoes

(Brummel, 2005): this hormone is also involved in plant response to both abiotic and biotic stresses. There are different antioxidant compounds in tomato berry tissues, thus it is relatively difficult and takes long time the measure of each antioxidant component separately. A very fast way to have general information about the content of antioxidants of a complex matrix is to evaluate its total antioxidant capacity, analysing all compounds at a time. In general, tomatoes have been considered as food plants with a medium content of flavonoids that although do not have nutritive value have a physiological effects in humans due to their function as antioxidants and free radical scavengers (Yu *et al.*, 2005). The term Vitamin E refers to a group of tocopherols (α-, β-, γ- and δ-), which differ in structure and biopotency: tocopherols are ubiquitous in higher plants and essential nutrients for animals. Vitamin E functions as a chain-breaking antioxidant that prevents the propagation of free radical reactions (Brigelius-Flohè and Traber, 1999) and the plants are the only organisms that produce tocopherols. It has been shown that salinity induces an oxidative stress, with changes in the amounts of antioxidant molecules involved in defence mechanisms (Meneguzzo *et al.*, 1999) and previously we found that salinity enhances antioxidant capacity of tomato berries (D'Amico *et al.*, 2003a, b). In the present study, the effect of the diluted sea water irrigation on the antioxidant capacity of tomato berry was evaluated and particular attention was focused on the comparison between a mutant genotype with long shelf-life and the wild type of Gimar.

MATERIALS AND METHODS

Plants of two different tomato (*Lycopersicon esculentum* Mill.) genotypes were grown: the genotypes consisted of near isogenic line (LIN) of the cv. Gimar, a tipical "salad" tomato, characterised by semi-determined growth and round, firm and medium-sized fruit. The genotypes differ in homozygous gene affecting ethylene metabolism (non-ripening, *nor*) in comparison with the wild type (WT). Non-ripening tomato (NOR) is characterised by a reduced biosynthesis of ethylene and consequently a reduced synthesis of carotenoids (lycopene and β-carotene), but a better berry shelf-life. Tomato plants were grown in rockwool slabs with recirculating nutrient solutions at a plant density of approximately 3 plants m^{-2} in a heated glasshouse at the University of Pisa (Italy) in the summer 2004. The experiment comprised a control at 3.0 mS/cm, corresponding to salinity level of local irrigation water and a treatment at 8.0 mS/cm, corresponding to 10% of sea water. The antioxidant capacity was measured on lipophilic and hydrophilic fractions of tomato berry and the total activity was the sum of the contribution of each fraction. Tomato samples were extracted as reported by Pellegrini *et al.* (1999) and assayed according to Re *et al.* (1999) using the ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid] method. Tocopherol was determined according to Sgherri *et al.* (2004). Flavonoids extraction and hydrolysis were as described by Crozier *et al.* (1997) and the analysis was performed by HPLC with UV detection at 365 nm (quercetin and kaempferol) and 290 nm (naringenin). The mobile phase was 55% of methanol and 45% of 0.05% trifluoroacetic acid, eluted at flow rate of 1 mL/min.

RESULTS AND DISCUSSION

It has been suggested that the total antioxidant capacity is an index of the healthiness of foods, nevertheless it is important to note that the ability to scavenge an artificial substance, such as the ABTS-radical cation, may not reflect the behaviour towards physiologically relevant radicals and that the radical-scavenging represents only one of the possible mechanisms of antioxidative defence. The antioxidant power can be considered an index of the level of potentially acting *in vivo* antioxidants and it may be considered an adjunctive quality parameter of tomato berry. The antioxidant activity of lipophilic and hydrophilic fractions changed depending on the genotype considered: the higher lipophilic antioxidant capability was found in WT while the higher hydrophilic one in NOR, so the opposite trends of the two fractions did not lead to significant variation in the total power (Fig. 1). In all the berries analysed the most abundant flavonoid was quercetin; kaempferol was present in smaller amount and was undetectable in the control of the WT. Naringenin was not present even in trace amount (Fig. 2). Genotype factor was significant (**), in fact mutant berries had more quercetin than the WT ones. Previous Authors (Crozier *et al.,* 1997; Martinez-Valverde *et al.,* 2002) found marked differences in the quercetin content of different types of tomatoes. In the present research salinity induced an increase of quercetin content both in mutant (30%) and in WT (19%). Kampferol too increased with salt treatment by 120% in mutant and from non detectable amount to 12.7 μg/g DW in the WT. Although kaempferol content of tomato has not been extensively studied, his presence was detected by Martinez-Valverde *et al.* (2002) only in two of the nine cvs they studied, suggesting that flavonoids composition is cv.-dependent. Major tocopherol determined were

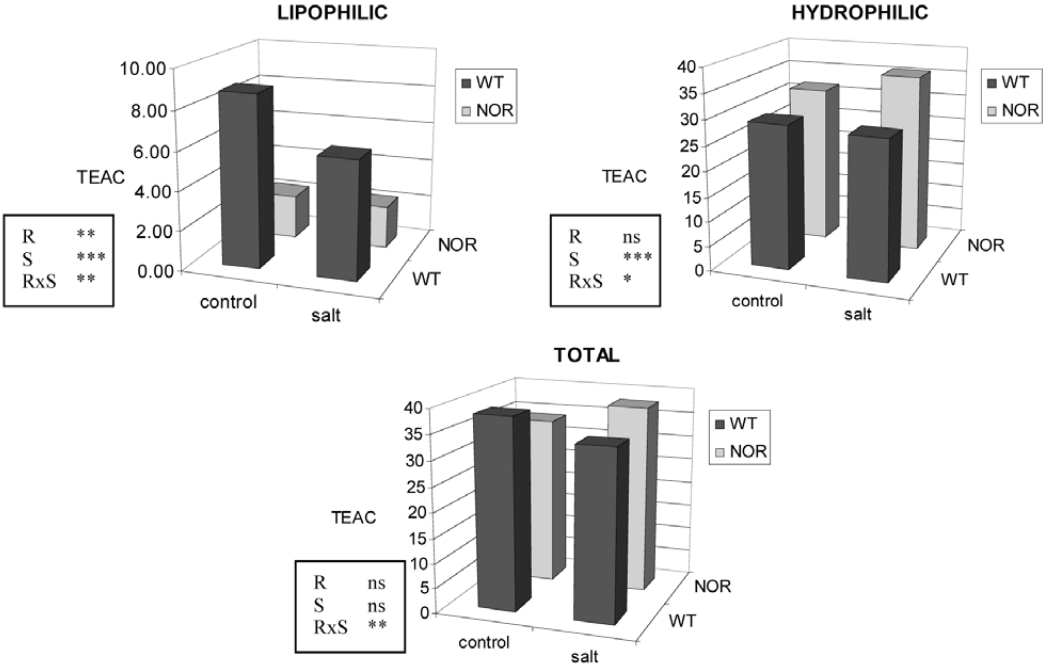

Fig. 1. Effect of genotype (WT and NOR) and sea waters (0, 10% corresponding to 3 and 8 mS/cm^{-1}, respectively) on antioxidant activity of lipophilic, hydrophilic and total extracts of tomato berries. Two-way analysis of variance (ANOVA) was applied to data regarding the interaction between two factors of variation (ripening stage - R and salinity - S). Significant differences between treatments are marked by asterisks: *, **, *** significant at P ≤ 0.05, 0.01, 0.001, respectively; ns= non significant.

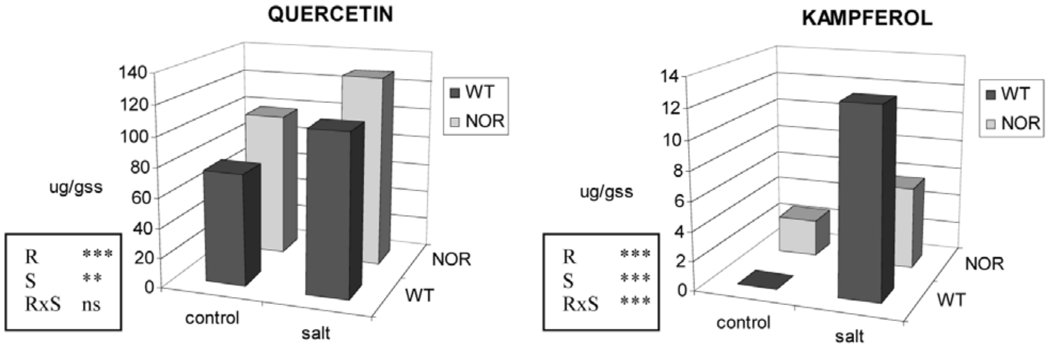

Fig. 2. Effect of genotype (WT and NOR) and sea waters (0, 10% corresponding to 3 and 8 mS/cm^{-1}, respectively) upon quercetin and kampferol content of tomato berries. Two-way analysis of variance (ANOVA) was applied to data regarding the interaction between two factors of variation (ripening stage - R and salinity - S). Significant differences between treatments are marked by asterisks: *, **, *** significant at $P \leq 0.05$, 0.01, 0.001, respectively; ns= non significant.

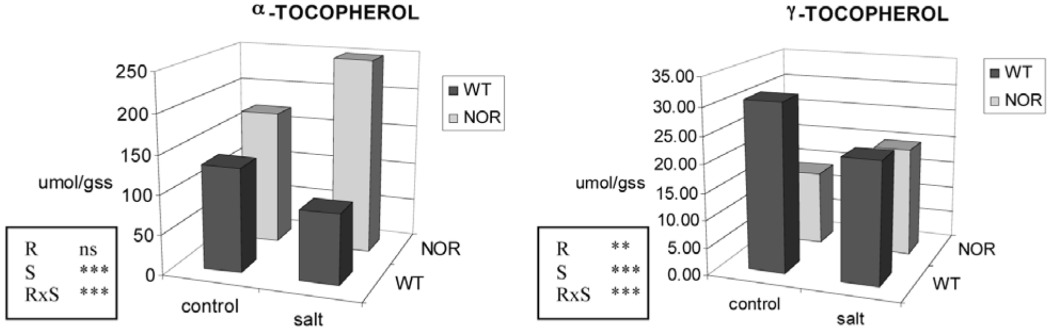

Fig. 3. Effect of genotype (WT and NOR) and sea waters (0, 10% corresponding to 3 and 8 mS/cm^{-1}, respectively) on α- and γ-tocopherol content of tomato berries. Two-way analysis of variance (ANOVA) was applied to data regarding the interaction between two factors of variation (ripening stage - R and salinity - S). Significant differences between treatments are marked by asterisks: *, **, *** significant at $P \leq 0.05$, 0.01, 0.001, respectively; ns= non significant.

α- and γ-tocopherol, whereas δ-tocopherol was found only in trace amount and no β-tocopherol was found. The amount of α-tocopherol was higher in NOR than in WT while the opposite was found for γ-tocopherol (Fig. 3). The cv.-dependence of the different forms of tocopherol found by Abushita *et al.* (2000) is confirmed also in the present study. The strong dominance of α-tocopherol is particularly important because it is considered as the most active form of vitamin E. Sea water irrigation effect showed an opposite trend in the two cvs: there was an increase in WT and a decrease in NOR of both tocopherol forms. In addition, we can notice that the trend of lipophilic antioxidant power does not follow the tocopherol one, likely because other lipophilic substances, such as lycopene and carotenoids do play a role in determining the lipophilic antioxidants amount. The response to salinity might be cv.-dependent, in fact in addition to the genetic background of the plants, even growing conditions represent a pivotal factor that affects the antioxidant content of the tomato berry. In the case of NOR genotype sea water improved flavonoids and α-tocopherol contents so that, keeping in mind the productive purpose of crops as well as the possible improvement of the shelf-life period, the possibility to use brackish water to improve antioxidants amount might be real.

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STUDY ON RELATIONSHIPS BETWEEN FOOD KINETIC STATE AND SOME REACTIONS OF FOOD DEGRADATION

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ABSTRACT

It is well known that food matrixes may exist as high viscous glass, rubbery or liquid states. Glassy state is defined as an amorphous very high viscosity and kinetically stable system; instead rubbery state is defined as an amorphous low viscosity and kinetically unstable system. Concerning food, moisture content is the most important component that can act as plasticizer, modifying the glassy transition temperature (Tg). Traditionally a food in glassy state ($T < Tg$) will be considered stable because the translational mobility of water is considered to be non-existent. Instead a food stored at $T > Tg$ will be in an unstable state and all reaction degradation will occur in short time. Some scientists studied the effect of moisture content on kinetic state of food and on degradation rate, but literature concerning the effect of glassy or rubbery state on the rate of degradation reactions is not exhaustive. We put a question: which would be the rate of reaction of some degradation if a food is at $T > Tg + T_i$°C? This work will attempt to answer, dealing with the eventual connections between food kinetic state and rate of some degradation reactions.

Key word: rubbery state, apples, diffusion coefficient, degradative reactions, NMR

INTRODUCTION

Moisture content is the most important factor that affects the functional cells, biological and chemical reactions of food. Usually, the state of water is measured by water activity value, but in 1991 Franks showed that this parameter cannot explain some important aspects like behaviour of some ions in aqueous solution, colligative properties, microbial growth. Also, with theoretical point of view BET's and GAB's equation cannot be used with water activity parameter because a lot of assumptions are not true for food. So, in the 1991 the importance of physical state of food such as crystalline state, rubbery and glassy state on reaction degradations was highlighted from Slade and Levine. This approach states that below glassy transition temperature a significant arrest of translational mobility occurs and degradation reactions cannot occur. In the rubbery state the viscosity of system is sufficiently low to allow chemical and biological reactions like Maillard reaction, enzyme inactivation, enzymatic reactions. After the publication of Slade and Levine's paper many researches concerning the effects of kinetical state of food on some reactions degradation were performed (Bell, 1995; Schebor *et al.,* 1999) but despite these papers, several questions are still open. For example, it is well known that Maillard reaction can occur at T-Tg < 0°C (Bell, 1995; Schebor *et al.,* 1999) but is not completely clear why it occurs. Some scientists supposed that in glassy state model system at obtained by free-drying an high local concentration of reactants exist and in this way translational motion of chemical compound is not necessary to obtain Maillard reaction. Instead other researchers supposed that the reactants can keep a sufficient level of mobility inside holes or defects of glassy state model systems. Also, it is well known the paradox of translational motion according to which a total decoupling between water diffusivity and macroscopic viscosity exists (Parker and Ring, 1995; Tromp, 1997; Hall *et al.,* 1997). Theoretically, in the rubbery state the viscosity should be sufficiently low to allow reactions degradation but many researchers showed different behaviour. Bell et al (1998) showed that glycine loss reduced during Maillard reaction when model systems goes through Tg and the rate of browning not significantly increases. Despite these papers, we were unable to find scientific papers regarding the effects of mobility on reaction degradation in real food.

Osmotic dehydration (OD) is a very important process to obtain the partial dewatering of vegetables. Industry use osmotic dehydration to obtain candy and as pre-treatment before freeze-drying and freeze in order to avoid aroma loss and textural changes during thawing. Rastogi et al. (2000) proposed a mechanism of mass transfer during OD according to which dehydration front moves toward the centre of sample; three regions with different coefficients diffusion and disintegration index exists. It is well known that OD produce some physical and chemical changes (Chiralt and Talens, 2005). For instance, Mavroudis et al (2004) showed that the first layers of osmotically treated apples cells in sucrose solution (50 % p/p) died after 20 minutes. The aim of this paper was to study the effect of kinetical state of osmotically treated apple samples on enzymatic browning in terms of mobility water by DSC and NMR techniques.

MATERIALS AND METHODS

Apples (Granny Smith) and commercial sucrose were purchased in local market. After washing apples were cut into cylindrical shape of 15x20 mm using a cork borer. Osmotic solutions were prepared dissolving sucrose in distillate water to obtain a concentration of 50% (p/p). A product: solution mass fraction of 20:1 was used. All experiments were carried out keeping an agitation rate of 400 rpm by using magnetic stirrers (Fisher Scientific mod. Isotemp). After the treatment, apples cylinders were washed with water for few seconds to remove the adherent osmotic solution and gently blotted with tissue paper. Moisture content expressed as g water/g dry solid was measured drying samples in a vacuum oven at 70°C till a weight constant was reached. All treatments were performed in triplicate and an average value was used. Colour parameters were measured by a colour meter Chromameter CR-300 (Minolta, Japan). Diffusion coefficients were calculated on the base of Fick's second law equation that, for finite cylinder of diameter 2r and eight 2l, can be solved by superimposition of the solution for an infinitive cylinder and a semi infinite slab (Crank, 1975; Rastogi, Raghavarao, Niranjan & Knorr, 2002). All data were analyzed using Matlab ver. 6.5 (The Mathworks, USA). DSC analysis were performed using Perkin Elmer series 7 calibrated with indium. Samples of 6-10 mg of apples were placed in aluminium DSC pans and a empty pan was used as reference. Sample were scanned from -60°C to 150°C at a heating rate of 10°C/min. For MRI experiments during osmotic dehydration time treatment, cylindrical apple samples were insert into a 15x45 mm glass vials (Fisher Scientific, USA) and cup was modified to allow recycle of osmotic solution. A variable flow mini-pump mod. 3386 (Control Company, Friendswood, USA) was used to recycle solution into a head space of vials to avoid change in osmotic solution concentration. Mini pump was connected to glass vials with plastic tubing with size 1/16x1/8 inch (Tygon, Saint-Gobain Performance Plastics, Akron, OH, USA). Two dimensional proton images from the centre region of the sample container were obtained using a 7T super-conducting magnet in conjunction with a Biospec console (Bruker Biospin MRI Inc., Billerica, MA). Spin-spin relaxation times were estimated from multi-echo image data sets with an echo time 5.5 ms. The spin-lattice relaxation times were determined from a saturation recovery experiment and a standard pulsed-gradient spin echo experiment was used to measure the diffusion coefficient. The field-of-view was 4.8 cm with a slice thickness of 4 mm, echo time was 5.5. The in-plane resolution was ~750 µm / pixel. All data were analyzed using the Curvefit Toolbox in Matlab® R2006a (The Mathworks, Natick, MA, USA).

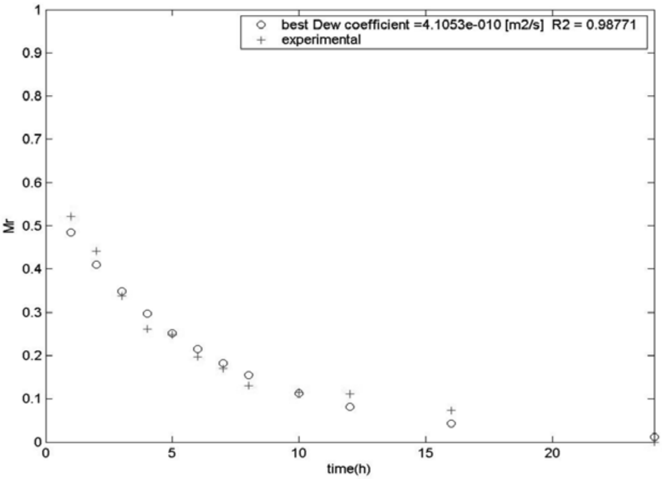

Fig. 1. Variation of moisture ratio values as a function of treatment time.

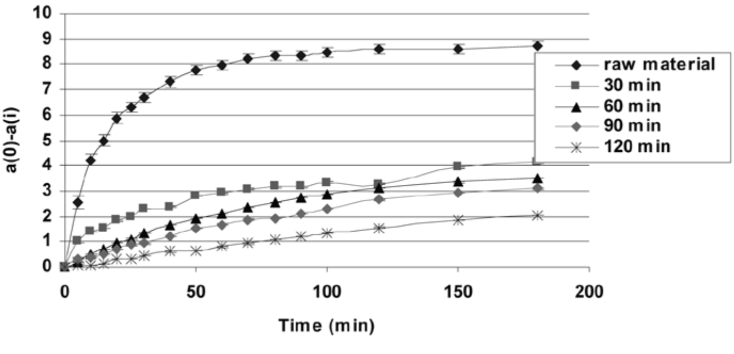

Fig. 2. Red index (a) trend of OD apple samples as a function of time.

RESULTS AND CONCLUSION

In figure 1 it is possible to observe the variation of moisture ratio of apple samples as a function of time treatment. A typical reduction trend of water loss is shown. In the same figure are present experimental and predicted values calculated by Fick's law equation. A significant coefficient regression ($r^2=0.98$) was obtained and we calculated a diffusion coefficient of water Dew = 4.1053e^{-10} m^2/s. In figure 2 is shown the trend of red index (a) of osmotically treated apple samples for the first 2 hours of treatment. It is clear that the rate of enzymatic browning reduces with the increase of OD time.

The constant rate of red index degradation for raw material was 0.2845Δamin^{-1}, 0.0834 Δamin^{-1}, 0.0476Δa*min^{-1}, 0.0301Δa*min^{-1} and 0.0148Δa*min^{-1} respectively for raw material and for 30, 60, 90 and 120 minutes. According with our results, Quiles et al (2005) showed that polyphenoloxidase activity of OD apples decreased from 0.43ΔAmin^{-1} of fresh apples, to 0.23ΔAmin^{-1} and 0.04ΔA min^{-1} for 2 h and 8 h respectively. Water activity values were ranged between 0.985 and 0.943 and all T-Tg values were inclusive between 61°C and 69°C (data not shown). The different rate of EB cannot be explain in terms of water activity and Tg approach in fact all samples during colour indexes determination (carried out at room temperature) were in the rubbery state with a difference of only 8°C. In terms of glassy state approach this low difference in Tg values had to shown a similar rate of EB. Moreover if the first layer of cells after OD treatment died (as reported by Mavroudis, 2004), a constant trend would be expected. In figure 4 the MR images of spin-spin relaxation time (T2) of OD apple samples are shown as a function of treatment time. It is clear that the surface in contact with sucrose solution decreases T2 values with increasing the treatment time. According to Rastogi *et al.*, (2000) we showed the existence of dehydration front that moves through sample during time treatment and a decreasing in terms of mobility water on the surface in contact with osmotic solution. In figure 5 are showed the T2 profile along the

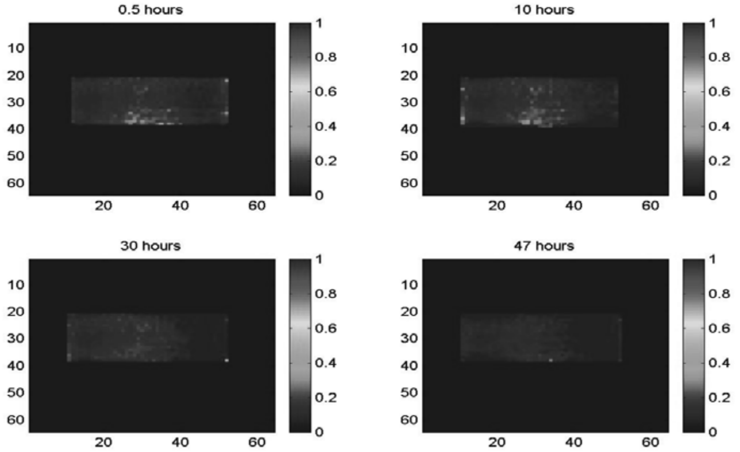

Fig. 3. MR images of OD apple samples after different time treatment.

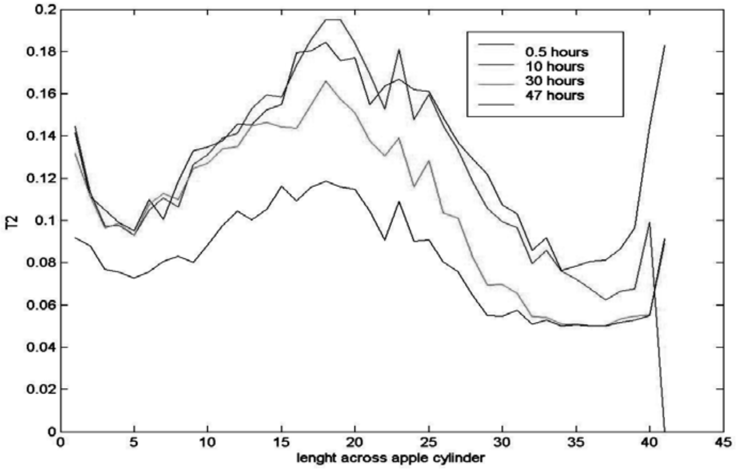

Fig. 4. T2 profiles along the length of OD apple samples.

length of OD apple samples at 0.5, 10, 30 and 47 hours of soaking time in sucrose solution. All samples show high T2 values at the centre of apple cylinder and low values at the edge. It can be observed that T2 values decrease with increasing the treatment time. Samples treated for 0.5 and 10 hours show significant difference only in the first few mm in contact with osmotic solution (between 35 and 40 mm). Samples treated for 30 hours show a significant reduction of T2 values between 15 and 40 mm while the surface far from the osmotic solution (from 0 to 15 mm) shows T2 values similar at 0.5 h and 10h OD apples. Samples treated for 47 hours show a similar value of 30 h samples in the first few millimeters (32 – 40 mm). These regions appeared completely dehydrated and shrunk. Starting to 30 mm it can be observed the decoupling of T2 values. According to this result we supposed that the reduction of mobility water at surface could explain the reduction of measured EB. Further investigation will be necessary to better understand the phenomena.

ACKNOWLEDGEMENTS

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MICROBIAL EVOLUTION IN VACUUM-PACKED MIXTURES OF GRATED PROCESSED CHEESES DURING COLD STORAGE

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ABSTRACT

The objective of this study was to evaluate the survival of *Escherichia coli* and Total Coliforms in 20 similar productions of grated processed cheeses after the introduction of a microbiologically contaminated dairy product in various concentrations. The obtained mixtures were vacuum-packed in thermosealable plastic films and subsequently stored at 2 ± 2°C for eight weeks. The results were used to obtain four linear expressions for the inactivation of Coliforms. In general, the survival of *E.coli* seemed to be extremely difficult in all the contaminated mixtures, while the speed of inactivation decreased when the chemical composition of grated cheeses was theoretically less favourable to Coliforms. The results related to Total Coliform counts seemed to follow the same pattern, but the inactivation was less evident. These findings seem to imply the crucial role of other competitive microorganisms. In fact, all the packed mixtures showed a number of fungal mycelia after 54 days at most. Consequently, it can be supposed that the higher the presence of spoiling moulds in the above-mentioned dairy products, the lower the temporal survival of *E.coli* and Coliforms. Further research is needed to verify this hypothesis.

Key words: processed cheeses, vacuum-package, microbial inactivation, food contamination

INTRODUCTION

At the present time, a great competition occurs between the traditional ripened cheeses and the processed dairy products. By the microbiological point of view, the "grated processed cheeses" should be very satisfactory. In fact, the microorganisms that are normally present in the employed raw materials are drastically reduced by means of a melting process (conditions: 90-100°C, 30 minutes) in appropriate steam mixers. The resulting melted mixtures are cooled and eventually dried to reach the desired dry matter content (60% or more on the total weight) and pH value (5.0). Normally, water activity (a_w) values are ≤ 0.90 in these processed cheeses. The final product, usually grated and packed in modified atmosphere, is particularly suitable for the production of prepared pasta plates, biscuits, dehydrated soups, and typical sauces. It can be inferred that grated processed cheeses are very unfavourable culture media for pathogen bacteria and spoiling microorganisms as moulds. However, grated cheeses are often contaminated by fungal spores (post-contamination events).

One of the more frequent causes for fungal contamination in grated processed products is the addition of different cheeses with high moisture ($\geq$ 60%), pH (6.2-6.7), a_w (0.97- 0.99) and Total Viable Counts (10^7 C.F.U./gram, excluding lactic acid bacteria). If the resulting mixture, prepared before the packaging or the food preparation (sauces, soups, etc.), shows sufficient pH and a_w values, the recovery of spoiling and pathogen microorganisms cannot be excluded.

The objective of this study was to evaluate the kinetics of inactivation for *E.coli* and Total Coliforms in a number of vacuum-packed mixtures of grated processed cheeses during cold storage. The creation of a detailed safety protocol for the production and the manipulation of grated dairy products is the final purpose.

MATERIALS AND METHODS

Experimental design. Two different typologies of dairy products (Hard Processed Cheese, HPC; Soft Processed Cheese, SPC) were weekly sampled in a dairy plant during a 6-months period. Hard cheeses had to show low moisture, pH and Total Viable Count values. On the other hand, Soft cheeses had to exhibit high moisture, pH and TVC values. Every week, four 1,000-g portions were obtained from the HPC sample and separately grated in an Österizer mixer (Sunbeam-Oster Co., Inc., Boca Raton, Illinois) with different quantities of the contemporarily sampled SPC. So, four mixtures of Grated Processed Cheese (GPC) with 5, 10, 20 and 30% (w/w) of SPC were obtained per week. All the mixtures were promptly divided in five 200-g subsamples. Each subsample was vacuum-packed from a commercial processor and immediately stored at 2 ± 2°C. Four of the subsamples representing a determined mixture were presented for microbiological analyses on days 14, 28, 42 and 54 from the date of production. The last package was refrigerated until the visible presence of fungal mycelia. In addiction, all dairy products and their related mixtures were presented for a number of chemical analyses.

Microbiological analyses. *Escherichia coli* (EC) and Total Coliforms (TC) were enumerated in duplicate on Petrifilm™ *E. coli*/Coliform Count (EC) Plate (3M Microbiology, St. Paul, MN, USA) according to AOAC 991.14 (AOAC® Official Methods℠). The colony counts were converted into log units and named Log EC and Log TC respectively.

Physicochemical analyses. The moisture content (MC) was analysed with an internally validated method by comparison to FIL-IDF 5A: 1969. The fat content (FC) was determined according to AFNOR NF V 04- 287: 1972. The pH was measured with the pH 538 pH-meter and the electrode Sentix 41(WTW GmbH, Germany). The fat in dry matter (FDM) was calculated. All the analyses were carried out in duplicate.

Statistical analyses. All the statistical analyses and other results were performed with the software Lotus 1-2-3, V.9 for Windows (Lotus Development Corporation, Cambridge, MA 02142).

RESULTS AND DISCUSSION

Table 1 shows the chemical data of the original processed cheeses, HPC and SPC, and their mixtures. Statistically significant differences were found (*t* test, $P < 0.05$) between the four blends with regard to their MC and pH values. However, the comparison between 5 % and 10 % SPC is not statistically relevant (*t* test, $P > 0.05$). From the microbiological viewpoint, all the Hard Processed Cheeses (HPC) samples showed no significant *E.coli* & Total Coliform contamination (< 10 C.F.U./gram), while the Soft Processed Cheeses (SPC) were highly contaminated (EC: 3,210 ± 627 C.F.U./gram; TC: 4,683 ± 585 C.F.U./gram).

Generally, a clear inactivation of *E.coli* was demonstrated in all the GPC subsamples (Table 2). The low moisture and pH contents allowed a limited survival of *E.coli* in anaerobic and refrigerated conditions. The linear correlation between the microbiological profiles of *E.coli* and the time of storage gave good R^2 coefficients (Table 3). The same situation was demonstrated for Log TC (Table 2) with different R^2 coefficients (Table 3). So, the linear equation described acceptably the expected decline. However, the slope showed by the eight equations in Table 3 tended towards more negative values with the increase of SPC in GPC blends. The more different slope was showed by the 30% SPC samples with reference to Log EC and Log TC. Two linear correlations were carried out between the obtained slope values and the quantity of Soft Processed Cheese in mixtures (data not shown). According to

Table 1. Chemical composition of original and grated dairy products (mean ± standard deviation).

Chemical features	Original Processed Cheeses		Vacuum-packed Grated Processed Cheeses (GPC)			
	Hard (HPC)	Soft (SPC)	5% SPC	10% SPC	20% SPC	30% SPC
MC	38.3 ± 0.5	63.2 ± 0.8	39.6 ± 0.5	40.8 ± 0.5	43.3 ± 0.5	45.8 ± 0.5
FC	18.0 ± 0.5	15.5 ± 0.8	18.0 ± 0.5	18.0 ± 0.5	17.5 ± 0.5	17.5 ± 0.5
FDM	29.2 ± 0.9	42.1 ± 1.5	29.8 ± 0.9	30.4 ± 1.0	30.9 ± 0.7	32.3 ± 0.8
PH	4.43 ± 0.08	6.76 ± 0.04	4.60 ± 0.07	4.69 ± 0.07	4.92 ± 0.06	5.16 ± 0.05

Table 2. Inactivation profiles of *E.coli* and Total Coliforms in vacuum-packed GPC derived by HPC and SPC (mean ± standard deviation).

Storage time, days	Log EC (C.F.U./gram of GPC) at 2 ± 2°C				Log TC (C.F.U./gram of GPC) at 2 ± 2°C			
	5% SPC	10% SPC	20% SPC	30% SPC	5% SPC	10% SPC	20% SPC	30% SPC
0	2.2 ± 0.1	2.5 ± 0.1	2.8 ± 0.2	3.0 ± 0.1	2.4 ± 0.1	2.7 ± 0.1	3.0 ± 0.1	3.2 ± 0.1
14	1.7 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	1.5 ± 0.2	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	1.8 ± 0.1
28	1.2 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
42	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
54	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0

Table 3. Linear correlations of inactivation profiles for EC and TC Table 2) in GPC products. All the interpolations are based on the first three points only.

GPC	Log EC: linear equation	R^2	Log TC: linear equation	R^2
5% SPC	Log EC = 2.20 - $0.0357^a \times$ days	1.000	Log TC = 2.47 - $0.0286^a \times$ days	0.923
10% SPC	Log EC = 2.52 - $0.0464^a \times$ days	0.998	Log TC = 2.73 - $0.0429^a \times$ days	0.991
20% SPC	Log EC = 2.83 - $0.0714^a \times$ days	0.997	Log TC = 3.07 - $0.0714^a \times$ days	0.987
30% SPC	Log EC = 2.88 - $0.0821^a \times$ days	0.970	Log TC = 3.10 - $0.0786^a \times$ days	0.976

the elaborated regressions, the maximum slope value is -0.0278 for Log EC ($R^2 = 0.970$) and -0.0219 for Log TC ($R^2 = 0.935$) if SPC is not present in grated products. These negative values demonstrate the inevitability of the Coliform decline in the examined blends. The exact meaning of the inverse relationship between slopes and SPC contamination remains to be discussed.

In fact, the modification of moisture and pH values aimed to turn the HPC samples into less unfavourable culture media (GPC) for these microorganisms. Consequently, the expected amelioration of temporal survival values for Coliforms and *E.coli* should have been confirmed by the contemporary rise of the slope towards positive values. In addition, the observed behaviour contradicts the possibility that the modification of GPC samples had not influenced the inactivation of Coliforms, with the consequent parallelism between the four lines. The significativity of differences between 30% SPC mixture and the other GPC samples (*t* test, $P < 0.0001$) appears evident. The same situation occurs for Total Coliforms.

So, the only unconsidered point remains the microbial competition. Basically, the chemical modification of GPC mixtures introduces other microorganisms that are able to take advantage of the semi-dry grated product. The presence of fungal mycelia after 30, 39, 45 and 51 days (average values) in the 30, 20, 10 and 5% SPC mixtures respectively suggests the competitive action of moulds, in spite of the very low oxygen quantity. It needs to be noted that the vacuum packaging cannot assure the complete absence of O_2, especially when granular or porous foods are sealed. Consequently, the fungal spoilage is not excluded in vacuum-packed dairy products (Hocking and Faedo, 1992).

CONCLUSIONS

This study demonstrated the inevitability of the inactivation for Coliform bacteria and particularly *E. coli* in vacuum-packed grated processed cheeses during the cold storage. The survival of these bacteria is certainly short even though melted cheeses are mixed with highly contaminated dairy products. The inactivation of *E.coli* is strongly dependent on the chemical composition and packaging parameters of grated cheeses. The same situation is highlighted by Total Coliform counts. However, the obtained results suggest the antagonistic role of spoiling microorganisms, as moulds. Further research is needed to verify the role of moulds.

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MINOR COMPONENTS WITH NUTRITIONAL VALUE, ANTIOXIDANT ACTIVITY AND ORGANOLEPTIC PROPERTIES STATUS THROUGH THE MARKET PERIOD OF COMMERCIAL OLIVE OIL TYPES

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ABSTRACT

The aim of this research was to describe the content of minor components in commercial Spanish extra virgin and olive oil categories, particularly with respect to the individual phenolic compounds, tocopherols (vitamin E) and volatile compounds and to analyze their evolution through the market period. The study was carried out classifying three different olive oil types: extra virgin olive oils (EVOO) with high phenols content (>250 mg/Kg; HP-VOO), EVOO with low phenolic compounds content (LP-VOO) and olive oil (OO), due to the relationship of biophenols towards oxidative stability, sensory properties and nutritional aspects. No statistically significant differences were observed in the oil characteristics between the three grades of olive oil, except for the K_{270} and the content of waxes. A slightly increase in the values of the oxidation indices was observed after one year, showing, as expected, a low oxidation rate and therefore a high stability of the products. An important decrease in the contents in α-tocopherol and some LOX volatile compounds, like E-2-hexenal, was observed during storage.

Key words: antioxidant, market period, minor components, virgin olive oil

INTRODUCTION

Olive oil is mainly composed by triacylglycerols, which are susceptible to oxidation, the prime cause of oil degradation, which decreases the sensory quality and the nutritional value of the product. However, virgin olive oil contains mainly monounsaturated fatty acids and minor compounds which greatly improve its stability during the market period.

Virgin olive oil natural antioxidants are important not only for their *in vitro* protection against rancidity, and therefore on the shelf life of the product, but also for their *in vivo* biological activity which participate to the nutritional value of this type of edible oil, together with its beneficial fatty acids composition (Visioli *et al.*, 1998). Moreover, several minor components, like volatile compounds and some individual phenols, also play an important role in positive sensory attributes, like fruitiness and bitterness, and hence in the consumer's preference (Angerosa *et al.*, 2002; Aparicio *et al.*, 2002).

The aim of this research was to describe the content of minor components in commercial Spanish extra virgin and olive oils categories, particularly with respect to the individual phenolic compounds, tocopherols (vitamin E) and volatile compounds and to analyze their evolution through the market period.

MATERIALS AND METHODS

Olive oil samples.

Two bottles of major Spanish extra virgin olive oils (n=26) and olive oils (n=13) brands were bought from different supermarkets. The oils were acquired at the beginning of 2005 with an estimated time in the market of approximately 2-3 months. The analytical determinations were carried out at that time using one bottle of each sample, while the second bottle was stored under similar conditions as in the market and analysed after approximately 9 months (total estimated market period 11-12 months, which is approximately 2/3 of the declared expiry date for the olive oils).

Analytical determinations in olive oils.

Quality indices were measured following the analytical methods described in European Regulation EEC 2568/91 and subsequent amendments. Phenolic compounds were extracted by solid phase extraction (SPE) using a diol cartridge and analyzed by HPLC using a Zorbax SB-C18 column (250 x 4.6 i.d. mm, 5 mm particle size) (Gómez-Alonso *et al.*, 2002). Volatile components were analyzed by SPME followed by GC, using a DVB/Carboxen/PDMS fiber (50/30 μm, 2 cm long from Supelco Inc.) and a Supelcowax 10 column (30 m x 0.25 mm x 0.25 μm, Supelco Inc.) (Vichi *et al.*, 2003). Tocopherols content were determined following the AOCS official method Ce 8-89. Oxidative stability was evaluated by the Rancimat method (Laübli & Bruttel, 1986). Antioxidant capacity was evaluated by DPPH assay (Brand-Williams *et al.*, 1995). Bitterness index (K_{225}) was determined by the method described by Gutiérrez-Rosales *et al.*, 1992.

RESULTS AND CONCLUSIONS

The distribution of the values of the quality and composition characteristics of extra virgin olive oil (EVOO) and olive oil (OO) commercial samples are reported in Table 1. The EVOO were classified on the basis of their phenolic content in two

Table 1. Characteristics of extra virgin olive oil (EVOO) and olive oil (OO) commercial samples (n=39) and their evolution during the market period.

Olive oil type	Percentile 10	MEDIAN	Percentile 90	Significant difference*	Monthly increase P10	Monthly increase P90
HP-VOO						
Acidity (% oleic acid)	0.10	0.20	0.25	ns	0.0028	0.0127
Peroxide value (meq/Kg)	5.4	7.1	9.9	ns	0.0166	0.2850
K_{232}	1.55	1.71	1.87	ns	0.0041	0.0181
K_{270}	0.11	0.15	0.22	a	0.0028	0.0062
Waxes (mg/Kg)	49	61	83	a	-----	-----
LP-VOO						
Acidity (% oleic acid)	0.13	0.19	0.32	ns	0.0081	0.0113
Peroxide value (meq/Kg)	7.28	8.04	10.35	ns	0.0511	0.2347
K_{232}	0.49	1.81	1.99	ns	0.0023	0.0428
K_{270}	0.11	0.14	0.19	a	0.0041	0.0076
Waxes (mg/Kg)	82	141	178	b	-----	-----
OO						
Acidity (% oleic acid)	0.08	0.23	0.48	ns	0.0038	0.0162
Peroxide value (meq/Kg)	5.71	7.99	9.71	ns	0.0617	0.3301
K_{232}	1.76	1.85	1.95	ns	0.0018	0.0081
K_{270}	0.24	0.32	0.37	b	0.0006	0.0036
WAXES (MG/KG)	153	170	234	C	-----	-----

*A different letter (a-c) indicates statistical significant difference (p<0.05) between the three olive oil types.
ns, not significant.

grades: HP-VOO, with a high phenolic content (> 250 mg/kg) and LP-VOO, with a low phenolic content due to its relationship with oxidative stability, sensory properties and nutritional aspects.

No statistically significant differences were observed in the oil characteristics between the three grades of olive oil, except for the K_{270} and the content of waxes which were statistically different in the three OO types. The quality and oxidative level of the commercial samples at the beginning of the market period (2-3 months) was quite satisfactory (i.e. acidity lower than 0.5% and peroxide value lower than 11 meq/kg). Only a slightly increase in the values of these indices was observed after one year, showing, as expected a low oxidation rate (Table 1) and therefore a high stability of the oils in the market.

Figure 1 shows the contents in natural antioxidants (total phenols, o-diphenols and α-tocopherol) and in volatile compounds (C5 and C6) in the three types of oils (HP-VOO, LP-VOO and OO) at the beginning of the study and after 11-12 months of storage. Statistically differences were found between LP-VOO and OO in the contents of total phenolic compounds and o-diphenols at 2-3 months (p= 0.001, in both cases) (Figure 1a and Figure 1b).

Small differences were observed in the α-tocopherol (vitamin E) content between EVOO and OO (Figure 1c), since it is allowed to add vitamin E to refined oils. However, a statistically significant difference was indeed observed between HP-VOO, OO and LP-VOO (*p*= 0.019). The α-tocopherol content fell significantly in all samples, and after approximately one year the total reduction in this vitamin

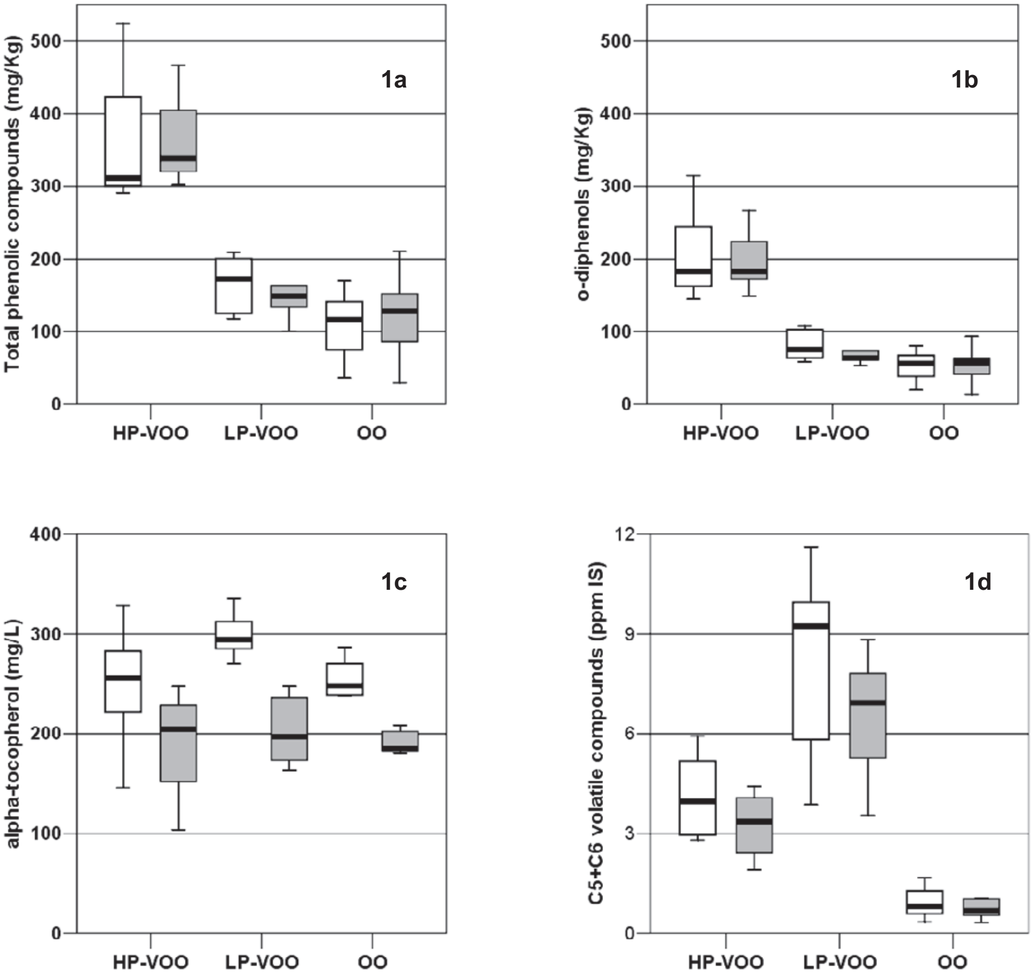

Fig. 1. Content in antioxidants (total phenols, o-diphenols and α-tocopherol) and volatile compounds at the beginning and after one year of market period. □, 2-3 months; ■, 11-12 months.

varied between 60 mg/Kg (25% of the initial content) in HP-VOO and OO samples and 88 mg/Kg (30%) in LP-VOO. On the contrary, the decrease of the total phenol and o-diphenols throughout the market period was not statistically significant in all the three different types of oil.

As expected, the antioxidant activity (evaluated by DPPH method) of HP-VOO was significantly higher, ranging from 785 μmol Trolox/Kg up to 1580 μmol Trolox/Kg, as 10th and 90th percentiles, while for LP-VOO and OO varied between 445-750 μmol Trolox/Kg and 197-500 μmol Trolox/Kg, respectively (p= 0.001). A similar trend was observed for the Rancimat oxidative stability of the oils (p= 0.002). Phenolic substances do not affect virgin olive oil stability only, since they also contribute to oil flavour and aroma, especially to the typical bitter taste of virgin olive oil (Gutiérrez-Rosales *et al.*, 1992). The K_{225} parameter, also called bitterness index, showed

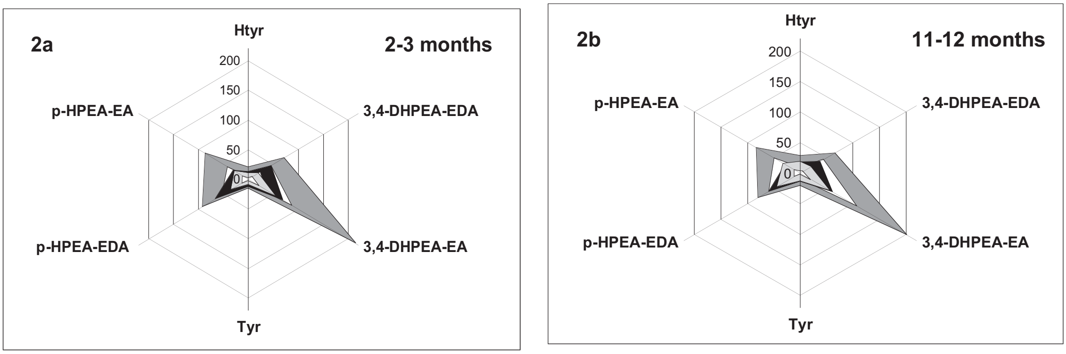

Fig. 2. Content and evolution of the hydroxytyrosol, tyrosol and their secoiridoid derivatives in HP-VOO, LP-VOO and OO. (The figures depict the values between percentile 10 and percentile 90).
▨, HP-VOO; ■, LP-VOO; □, OO.

statistically significant differences between the three groups of oils, varying from 0.20-0.36 for HP-VOO, 0.14-0.20 for LP-VOO and 0.05-0.14 for OO, as 10th and 90th percentiles respectively (p= 0.001). In addition, the intake of biophenols, especially o-diphenols (hydroxytyrosol and its derivatives, 3,4-DHPEA-EDA and 3,4-DHPEA-EA), is beneficial for the human health and one of the nutritional positive aspects of virgin olive oil consumption (Visioli *et al.,* 1998). The estimated vitamin E intake (α-tocopherol) proceeding from the EVVO and OO consumption achieves the recommended daily allowances (RDA, 8-19 mg/d in adults) in Greece, Italy and Spain. On the contrary, the β-carotene consumption only reaches the 5-7% of the RDA for vitamin A (data not shown).

The content and evolution of the main individual phenolic compounds is depicted in Figure 2, in which the great difference between the three olive oil types, especially in the dialdehydic forms derivatives of hydroxytyrosol and tyrosol (3,4-DHPEA-EA and p-HPEA-EA) is observed.

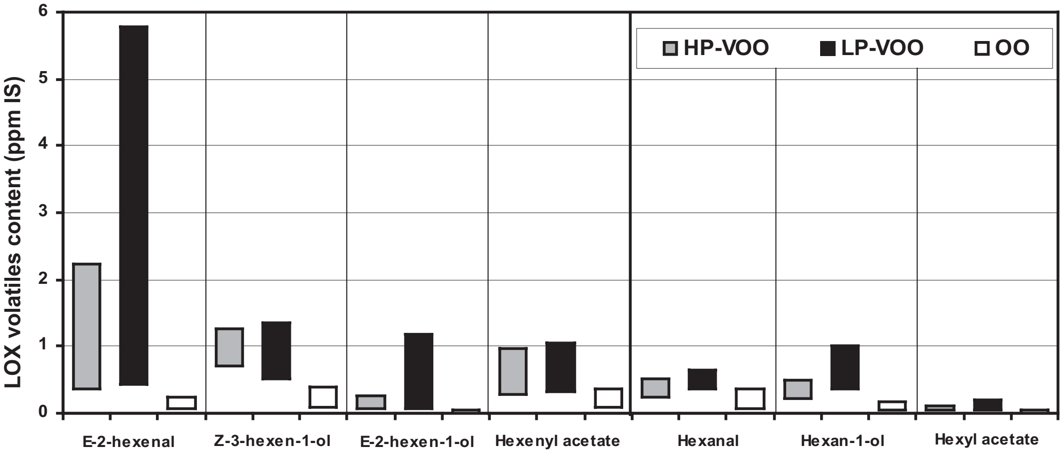

Fig. 3. Content of the individual LOX volatiles in HP-VOO, LP-VOO and OO at the beginning of the market period. (The figure depicts the values between percentile 10 and percentile 90).
▨, HP-VOO; ■, LP-VOO; □, OO.

As concerned the LOX volatiles content, it was significantly higher in LP-VOO than in HP-VOO and lower in the OO (Figure 1d). Moreover, the sum of C6 and C5 volatile compounds slightly decreased along approximately one year of market period, so probably the green odour notes in the olive oils would diminish. Only the hexanal content increased in this period, this could be due to the fact that the hexanal is a product of the oxidation of the linoleic acid that takes place along the shelf life of the olive oils.

Finally, Figure 3 shows the individual LOX volatiles contents in the oils at the beginning of the market period. Statistical significant differences were observed in the LOX volatiles from the linoleic acid (hexanal, hexan-1-ol and hexyl acetate). Moreover, the figure shows the wide range found for the major LOX volatile, the E-2-hexenal, in the two different types of EVOO samples, established on the basis of their biophenols content.

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IMPACT OF THE TYPE OF PACKAGING ON THE SCOPE OF FLUCTUATIONS OF THE QUALITY OF THE PACKAGED PRODUCTS

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ABSTRACT

The purpose of the author's own research was to determine the intensity of the quality fluctuations in a selected product from the group of confectionery bread, offered in various types of packaging. The examined product was packaged in bags with lateral folds, made of laminate OPP/PE, with a mass of 180g, and moulded plastic recipient with partitions OPP, closed with a cover foil OPP/PE with a mass of 200g.

The assessment of the scope of fluctuations of the water content in the packaged product and of the index of the overall sensorial quality was made on the basis of the indices of the dynamics of change.

Key words: packaging, the water content, sensory quality of product

INTRODUCTION

The quality of food can be defined as the sum of the properties of the food product, critical for the assessment of such product as apt to satisfy the needs of the contemporary consumer. Such needs can be met if the product fulfils the requirements of the legislation in force, has the nutritive value desired by the consumer, is consistent with the health-related requirements, has the expected technological value and the desired sensorial properties during the period of durability of the product (ESKIN *et al.* 2001; MAN *et al.*, 2000).

Many factors influence the durability of food products. Their aggregate impact has to be taken into account in the research intended to ensure and extend the durability of packaged products, and forecast the durability period. It is a system involving: a product with specific properties, packaged in a packaging having a certain construction, size, barrier qualities, mechanical resistance and other known quality parameters and a cryptoclimate filling the space between the product and the packaging, and the environment where the packaged product is located. The effects of the composite interactions of all the factors influencing the product determine the period of durability of the product (LISIŃSKA-KUŚNIERZ *at al.*, 2003; TAUB *at al.*, 1998).

The publications on the subject show a significant diversity in the approaches to the systematisation of the above factors and to the place of the packaging among them. According EN 14182 "packaging shall mean all products made of any materials of any nature to be used for the containment, protection, handling, delivery and presentation of goods, from raw material to processed goods, from the producer to the user or to consumer. Non-returnable items used for the same purpose shall also be considered to constitute packaging" (EN 14182, 2002).

That is the result of the diversification of the tasks and functions fulfilled by the contemporary packaging. The role of the packaging in the preservation and extension of the durability the product is therefore unquestionable.

MATERIALS AND METHODS

The subject of this investigation was product made of dough steamed in a sodium hydrante solution. For these products, two kinds of packaging were used:

– bag with a side fold OPP/PE which were moulded in v.f.f.h (vertical form-fill-seal) system - weight 180 gramme,
– moulded plastic recipient with partitions OPP, closed with a cover foil OPP/PE - weight 200 gramme.

In order to determine the influence the kinds of packaging on the shelf life of product, the research was carried out in a air conditioning chamber, as following: T= 40°C ± 1°C, R= 80% ±2% (D/NI-04-04-01, 1997).

These products were used as a basis for the sensory evaluation of changes in the products and of the water content in the product. The water content was determined using Electronic Weighting Equipment, Type WPS 110S Radwag: mod E1 (SERVICE MANUAL OF ELECTRONIC WEIGHTING EQUIPMENT, 2002).

The sensory tests were performed in a special sensory laboratory with separate testing and product- preparation areas. Products were assessed by a trained panel consisting of 6 persons (ISO 8586, 1987). The scoring method applied to sensory estimation of product was a 5 point scale. Finally, an index of total sensorial quality (ITSQ) was determined (ISO 4121, 1987).

RESULTS AND DISCUSSION

In order to verify the fitness of the proposed models to the empirical data, certain measures of the accuracy of estimation of the model parameters were used, including: index of determination (R^2). The determined models reflect the scope of changes in the specific quality parameters of the products packaged in various packaging types, while in order to determine the intensity of the above mentioned changes, the indices of dynamics of the changes were estimated. The initial value of the analysed quality index was adopted as the basis for the setting of the above indices of dynamics (ZELIAS *et al*, 2004).

The functions describing the changes of the individual X and ITSQ are shown on fig. 1 and 3, and the indices of the dynamics of the above changes – on fig. 2 and 4. The obtained values R^2 are the proof of the good fitting of the function models.

Both in the case of the water content and ITSQ changes with respect to a product packaged in moulded forms the dynamics of adverse changes is lower.

With respect to X changes, the indices of dynamics for the products packaged in sachets show a consistent growth compared to the indices of dynamics for the products packaged in moulded forms (for instance, starting from the 3 week of storage, the differentiation of the indices is already significantly greater) (fig. 2).

Taking into account the results obtained from the analysis of the second basic quality parameter which is ITSQ, we can state that in the case of the products packaged in sachets the dynamics of adverse changes is clearly greater starting from the 1 week of storage (fig. 3).

During the 8 week of storage in dramatic microclimate conditions the index of the dynamics of ITSQ changes for the products in sachets changed to 32% compared to the initial value. In the case of moulded forms, that index dropped only to 58% $ITSQ_0$ (fig. 4).

CONCLUSION

The average values, calculated on the basis of the results showing the changes of specific quality parameters of the analyzed products, were used to calculate the

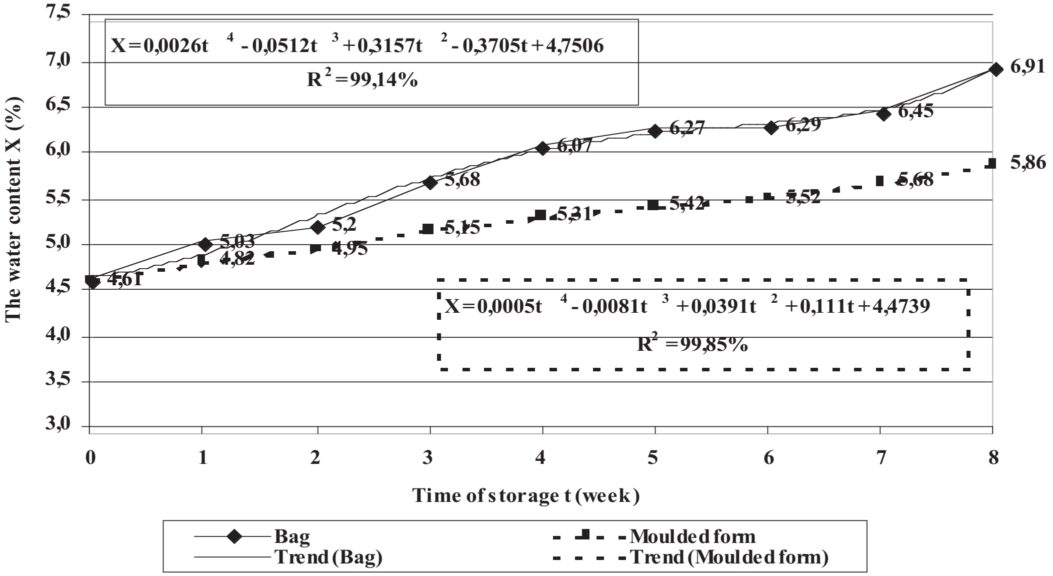

Fig. 1. Changes of the water content (X) in the products packaged in different kinds of packaging during storage (t), in %.
Source: own's work.

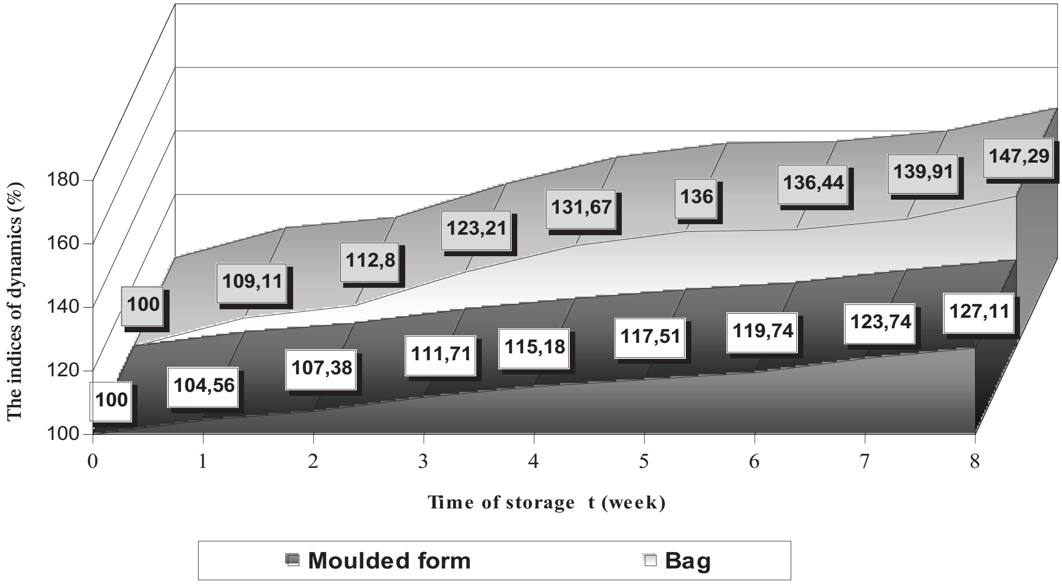

Fig. 2. The indices of dynamics of the changes of the water content (X) in the products packaged in different kinds of packaging during storage (t), in %.
Source: own's work.

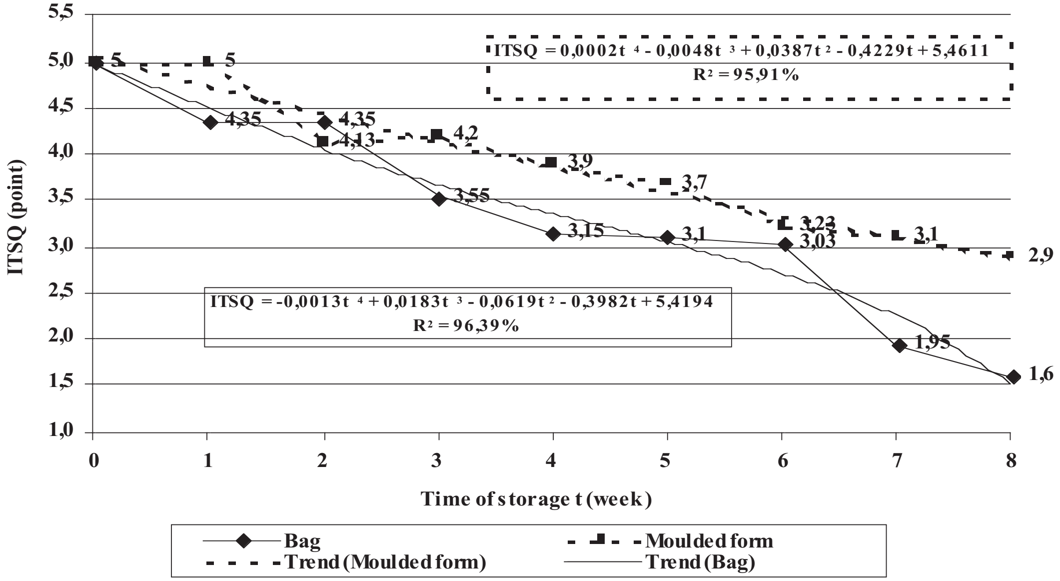

Fig. 3. Changes of the of the index of total sensorial quality (ITSQ) in the products packaged in different kinds of packaging during storage (t), in point.
Source: own's work.

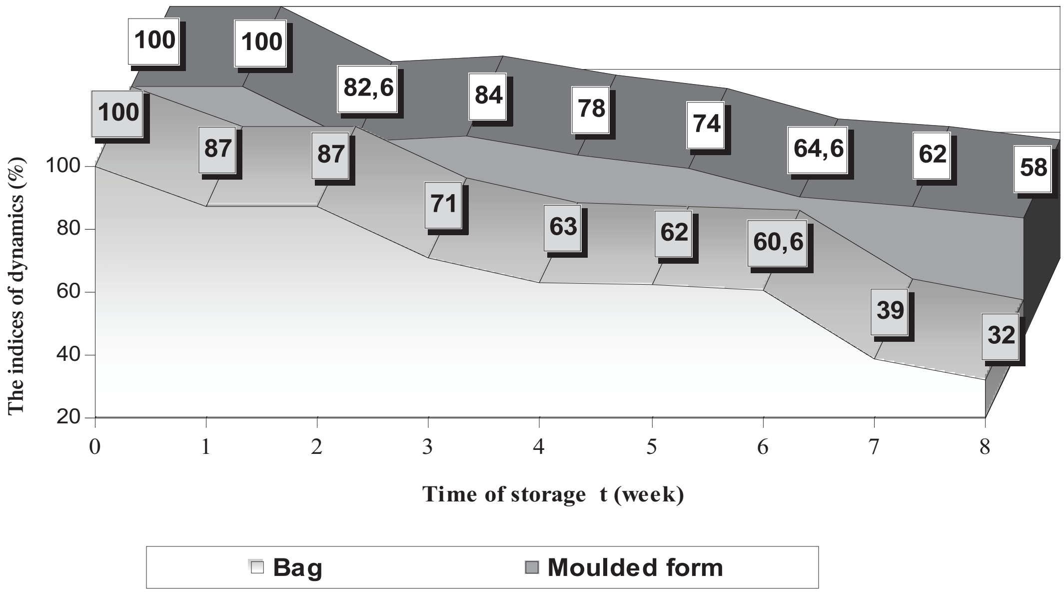

Fig. 4. The indices of dynamics of the changes of the index of total sensorial quality (ITSQ) in the products packaged in different kinds of packaging during storage (t), in %.
Source: own's work.

functions mapping the development of such changes during storage. On the basis of the outcome, we can state that the kind of packaging has a significant influence (including the construction and packaging material properties) on the extent of the adverse changes occurring in the course of the product storage.

The above research conducted as implementation research for a manufacturer were performed on the basis of the procedure recommended for the given product.

We should like to point out the need to conduct accelerated tests and verify the same in a situation of a long term storage in standardised conditions, with every change of the packaging construction and packaging material for any given product.

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ACCELERATED TESTS IN ESTIMATION OF THE SHELF-LIFE OF BAKERY PRODUCT

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ABSTRACT

The purpose of the author's own research was to analyse the changes in the quality of bakery product stored in an environment of accelerated ageing.

The study took into account the impact of the storage temperature on the intensity of variation of the quality parameters in the packaged product.

The scope of specific changes of quality occurring in the accelerated ageing environment was subsequently compared to the dynamics of those changes as occurring in the course of long term storage.

Key words: shelf-life, accelerated ageing, dynamics of quality changes

INTRODUCTION

In the publications on the subject, several different approaches are described with respect to the determination of the durability of the packaged food products. Among them, the following methods were distinguished (LABUZA, 2000; MAN *et al.*, 2000):

– analysis of the data in the publications,
– long term storage tests in standardised conditions,
– accelerated shelf life test (ASLT).

The durability of food can be determined on the basis of the data contained in the publications and related to similar kinds of products and suitable packaging. However, the scope of application of the above method is usually limited to the products offered by one producer, therefore it is used rather infrequently UCHEREK, 2002).

The most reliable results can be obtained when the long term tests are conducted in standardised conditions, with the levels of temperature and relative humidity of the air equal to those required in the distribution of the given product. They include a long term storage of a originally packaged lot of product, taking samples for test in regular time intervals, and marking the progress of the degradation changes using the indicators which are related to the quality condition of the examined product. A correctly planned experiment in standardised conditions leads to accurate results. And yet such research has an important flaw – the time of waiting for the final outcome.

The combination of the long duration of the storage experiment in a standardised environment with an economically justified life span of the product on the market encouraged the researchers to seek for research methods enabling them to obtain sooner the information about the durability of food products. Using accelerated storage tests ASLT is such a solution for certain products ASLT.

The accelerated tests methods (ASLT) usually include testing the stability of the packaged product in the conditions of more intense operation of the environment factors (e.g. high temperature and/or high relative humidity of the air), and the obtained results are extrapolated to the conditions of the standardised storage (HINE, 1987; LABUZA, 2000).

MATERIALS AND METHODS

The research focused on biscuits packaged in bag with a side fold OPP/OPP which were moulded in v.f.f.h (vertical form-fill-seal) system - weight 180 gramme.

In order to determine the influence of the storage conditions on the durability of the products packaged, the research was carried out in a special microclimate, using air conditioning chamber, as following: the standardised conditions (temp. $18^0C\pm 1^0C$, R=75% ±2%) and the accelerated ageing environment conditions (temp. $40^0C\pm 1^0C$, R=80% ±2%) (MAN *et al.*, 2000; ISO 554, 1995).

The storage tests were conducted during 36 weeks on a weekly basis (standardized conditions) and 6 weeks, on a weekly (1 week) basis. The research pertained to the following quality parameters of the product (ISO 4121, 1987; SERVICE MANUAL OF ELECTRONIC WEIGHTING EQUIPMENT, 2002):

– an index of total sensorial quality (ITSQ),

– the water content (X), using Electronic Weighting Equipment, Type WPS 110S Radwag mod E1.

The sensory tests were performed in a special sensory laboratory with separate testing and product- preparation areas. Products were assessed by a trained panel consisting of 6 persons (ISO 8586, 1987). The scoring method applied to sensory estimation of peanuts was a 5 point scale test with gradation of validity (5-very good quality, 4-good quality, 3-sufficient quality, 2-tolerance quality, 1-poor quality). The valuation cards with coefficients of importance for quality factors were created by experts. Finally, an index of total sensorial quality (ITSQ) was determined.

RESULTS AND DISCUSSION

Statistical and mathematical calculations involved a regression analysis where the period of product storage was the independent variable (in weeks) (t), and the specific quality feature of the product – the dependent variable “y”.

In the case of the variations of water content and ITSQ it was recognised – on the basis of the dispersion of the empirical points in the co-ordinate system, that the linear function: y = a + b*t. shall be that function which shall reflect the related trends in a sufficiently accurate manner. The R^2 values calculated for all the estimations were very high (above 90%), which is a proof of a very good accuracy of the estimates (ZELIAS *et al.*, 2004).

Using the straight line function model allowed also for a comparison of the values of suitable indices of regression "b", in order to verify in which set of storage conditions the changes of the examined parameter occur at the higher rate (the higher absolute value of the "b" index is a signal of a higher rate of change within the examined period of time).

The changes in the water content (X) in the product and ITSQ during the storage in different microclimatic environment were shown on fig. 1 and 3, and the indices of the rate of the above changes, on fig. 2 and 4, respectively.

For the changes in the water content the determined index of test acceleration by a comparison of the regression indices "b" exceeds 6, while for ITSQ the value obtained was in excess of 5,5 (fig. 1 and 3). For that reason it should be assumed that the organoleptic changes expressed by ITSQ are more degrading than the changes in the dry mass content. Thus, they should serve as basis for the determination of the durability of packaged products.

The kinetics of the deteriorating quality of the product are corroborated by the scope of decrease of the indices of dynamics of ITSQ and the scope of growth of the water content (X) in the product, shown on fig. 2 and 4.

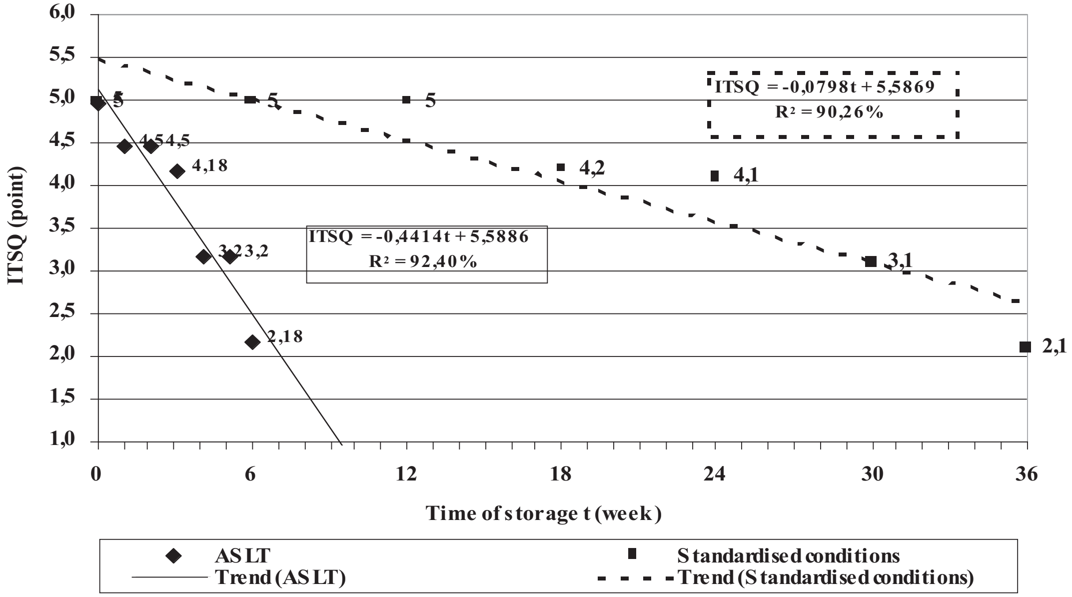

Fig. 1. The changes of the index of total sensorial quality (ITSQ) of the product during the storage in different microclimatic environment, in points.
Source: own's work.

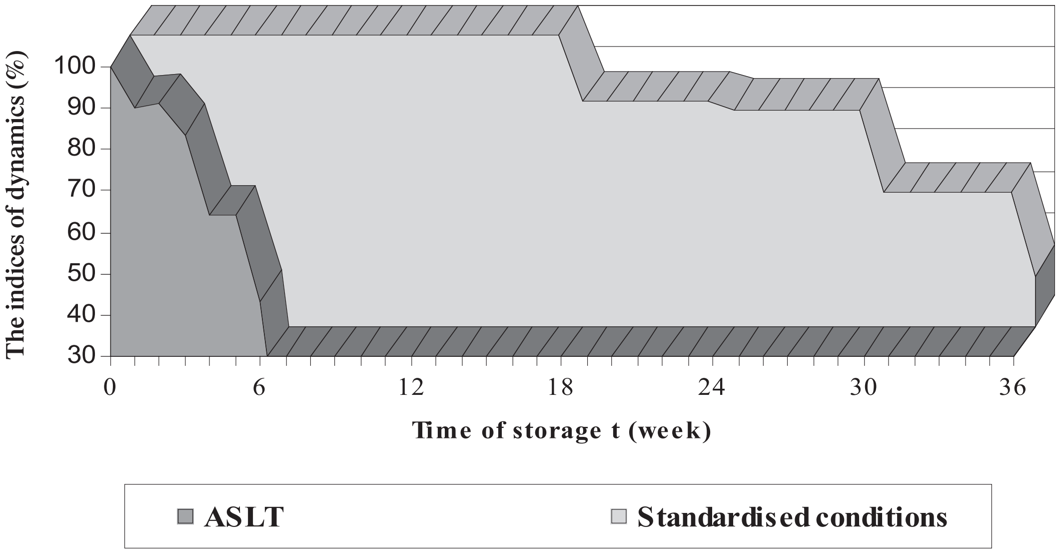

Fig. 2. The indices of dynamics of the changes of the index of total sensorial quality (ITSQ) of the product during the storage in different microclimatic environment, in %.
Source: own's work.

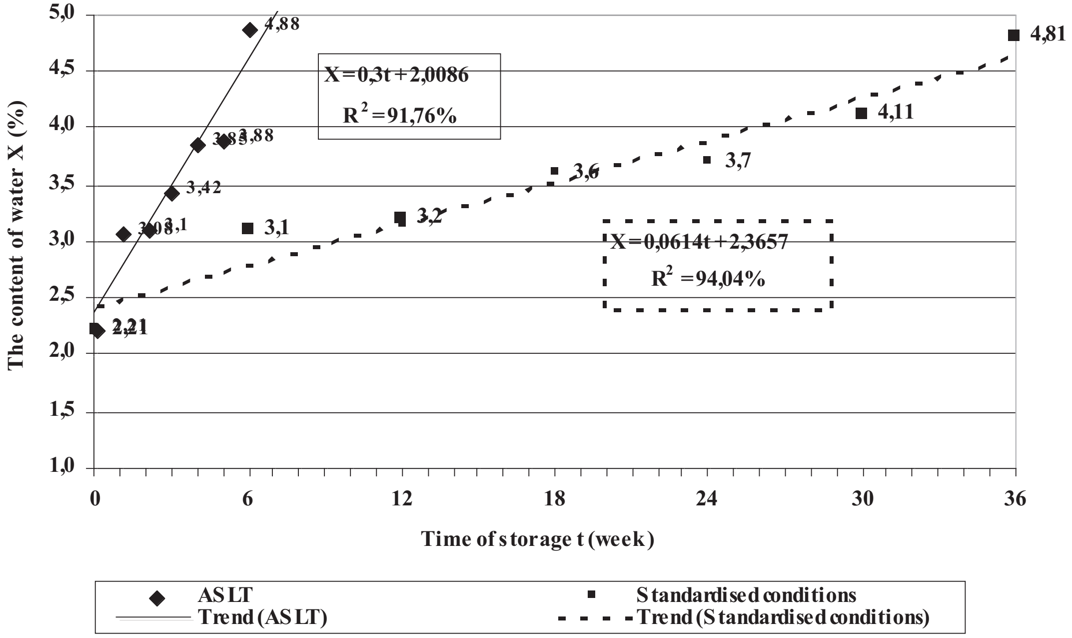

Fig. 3. The changes in the water content (X) in the product during the storage in different microclimatic environment, in %.
Source: own's work.

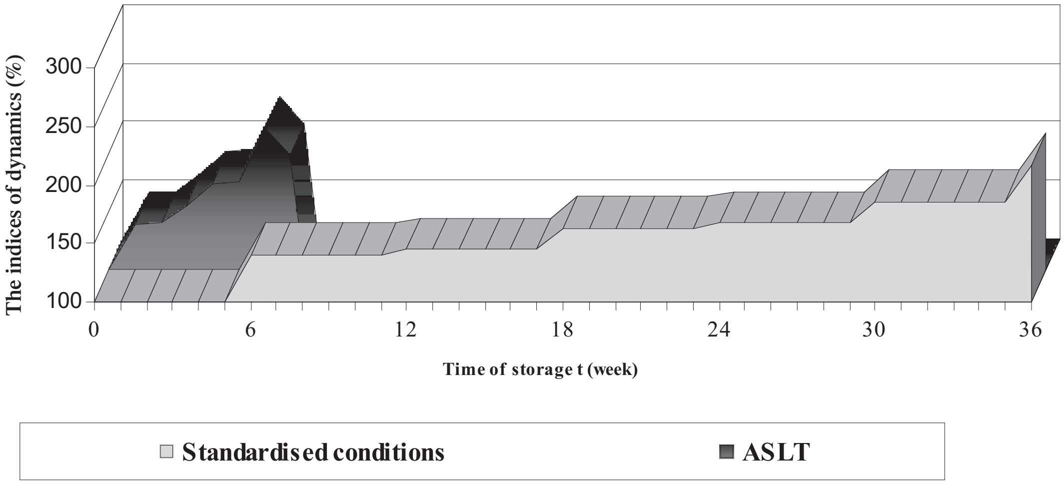

Fig. 4. The indices of dynamics of the changes in the water content (X) in the product during the storage in different microclimatic environment, in %.
Source: own's work.

CONCLUSION

In the case of accelerated tests, no general theory of acceleration has been developed, nor a standardised set of concepts. It should be emphasised that the accelerated tests can only be used for the determination of durability of the products which in the standardised and accelerated conditions show the same mechanism of quality changes, and the rate of durability loss can be characterised by measurements of distinctive features having a reference to the organoleptic properties and to the microbiological condition of the samples.

It is also worth noting that in the course of the accelerated tests the food is submitted to the influence of conditions which are not admissible for the storage of such food products, and such conditions can initiate quite untypical degradation mechanisms, leading to an erroneous prognostic.

For that reason, many authors, including T. Labuza (LABUZA, 2000), S. Gyeszly (GYESZLY, 1980) and D.J. Hine (HINE *et al.*, 1987) recommend a verification of the results obtained in accelerated tests in the course of a standardised storage. The above standpoint found its confirmation also in the described tests of confectionery products.

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INFLUENCE OF STORAGE TEMPERATURE AND ETHYL ALCOHOL CONTENT ON THE SHELF-LIFE OF ASTI SPUMANTE DOCG

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ABSTRACT

'Asti' or 'Asti Spumante' is an aromatic Italian sparkling wine produced exclusively with 'Moscato bianco' grapes growing in Piedmont (North West Italy) and commercialized as a sweet *dessert* wine. In this work, relationships among alcohol concentration, storage temperature and shelf-life of product have been defined. Three 'Asti' wines produced from the same must but having different alcohol concentrations and stored at three temperatures were used for the trial. Colour, acetaldehyde, volatile compounds and sensory analysis were used for defining the effect of storage. The results show that product shelf-life is directly correlated to the alcohol concentration of wine and inversely correlated to the storage temperature.

Key word: Asti Spumante, storage temperature, volatile compounds, dessert wine

INTRODUCTION

The 'Muscat' family of aromatic grapes grow all over the world and are largely used in winemaking, as table grapes and raisins. Muscat white is the most important 'Muscat' grape variety cultivated in Italy and is used for Asti DOCG sparkling wine and Moscato d'Asti DOCG wine production.

The shelf-life of these products is very short (ca. 1 year) and aged products show an intense yellow colour, low odour and oxidised aroma. Previous researches showed that high acidity, high temperature and exposure to light could accelerate the terpenoid chemical degradation and reduce the shelf-life of white wines (Oliveira *et al.*, 2002; Silva Ferreira *et al.*, 2003a, 2003b; Gonzalez-Viñas *et al.*, 1998; Sivertsen *et al.*, 2001), but little data about Muscat wines shelf-life have been published (Castino and Di Stefano, 1981; Di Stefano and Ciolfi, 1983).

In this work, relationships among alcohol concentration, storage temperature and product shelf-life have been determined using sensory analysis.

MATERIALS AND METHODS

Samples

Winemaking (10000 L) was performed in autoclaves, with a homogeneous Muscat must clarified and filtrated and three wines with 6.5, 7.5 and 9.5 % of alcohol concentration were obtained. Bottles were stored at three temperatures (5, 15 and 22 °C) for one year and analysis was carried out every four months.

Chemical analysis

The optical absorbance of wine at 420 nm (10 mm of optical path) was used as to quantitatively evaluate colour. The concentration of acetaldehyde was determined with an enzymatic test (R-Biopharm, Cerro al Lambro, MI, Italy). Free terpenoidic compounds were determined according to Di Stefano (1991). Compound identification was achieved with a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP-5000 quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). Three analyses were performed for each sample.

Sensory analysis

A ranking test (ISO 8587) with twenty-five selected and trained tasters was performed. For each sample only the overall evaluation was defined. Three replicates of each tasting session were performed.

Statistical analysis

Data analysis was performed with the Statistica software package (ver. 6.0; StatSoft Inc., Tulsa, OK, USA).

RESULTS AND CONCLUSIONS

Factorial analysis of variance shows a significant effect of temperature and ethanol concentration for the overall evaluation. Also interaction between these two factors is significant for overall evaluation at each storage time. As shown in Figure 1, samples stored at 22 °C were less appreciated than others even after only four months after bottling. For the other two examined temperatures an ethanol effect can be highlighted. With a temperature of 15 °C but ethanol concentration of 9.5% the product shelf-life is longer than with an ethanol concentration of 6.5%. Storage also caused a change in the terpenoidic profile of wines. Linalool decreases due to transformation into α-terpineol, while furanic linalool oxides increase along with Ho-trienol according to Di Stefano (1989). At the time of bottling all samples showed a similar linalool content (about 500 μg/L), but wines with an ethanol concentration of 9.5% and stored at 15 and 5 °C show higher linalool concentrations at the end of the test. A correlation among the terpenoidic profile and sensory evaluation was then highlighted (Table 1). Also for colour intensity a correlation with overall evaluation was determined. No such correlation was observed between overall evaluation and acetaldehyde concentration. It is generally believed that acetaldehyde

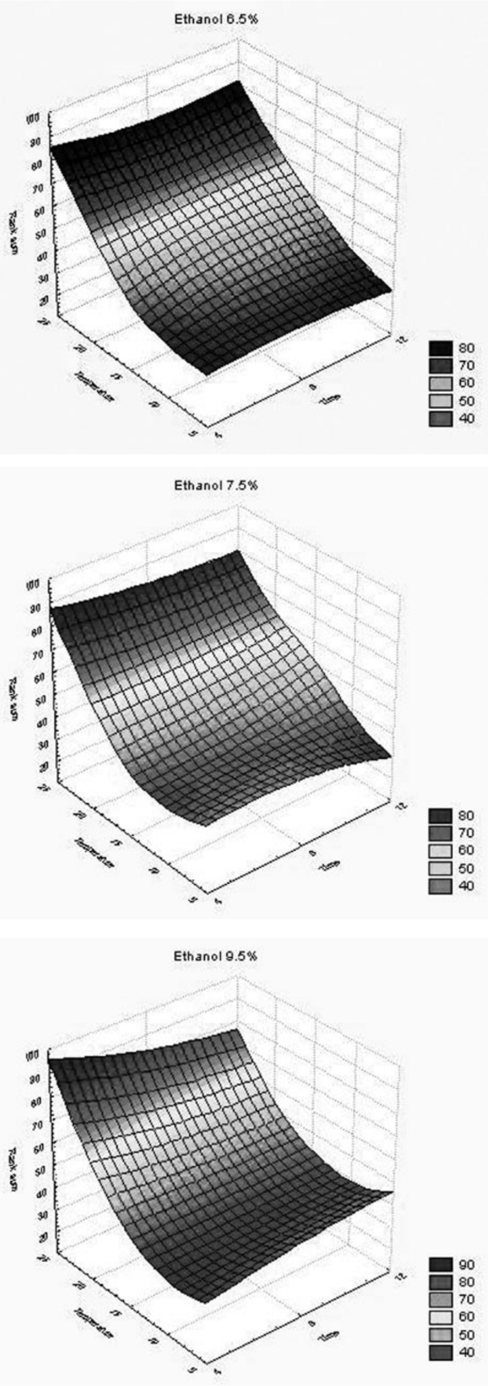

Fig. 1. Surface plots for rank sum computed for overall evaluation defined at each ethanol concentration related to storage times and storage temperatures.

Table 1. Correlation coefficient between overall evaluation defined as rank sum and chemical parameters.

Acetaldehyde mg/L	0,0924
Color intensity	0,5012**
Geraniol µg/L	0,0284
Ho-trienol µg/L	0,5176**
Oxide A µg/L	0,7227***
Oxide B µg/L	0,7200***
Oxide C µg/L	0,0821
Oxide D µg/L	0,3007
Geranyl Acetate µg/L	0,4549*
Linalool µg/L	-0,7555***
Diol 1µg/L	-0,1371
Diol 2 µg/L	0,1577
Citronellol µg/L	0,0725
α-terpineol µg/L	0,5760**
2-Phenyl ethyl acetate µg/L	-0,1658
2-Phenyl ethanol µg/L	-0,2164
2-Ethyl phenol µg/L	0,2836
Nerol µg/L	-0,3235
1-Hexanol µg/L	0,1945

* Significant at $p \leq 0,05$; ** significant at $p \leq 0,01$; *** significant at $p \leq 0,001$.

is the main aroma generated during wine oxidation and so this compound is often used to describe the loss of wine aroma by oxidation (Noble *et al.*, 1987; Halliday and Johnson, 1992). For Moscato sparkling wine this relation was not demonstrated.

In conclusion, our results demonstrate that storage conditions are also very important for the shelf-life of 'Asti'; The storage time is >1 year only with storage temperatures <5 °C . The shelf-life is also directly correlated to the ethanol concentration.

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POLIMERIC MATERIALS AS PACKAGING FOR APULIA TABLE WINES

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ABSTRACT

In this study, preliminary results on the preservation of red, rose and white table wine in polymeric packages (Polyethylene terephthalate (PET); PET with oxygen scavenger; PET with external coating) compared to that of the same wines in glass bottles are reported. Analytical parameters measuring preservation state, including amount of oxygen dissolved, sulfur dioxide, volatile acidity, titratable acidity, absorbance at 420 nm, at 520 nm and 620 nm, alchol content, pH and total phenols have been considered. Furthermore, volatile and polyphenolic fraction profiles have been evaluated by means of GC/MS and HPLC/DAD/MS techniques, respectively. All the results have confirmed that the alternative containers are efficient for preserving red, rose and white table wines up to the storage time that was at the moment considered.

Key words: wine, preservation, polymeric materials, packaging

INTRODUCTION

The shelf-life of wine is a primary concern of the wine industry and it is directly related to its oxidation, indeed, both red and white wines undergo oxidative deteriorations and colour alterations. In recent years, active materials have been increasingly applied to food packaging in order to extend the product shelf-life maintaining its quality (Strathmann *et al.*, 2005). In particular, polymeric materials with oxygen scavengers have been demonstrated to be very efficient in food preservation from oxidation (Zerdin, 2003). Packages can have a significant effect on the quality of wine, especially with regard to oxidative degradation and scalping processes (Frank *et al.*, 1999) that could produce organoleptic changes resulting in consumers rejection. Therefore, the monitoring of phenolics fate in the packages appears to be fundamental for the study of wine preservation. Wine quality can be also lost as a consequence of wine aroma oxidation resulting in oxygen-related off-flavours formation (Escudero *et al.*, 2002). Hexanal, t-2-octenal, t-2-nonenal, benzaldehyde, furfural, 4-hydroxy-4-methylpentanone, 2-nonanone, eugenol and high concentrations of acetaldehyde (Usseglio-Tomasset, 1985) could be considered as chemical markers for wine oxidative deterioration (Escudero *et al.*, 2002).

The purpose of this investigation is the study of the preservation of white, rose and red table wines in containers other then glass (Polyethylene terephthalate (PET); PET with oxygen scavenger; PET with external coating) by a twofold strategy: 1) the monitoring in the time of the main wine analytical parameters such as amount of oxygen dissolved, absorbance at 420 nm and 520 nm, tonality, total phenols, volatile acidity and sulfur dioxide, 2) the study of the qualitative and quantitative composition of polyphenolic and volatile fractions in the time by GC/MS and HPLC/DAD/MS techniques, respectively.

MATERIALS AND METHODS

Polymeric packages. 1 L bottles of glass, PET (thickness 0.3 mm), PET (thickness 0.3 mm) with external coating (epossidic resin thickness 10 μm) and PET (thickness 0.3 mm) with oxygen scavengers were used.

Measurement of oxygen permeability (O_2TR). The measurement of oxygen permeability related to packaging materials has been performed according to ASTM F1307-02 Regulation, at 23 °C and ambient humidity (40-50%).

Analyses of wine analytical parameters. Alcohol content, pH, titratable and volatile acidity, sulfur dioxide, dissolved oxygen, absorbance values at 420 nm, 520 nm and 620 nm have been evaluated according to Official Gazette of EEC (1990). The analyses were carried out at regular intervals: at the moment of packaging (T0) and after 1, 2 and 3 months (T1, T2, T3). All analyses were performed in triplicate. The samples were stored at room temperature and in the dark.

Extraction of volatile components. A 1 ml alcoholic solution of 2-methyl-penthanol (200 mg/l, internal standard) and 40 ml of dichloromethane were added into 200 ml of wine. The mixture was stirred by magnetic agitation at 10 °C for 1 h and cooled at -20 °C overnight. The separated organic phase was then dried over sodium sulphate and concentrated to a volume of 200 μL with nitrogen flow. A 1 μL sample was injected in the gas chromatographic system.

GC/MS analyses. A 6890 N series gas chromatographic system (Agilent Technologies) was interfaced with Agilent 5973 mass selective detector. A fused-silica capillary column DB-Wax (60 m x 0.32 mm I.D; 0,5 μm film thickness, J&W Scientific Inc., Folsom, USA) coated with bonded methylpolysiloxane was used. The column oven temperature was initially at 40 °C and then raised to 220 °C at 4°C/min, with a final holding time of 25min. The injector temperature was 250°C in splitless mode. The carrier gas was helium at 1.5 ml/min. The mass spectrometer was operated in electron impact mode (EI, 70 eV) and the masses were scanned over a m/z range of 30–500amu at 3,2scans/s. A solvent delay time of 8 min was used, to avoid overloading the mass spectrometer with dichloromethane.

HPLC/DAD/MS analyses. The HPLC-DAD-MS system consisted of a HPLC 1100 with a DAD and a G2447A XCT MSD Plus mass spectrometer (Agilent Technologies) in a series configuration. A reversed phase Phenomenex C18 column (150 x

2.0 mm i.d., 5 μm packing) protected by a C18 Guard Cartridge (4 mm x 2.0 mm i.d., Phenomenex) was used. The solvents used were (A) water/acetonitrile (95/5% v/v) containing 0.1% (v/v) trifluoroacetic acid and (B) water/acetonitrile (10/90% v/v) containing 0.1% (v/v) trifluoroacetic acid, and the following gradient was established: isocratic 2% B for 2 min, from 2 to 10% B in 6 min, from 10 to 13% B in 22 min, from 13 to 20% B in 20 min, from 20 to 30 % in 25 min, followed by washing and re-equilibrating the column. The flow rate was of 0.2 mL min^{-1} and temperature was ambient. UV detection was carried out at a 520 nm wavelength. HPLC-ESI-MS^n data was acquired in the positive ion mode using the Chemstation (Agilent) software. The analyses were carried out at the moment of packaging (time 0) and after 1 month.

RESULTS AND DISCUSSION

Oxygen permeability of polymeric packages. As expected, the measured permeability values were 0.088-0.090, 0.002-0.002, <0.001 for PET, PET with coating and PET with 4% oxygen scavengers, respectively.

Wine analytical parameters. The main analytical parameters related to oxida-

Table 1. Free sulfur dioxide (mg/L), dissolved oxygen (mg/L), absorbance at 520 nm (mAU) and absorbance at 420 nm (mAU) for red table wine in GLASS, Polyethylene terephthalate (PET); Polyethylene terephthalate with external coating (PET+COAT); Polyethylene terephthalate with oxygen scavenger (active PET).

RED	free sulfur dioxide (mg/L)		dissolved oxygen at 520 nm (mg/L)		absorbance at 420 nm (mAU)		absorbance (mAU)	
	T0	T3	T0	T3	T0	T3	T0	T3
GLASS	49 ± 13	23 ± 2	8,6 ± 0,2	1,4 ± 0,1	261± 6	306 ± 1	230 ± 2	253 ± 2
PET		14 ± 2		1,2 ± 0,2		353 ± 4		281± 2
PET+COAT		15 ± 4		1,4 ± 0,1		358 ± 9		280 ± 5
ACTIVE PET		16 ± 3		1,8 ± 0,3		400 ± 50		300 ± 40

T0 = analysis time at the moment of packaging; T3 = analysis time after 3 months.

Table 2. Free sulfur dioxide (mg/L), dissolved oxygen (mg/L), absorbance at 420 nm (mAU) for white table wine in GLASS, Polyethylene terephthalate (PET); Polyethylene terephthalate with external coating (PET+COAT); Polyethylene terephthalate with oxygen scavenger (active PET).

WHITE	free sulfur dioxide (mg/L)		dissolved oxygen (mg/L)		absorbance at 420 nm (mAU)	
	T0	T3	T0	T3	T0	T3
GLASS	32 ± 6	17 ± 2	6,2 ± 0,7	1,5 ± 0,2	120 ± 30	121 ± 1
PET		11 ± 2		2,3 ± 0,4		119 ± 1
PET+COAT		12 ± 2		2,1 ± 0,1		120 ± 1
ACTIVE PET		17 ± 2		1,1 ± 0,3		113 ± 2

T0 = analysis time at the moment of packaging; T3 = analysis time after 3 months.

Table 3. Free sulfur dioxide (mg/L), dissolved oxygen (mg/L), absorbance at 520 nm (mAU) and absorbance at 420 nm (mAU) for rosè table wine in GLASS, Polyethylene terephthalate (PET); Polyethylene terephthalate with external coating (PET+COAT); Polyethylene terephthalate with oxygen scavenger (active PET).

ROSE'	free sulfur dioxide (mg/L)		dissolved oxygen (mg/L)		absorbance at 520 nm (mAU)		absorbance at 420 nm (mAU)	
	T0	T3	T0	T3	T0	T3	T0	T3
GLASS	32 ± 3	13 ± 3	7,2 ± 0,9	1,7 ± 0,1	47 ± 1	482 ± 2	47± 2	482 ± 8
PET		8,5 ± 1,8		1,7 ± 0,3		550 ± 50		540 ± 40
PET+COAT		7,5 ± 1,8		1,6 ± 0,1		530 ± 20		530 ± 10
ACTIVE PET		16 ± 1		1,6 ± 0,1		590 ± 50		570 ± 50

T0 = analysis time at the moment of packaging; T3 = analysis time after 3 months.

tion for red, rose and white wines are summarized in Table 1, Table 2 and Table 3, respectively. Slight increase of the 420 nm absorbance together with a slight decrease of free sulfur dioxide content were observed for polymer packaged red and rose wines with respect to glass bottled ones, suggesting a beginning of oxidation processes in the wines bottled in the alternative materials. On the other hand, the increase in the 520 nm absorbance values could indicate a color stabilization by the conversion of anthocyanins into more stable pigments favoured by a slight oxygenation of wines (Castellari *et al.*, 2000). No significant difference has been observed for the content of dissolved oxygen, suggesting that the oxygen eventually

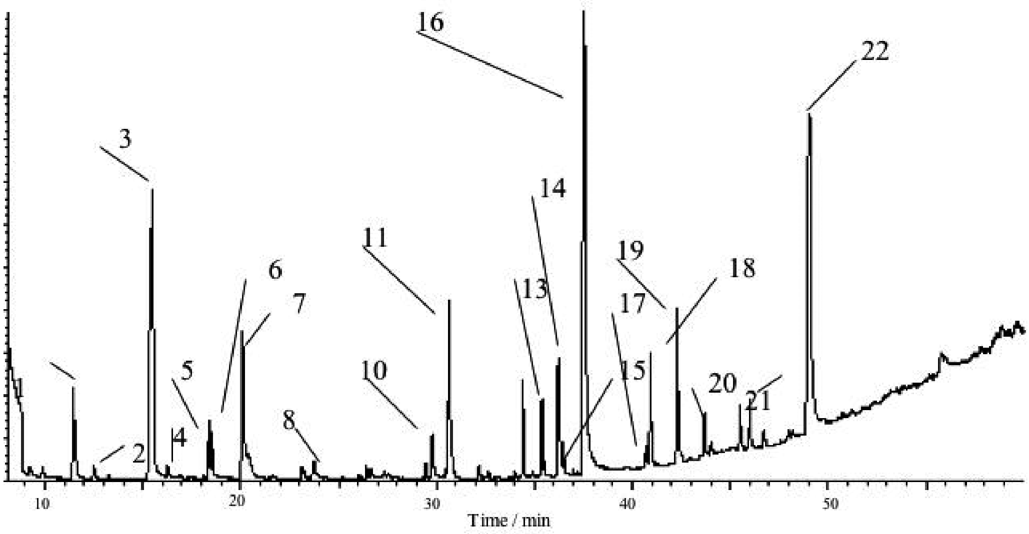

Fig. 1. Chromatogram GC-MS of a dichloromethane wine extract. Mean peak identification: (1) 2-methyl-1-propanol; (2) 2+ 3-methyl-1-butanol; (3) 3-methyl-1-butanol; (4) ethyl hexanoate; (5) 3-hydroxy-2-butanone (acetoin); (6) IS: 2-methyl-1-pentanol; (7): 2-hydroxy-ethyl-propanoate acid; (8) acetic acid: (9) benzaldehyde; (10) 4-hydroxybutanoic acid; (11) butanoi acid diethyl ester; (12) noedecanoic acid; (13) hexanoic acid; (14) N-(3-methylbuthyl) acetammide; (15) benzoic acid; (16) phenylethyl alcohol; (17) hydroxybutandionic acid, diethyl ester; (18) ottanoic acid; (19) 2-1-dimethylphenol; (20) 2-methoxy-4-penthenoic acid; (21) N-decanoic acid; (22) hydroxy ethyl succinate.

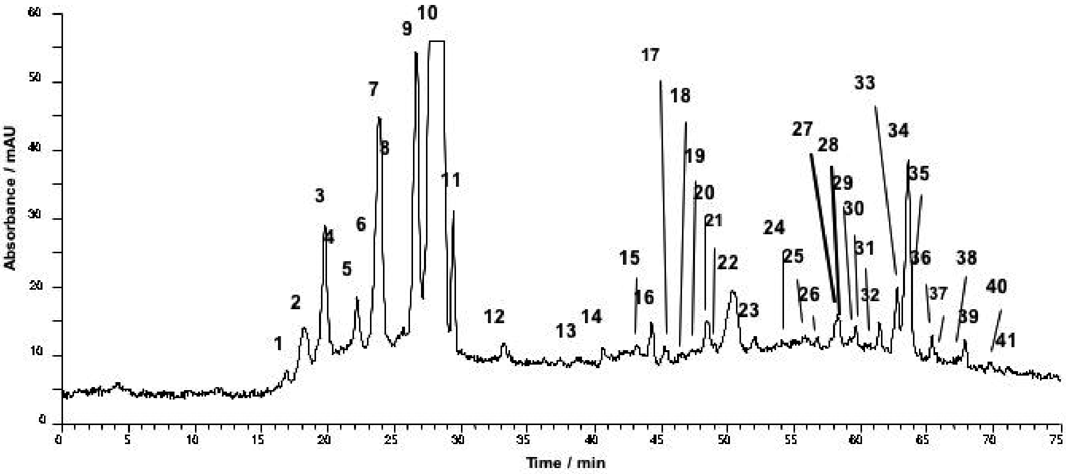

Fig. 2. HPLC-Uvtrace relevant lo HPLC-DAD-MS analysis of wine samples.

permeated through the polymeric packages was consumed by oxidation or condensation processes. Moreover, alcohol content, pH, titratable and volatile acidity, total phenols and tonality showed no significant difference among wines bottled in polymeric and glass containers suggesting that, regarding the above quality indeces, the alternative containers tested guarantee an acceptable 3 month-preservation of investigated wines.

Volatile fraction. 21 volatile compounds were identified as reported in figure 1. No significant difference was found in the aroma profile between glass packaged wine samples and polymer packaged ones. The identification of minor species is, at the moment, under investigation.

Anthocyanin-derived pigments. The detection of 41 pigments absorbing at 520 nm has been achieved according a HPLC-UV-MS method previously optimized in our laboratory (Pati *et al.*, in press). Figure 1 is representative of the 520 nm UV trace relevant to the chromatogram obtained for a glass bottle wine after 3 month packaging. Identified compounds can be grouped into five structural classes: anthocyanins (peaks 3,5,8-10,21-23,25-28,33,34), pyranoanthocyanins (peaks 11,12,20,35,39,40), vinyl-linked anthocyanin-flavanol pigments (peaks 15,17,24,29,30,36-38), flavanol-anthocyanin compounds (peaks 1,2,4,6,7,19), and ethyl-bridged anthocyanin-flavanol pigments (peaks 13,14,16,18,31,32,41). No significant difference between the samples packaged in polymeric and glass bottles in relation to the detected pigments was found after 3 month packaging.

All the results have confirmed that the alternative containers are efficient for preserving red, rose and white table wines up to the storage time that was at the moment considered, although some slight oxidation processes appear to occur for wines bottled in plastic container. A longer preservation time is still under investigation.

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LISTERIA INNOCUA DYNAMIC IN MINIMALLY PROCESSED VEGETABLES PACKAGED IN ORDINARY AND MODIFIED ATMOSPHERES

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ABSTRACT

This work was carried out to study the microbiological and safety aspects of Minimally Processed Vegetables (MPV) packed under different conditions. The type strain *Listeria innocua* DSMZ 20649 was used to study *in vitro* the growth/survival at different initial parameters. The bacteria was cultured in TSB (Tryptic Soy Broth) using a 3x3x3 complete factorial design to assess the impact of temperature (15, 25, 37 °C), initial pH (5.2, 5.7, 6.0) and oxygen (aerobic, microaerophilic and anaerobic conditions) on the microrganism's growth.

In order to verify the behaviour of *Listeria innocua* DSMZ 20649 in the MPV artificially inoculated, packaged both in ordinary and modified atmospheres, the PCR-DGGE analysis was used. Moreover, the same approach was carried out in order to follow microbial dynamics of MPV products without inoculum. *Listeria innocua* DSMZ 20649 showed the highest growth in microaerophilic conditions, at 25 °C and at highest pH value. In aerobic condition the strain grew better at low temperature (15 and 25 °C) and in anaerobic condition at 37 °C showed at the lowest pH value the highest growth.

The *in vivo* results showed that *Listeria innocua* became one of the dominant species at the end of the storage time and that indigenous microbial population highlighted slight shifts during storage.

Key words: *Listeria* spp., minimally processed vegetables, PCR-DGGE, shelf-life

INTRODUCTION

Minimally Processed Vegetables (MPV) are fresh products that have been have expanded tremendously in the past decade becoming always more popular. The MPV products may harbour different microrganisms including pathogens such as *Salmonella, Shigella* spp., *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Listeria monocytogenes.* Contamination of MPV with *Listeria monocytogenes* is a concern that needs to be addressed in order to increase the safety of these products. Previous works have showed that *L. monocytogenes* can grow onto vegetables (Carlin and Nguyen-the, 1994; Carlin *et al.,* 1995) in a wide range of CO_2 concentrations even in modified atmosphere (Carlin *et al.,* 1996). The pathogen can increase to high numbers in food, during refrigerated storage, due to its ability to multiply at low temperature. Moreover *L. monocytogenes* is able to survival/growth within modified atmosphere packages (Farber *et al.,* 1998; Berrai *et al.,* 1989) for being tolerant to low O_2 atmospheres. Recently to detect microbial population from complex communities many methods cultivation independent have been proposed (Rudi *et al.,* 2002) and several papers report the PCR-DGGE analysis as able to study bacterial communities in food samples (Randazzo *et al.,* 2005; Rantsiou *et al.,* 2005).

The aim of the present work was to study *in vitro* the behaviour of *Listeria innocua* (type strain) at different pH, temperatures and oxygen conditions and to study *in vivo* the microbial dynamics of *Listeria* onto the MPV products by using PCR-DGGE approach.

MATERIALS AND METHODS

In vitro experiments

The *L. innocua* DSM 20649 was cultured in Tryptic Soy Broth plus 0.6% of Yeast Extract (TSB+YE). The suspension (2%) was inoculated into 200 ml of TSB+YE at pH 5.2, 5,7 and 6.0, obtained by adding lactic acid. The OD values were determined at initial time and after 24 h. For each trial of pH value, different tubes were prepared and incubated at different oxygen concentration (aerobic, anaerobic and microaerophilic conditions) and at different temperatures (5, 25 and 37 °C).

In vivo experiments

The MPV samples were taken from the regular farming production run. Twenty-four MPV samples were packaged in ordinary atmosphere (OA), and 24 MPV samples were packaged in a 90 % nitrogen, 6 % carbon dioxide, 3 % oxygen, modified atmosphere (MA). The samples for each treatment were analysed in triplicate the same day of production, and after 3, 5 and 7 days of storage at 4°C.

Cells of *L. innocua* DSM 20649 were sprayed onto MPV samples, at a final concentration of 10^3 CFU/g and 10^5 CFU/g onto samples packaged in OA and in MA, respectively. The MPV samples were analyzed for the mesophilic aerobic count (MAC), onto TSA medium incubated at 32°C for 24-48h and for the Listeria count onto LSB medium.

PCR-DGGE analysis

The same DNA isolation procedure was used to isolate DNA from the bacteria and from the MPV products. PCR amplification was performed using *Taq* DNA polymerase (Invitrogen), and the universal PCR primers U968-GC and L1401-r in order to amplify the V6 to V8 regions of eubacterial 16S rDNA (Nubel *et al.,* 1996). The cycling program was: 5 min at 94°C; 35 cycles each consisting of 30s at 94°C, 30s at 56°C and 40s at 68°C; and 7 min at 68°C. PCR products were quantified by conventional electrophoresis procedure.

The DCode Universal Mutation Detection system (Bio-Rad) was used for the separation of the PCR amplicons. Electrophoresis was performed as previously described (Muyzer *et al.,* 1993). The DNA bands were visualized by silver staining.

RESULTS AND CONCLUSIONS

In vitro experiments

The in vitro results are showed in the figures 1, 2 and 3, where the OD (as difference between the OD detected at 24 hours and initial time) for *L. innocua* DSMZ 20649 at different conditions of pH and temperature, in presence of oxygen, in microaerophilic and in anaerobic conditions, respectively, are reported.

The lowest values were detected at pH 5.2, for all temperatures tested, in aerobic

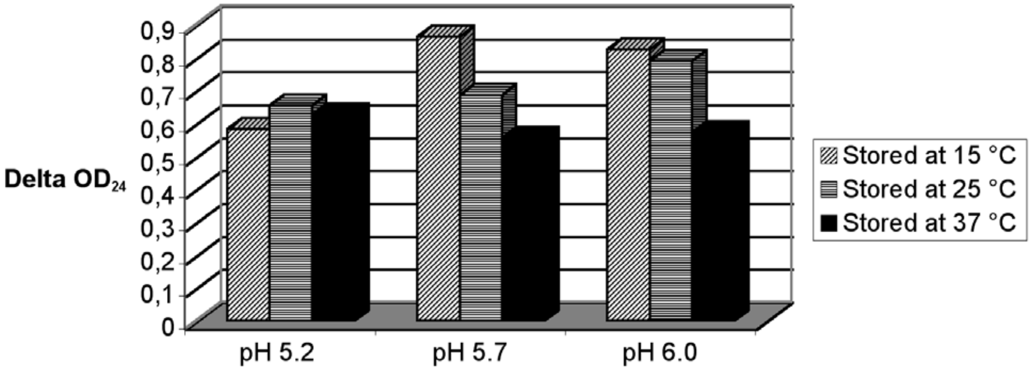

Fig. 1. Growth/survival curves of *Listeria innocua* DSM 20649 in TSB medium at different pH values and temperatures, in aerobic condition.

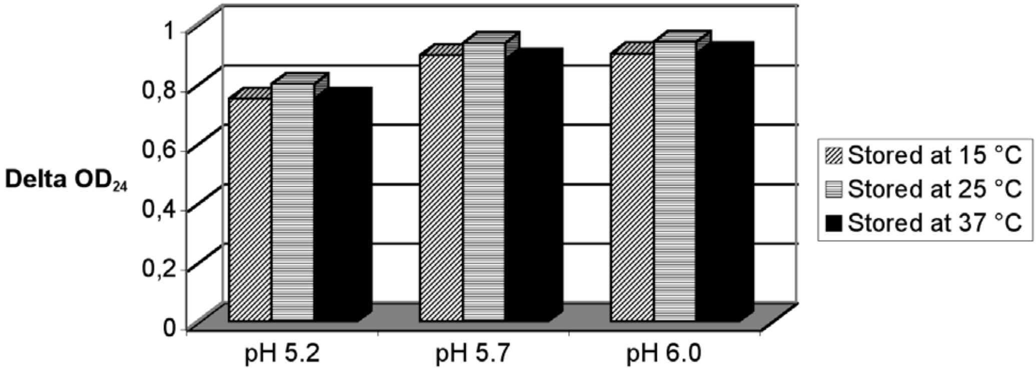

Fig. 2. Growth/survival curves of *Listeria innocua* DSM 20649 in TSB medium at different pH values and temperatures, in microaerophilic condition.

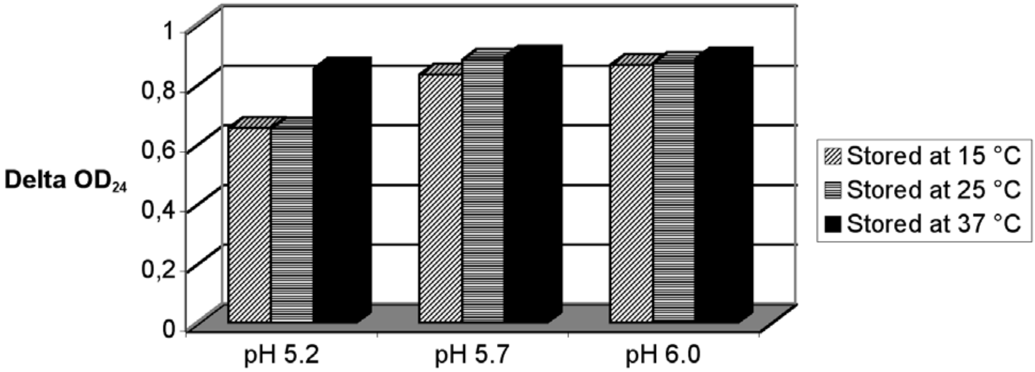

Fig. 3. Growth/survival curves of *Listeria innocua* DSM 20649 in TSB medium at different pH values and temperatures, in anaerobic condition.

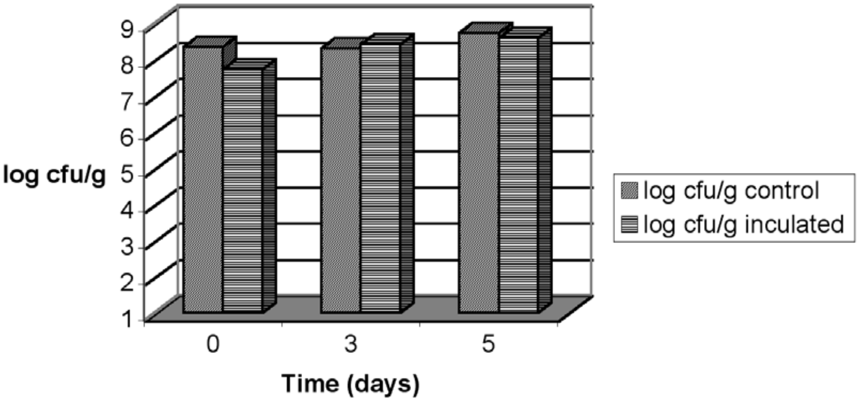

Fig. 4. Microbial counts in TSA medium of minimally processed vegetable samples inoculated with *Listeria innocua* DSM 20649 and packaged in ordinary atmosphere.

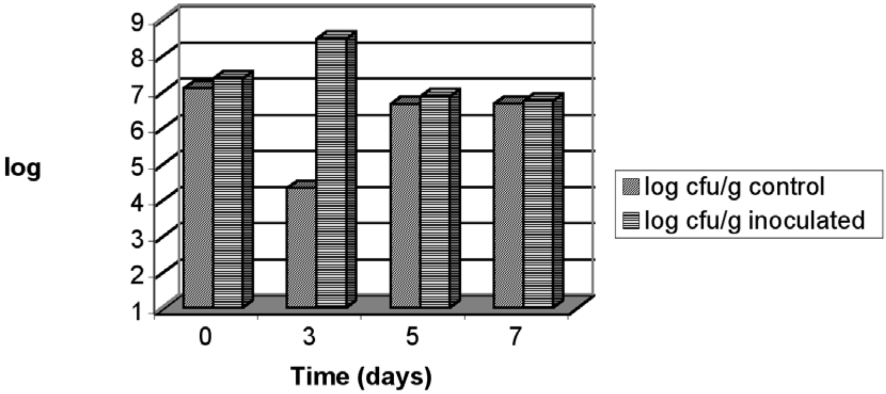

Fig. 5. Microbial counts during storage at 4°C in TSA medium of minimally processed vegetable samples inoculated with *Listeria innocua* DSM 20649 and packaged in modified atmosphere.

conditions (Fig.1). In microaerophilic condition the growth bacteria was not effected by the combination of pH and temperatures values, even at the lowest pH (Fig. 2). In anaerobic conditions (Fig. 3) the general trend is an increasing of OD detected, proportional to the increasing of temperature. This is in agreement with the synergic effect of low oxygen presence, low temperature and low pH.

In vivo experiments

The MAC obtained in TSA medium of treated MPV, packaged in OA, are showed in Figure 4, where it can be seen that counts are on the order of 8.34 and 7.73 log CFU/g. The MAC counts obtained in the MPV packaged in MA showed different trend during time (Fig. 5). After 3 days the treated samples highlighted an increasing of 1 log unit of count, and a decreasing (about 2 log unit) after 5 days of storage. No correspondence has been found between the Listeria selective base count and PCR-DGGE results. The absence of colony in LSB proves that culturing approach and/or selective media under-estimate the survival of the inoculated *Listeria*, as previously reported (Jacxsens *et al.*, 2002). The reason can be due to the high load of background population on vegetables. In fact, in the present study, the counts obtained in TSA medium are high also for non inoculated samples.

The PCR-DGGE analysis of samples, at first day of packaging, showed different profiles, with bands at different position (Fig. 6, lanes: 1, 4, 9, 12), suggesting a variability in the microbial communities. Regarding inoculated samples, it can be seen that all samples in MA (Fig. 6, lanes: 12, 13, 14 and 15), showed the intense band *L*, at the same position of the amplicon detected for *L. innocua* strain (Fig. 6, lane 8), while the inoculated samples packaged in OA showed a weak band L (Fig. 6, lanes 9, 10, 11).

Although the MA reducing level of O_2 and increasing levels of CO_2 is generally reported as able to reduce or inhibit the microrganisms growth when is combined with refrigeration (Francis *et al.*, 1999) the results of present work can be considered

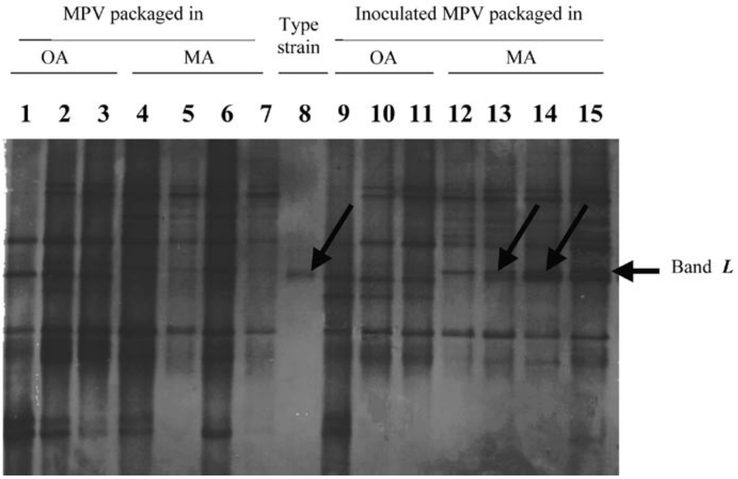

Fig. 6. DGGE profiles of amplicons generated by PCR of the V6 to V8 regions of the 16S rDNA of minimally processed vegetable samples inoculated with *Listeria innocua* DSM 20649 packaged in ordinary and modified atmosphere, during storage at 4°C. Lines 1 to 3: MPV samples, packaged in ordinary atmosphere, at 0, 3, and 6 days of storage; lines 4 to 7: MPV samples packaged in modified atmosphere at 0, 3, 5, and 7 days of storage; line 8: *Listeria innocua* DSM 20649; lines 9 to 11: MPV samples inoculated with *Listeria innocua* DSM 20649 packaged in ordinary atmosphere at 0, 3, and 6 days of storage; lines 12 to 15: MPV samples packaged in modified atmosphere inoculated with *Listeria innocua* DSM 20649 at 0, 3, 5 and 7 days of storage.

as a warning indicator, which reinforces the necessity for corrective measures to avoid contamination of vegetables, plant and processing machinery.

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TIME-TEMPERATURE EXPOSURE OF FRESH FISH IN THE COMMERCIAL CHAIN

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ABSTRACT

Fresh fish is a very precious and delicate commodity. Testing/modeling of the shelf life of fresh European Sea bass (*Dicentrarchus labrax*), with reference to its thermal history in the commercial chain, was approached by means of a new kind of small temperature recording devices, directly placed in the fish meat and/or in the storage rooms. Twenty selected "real" thermal histories were so collected and split into the different steps experienced during the commercial life (storage at manufacturer, delivery, storage in retail cells, exposition in retail counters).

The fish remains at temperatures lower than 1°C during the first steps of the commercialization and reaches 5°-8°C during the exposition at the sale point: this step is also the longest one (3-5 hours). The obtained data were statistically analyzed and plotted on time-temperature exposure probability charts (50%, 75%, 90% probability values were considered). Delivery at the sale points resulted as the critical step because of the poor control of product temperature and the influence of ambient conditions. Collected data were also employed to parameterize the shelf life: a suitable hypothesis was applied to work out the integration of the most probable time-temperature exposure history. Our findings (75% of the cases) showed that the expected shelf life (or "freshness") just after the purchase of fresh fish was 60% of the whole one. This condition can be related to an integration average temperature of 4°C, considering 2 days of commercialization time. Furthermore, our results showed that freshness can be maintained for 1-2 days more during the storage at home. The average exposure temperature could be then reduced by 1-2°C with a joined effort (manufacturer, sale point management, consumer advertising) thus extending the fresh fish shelf life up to 4-5 days.

Key words: European Sea bass, *Dicentrarchus labrax*, shelf life, temperature

INTRODUCTION

European sea bass is traditionally one of the preferred finfish species by the Italian consumer and represents half of the national eurialine fish-farming product. The white flesh, mild flavor and low fat content of sea bass are the major attributes sought by the consumer.

A review of the scientific and technical literature revealed some information about the composition of European sea bass, and the effects of farming practices on the body composition of the growing fish . However, little information is available on quality changes occurring in European sea bass during post harvest handling, distribution and retail, or on the most effective methods of assessing quality changes and storage stability of this marine species (Poli et al. 2002).

This fish is a very safe and delicate commodity, and its shelf life is affected by spoilage related to chemical and microbiological changes, with lowering of sensorial properties. Temperature and handling practices are the most important factors in determining the shelf life of any species of fish. If a fish product is handled carefully, the temperature at which it is held controls its shelf life, i.e. the rate of bacterial spoilage and enzyme breakdown. An indisputable fact is that the higher the temperature the faster fish spoil.

In the commercial chain, temperature exposure and its fluctuations are often unpredictable and higher then recommended tolerances (Italian DL 30/12/1992 and other regulations recommend the melting point of ice as the ideal storage temperature for fresh fish products).

Moreover, the classical temperature measures and recording devices (i.e thermocouples) are not efficient tools to monitor the commercial chain, because they reflect the environment temperature instead that of the product (Riva and Crepaldi 2004).

The purpose of this study is to define the reliable time-temperature exposure conditions for fresh European sea bass during its marketing. We have applied a new small device to record temperature, which is directly placed in the fish meat and/or in the storage rooms, in order to obtain a number of records of the overall thermal history.

MATERIALS AND METHODS

Time-Temperature recording devices (TB Econorma SAS), water proof, +/- 0.5°C temperature tolerance, were employed. Figure 1 shows the features and the size of the device. Each device was set and read by a PC. The devices were placed under the gill of a fresh fish at the beginning of its commercial life.

Observations were collected during summer 2005, in collaboration with a retailer operating in the North Italy. He takes care of receiving the fish which comes from a Tuscany fish-farm, in refrigerated trucks, where the fish is kept on melting ice, 6-8 hours after catching. The retailer is responsible for inspecting, weighting and settling the fish in polystyrene boxes (always on melting ice). After a short rest in a storage room, boxes are taken to the sale points, where they rest in a storage cabinet, and finally are exposed on the sale bench.

Samples of the fresh fish is delivered through the commercial chain with the measuring device, which is recovered either at a given step of the chain or at the exposure bench. Time-temperature data were downloaded into a PC. Twenty thermal histories related to 5 sales points were so collected.

Recorded data were segmented into five periods: 1) storage at the retailer, 2) rest in the truck (before transport), 3) delivery, 4) storage at the sale point, 5) rest on the exposure bench. For this purpose, the time span of each period was assessed through official register records related to each box, whereas the total exposure time was recovered from each device.

For each step and for all of the time-temperature histories a couple of exposure probability charts was prepared (Riva and Crepaldi 2004).

Figure 2 shows a record of a complete thermal history.

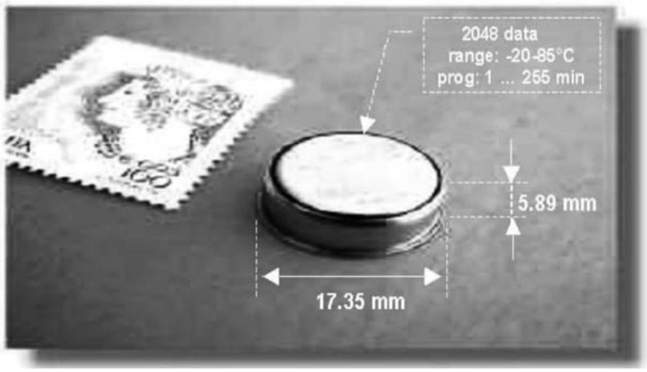

Fig.1. Picture of feature and size of time temperature recording devices.

RESULTS

For each step of the commercialization life, the data of the twenty records were reported in a table, so as to compute the average time and temperature and their standard deviation. Raw data were also analyzed in order to compute their cumulative frequency distributions and plotted in time-temperature exposure probability charts (50%, 75%, 90% probabilities were considered). The 50% probability (or cumulative frequency) refers to an average exposure, whereas the 75% probability is used to "really" describe the thermal history of the product and the 90% probability to extrapolate a more severe hypothesis (Jul 1984).

Figure 3 shows the raw records (on the left) of the time-temperature histories during the critical step of the bench exposure. At the sale point, fish is taken out from the polystyrene box and distributed over the exposure bench in the presence of melting ice. The figure shows a great variability of the thermal histories due to the casual location of fish on the bench and to the slow

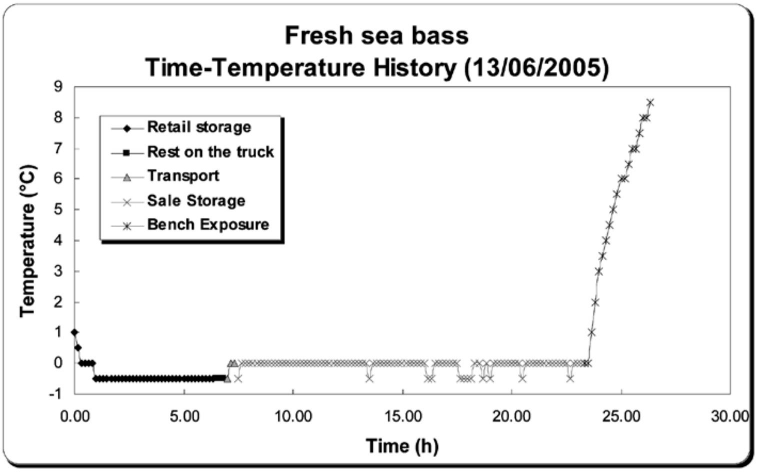

Fig. 2. An example of complete recording of a thermal history. The insert shows the average data for each commercialization stage.

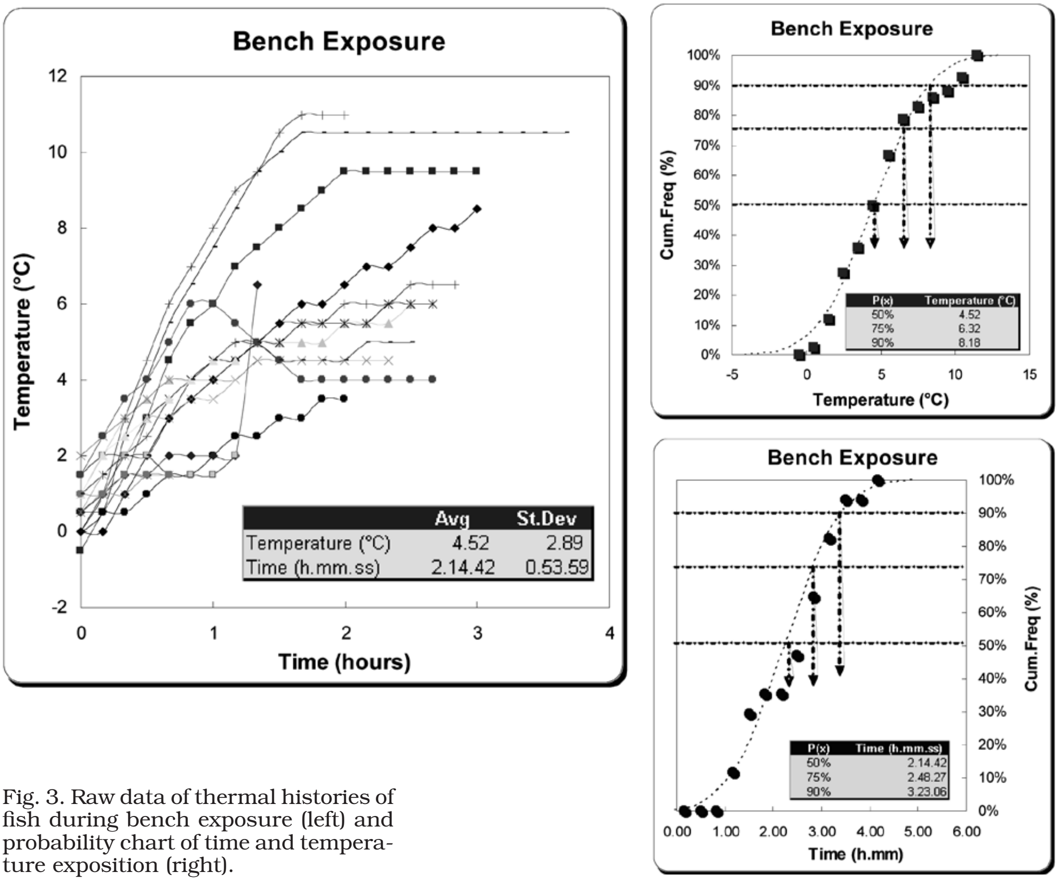

Fig. 3. Raw data of thermal histories of fish during bench exposure (left) and probability chart of time and temperature exposition (right).

disappearance of the ice covering. Figure 3 shows also the temperature and the time exposure probability charts (on the right) for this commercialization step: experimental data were fitted with a statistical distribution function, whereas the insert in each chart reports the relevant data.

Table 1 summarizes the results obtained for all the considered commercialization steps.

Table 1. Time-Temperature reference data.

	Time (h.mm.ss)			Temperature (°C)		
Step	p = 50%	p = 75%	p = 90%	p = 50%	p = 75%	p = 90%
Storage at the retailer	6.25.18	6.56.24	7.28.21	0.240	0.587	0.944
Rest in the truck (before transport)	0.21.46	0.26.49	0.32.01	0.081	0.331	0.588
Delivery	0.25.18	0.29.47	0.34.23	0.105	0.346	0.595
Storage at the sale points	27.11.46	38.15.41	49.37.40	0.184	0.468	0.761
Rest on the exposure bench	2.14.42	2.48.27	3.23.06	4.515	6.324	8.182
CUMULATIVE	1.53 (°)	2.04 (°)	2.57 (°)	0.46	0.82	1.19
(°): days						

DISCUSSION

Time-Temperature reference data (table 1) show that temperature control assured by the melting ice is optimal during the first 4 steps of the commercialization and only in the most severe hypothesis (1 out of 10 times) the temperature reach 1°C. In the first 4 step, the time span is longer for the storage at the sale points, where the boxes are stored for 1 (minimum) to 2 days (maximum) before exposure, so as to assure a proper and continuous supply to the bench. The critical point (for temperature) is the rest on the exposure bench, were the fish are out of the boxes, the ice melts and the room temperature is higher. This step is not longer than 3.5 hours (9 out of 10 times), because normally the amount of fish exposed is planned. Considering the cumulative history, our data support the current practice of delivering the fresh fish 2-3 times a week at each sale point.

The expected shelf life was then tentatively determined on the basis of time-temperature history data. According to the scientific literature (Labuza 1982, Singh and Heldman 1993) the following equation can be applied:

$$\text{RSL } \textit{(Remaining Shelf Life)} = 1 - \sum_{i=0}^{n} \left(\frac{t_n}{t_s} \right)_{Tn}$$

where t_n is the time lapse of the n^{th} stage and t_s is the corresponding stability time at the temperature T_n.

The stability time (t_s) depends on temperature through the following classical relationship:

$$t_s = t_o \exp [T_n - (\ln Q_{10} / 10)]$$

with Q_{10} = increase of a degradative reaction rate for a 10°C temperature increase.

Few data are available in the scientific literature about the temperature dependence of the shelf life of European sea bass. Concerned researchers are normally interested only in the definition of freshness decay at 0-1°C, i.e. in the presence of melting ice.

In few works, with reference to different freshness indices, the following shelf life values have been reported: 12 days (total volatile basic nitrogen content, TVBN, Castro *et al.*, 2006), 16 days (sensory, Ozogul *et al.*, 2005), 9 days (biogenic amines and *Pseudomonas* population, Paleologos *et al.*, 2004), 8-9 days (microbiological counts, Taliadourou *et al.*, 2003), 16-19 days (sensory and microbiological counts, Kyrana and Lougovois 2002). Our opinion is that only the lowest values are reliable.

Figure 4 reports data related to the dependence of shelf life on temperature for Sea bream (Koutsoumanis 2001), Sea bass (Poli *et al.*, 2002) and Sea bass (Riva, unpublished results): the relevant Q_{10} values, 3.44, 3.86, 3.31, respectively, allow a safe choice of 4 as a reliable reference (with shelf life equal to 4 days at 4°C).

The most reliable (75% probability) time-temperature exposure data recorded in our survey were integrated with an appropriate routine calculation based on the previous relationships, in order to define the remaining shelf life (RSL) (Riva and Piergiovanni 2002). Results of this approach are shown in figure 5.

When fresh European sea bass has been purchased, the remaining shelf life is more than 75% 3 out 4 times. This condition is related to an integrated average temperature of 0.8°C and to a 2.57 day overall commercialization time.

The application of the most severe hypothesis (90% probability) allow to compute a 35% decay of freshness.

In our survey, the step of the fish transport from fish farm to retailer was not considered. A reliable hypothesis (based on retailer experience and information) is that fish is delivered in a day (maximum) in conditions of ice covering (T=1°C).

Also the ending period (purchase by the consumer, transport to home, home storage) of the product history was not considered: these steps are very unpredictable!

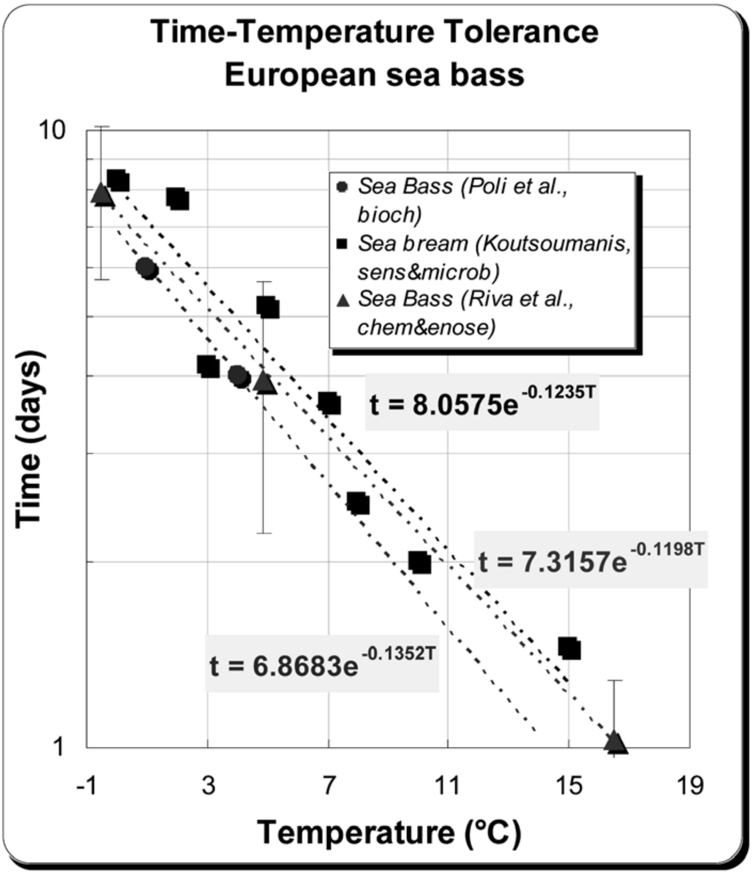

Fig. 4. Time-Temperature Tolerance chart of fresh fish based on different shelf life studies.

In a previous work (Riva and Crepaldi 2004), however, data of a survey related to another fresh product (pasteurized milk) have revealed that transport to home is a very short step (less than 1 h, with temperature lower than 15°C, 3 out 4 times). The critical point is the home storage: normally the temperature of the refrigerators is higher than the planned temperature of 4°C and, in 1 out of 4 times, was higher than 10°C.

The remaining shelf life plot and related prediction reported in figure 5 are accordingly “corrected”, taking into account these three hypothesis: figure 6 results support the conclusion that, in this case, 50% of the fish freshness is “wiped” during commercialization; the home storage can consequently be extended to only 1 day longer.

Another post-simulation shows that a 2°C reduction of the refrigeration temperature at home (with an appropriate advertising campaign) could increase the shelf life to 5-6 days and further day could be gained with a better handling at sale point (employ of refrigerated cabinets) and delivery (transport with melting ice).

CONCLUSIONS

In the shelf life surveys, as demonstrate by our observations, a very accurate time temperature history can be obtained by an "*in situ*" recording with micro-devices. The shelf life of fresh European sea bass at 4°C can be supposed to last 4 days at 4°C. The average exposure temperature in commercial chain is correct (0-1°C) during storage and delivery. Exposition at the sale point and household storage (probably) are the critical stages. The average time or the most probable commercialization time can clearly assure the freshness of fish for 3-4 days. A joint effort (sale point management, consumer advertising) could reduce the exposure temperature by 1-2 °C and justify an extension of the shelf life of fresh fish to 2-3 days after purchase. Obviously, this last point should be investigated further: our study suggests only a methodology.

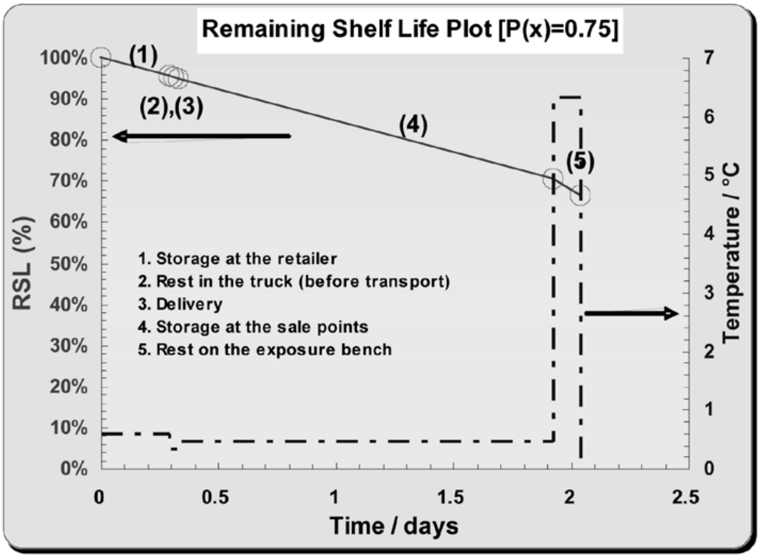

Fig. 5. Remaining Shelf Life parameterization of fresh fish shelf life during commercialization.

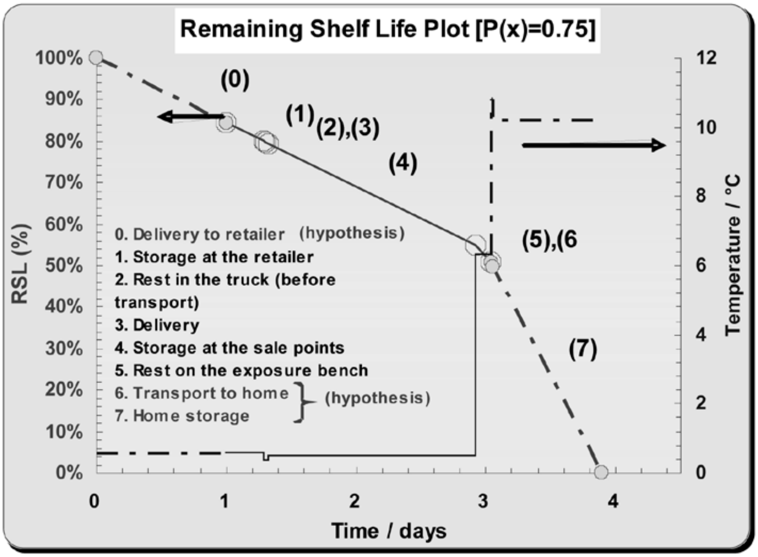

Fig. 6. Remaining Shelf Life parameterization of fresh fish shelf life during commercialization, considering the transport from fish farm to retailer and the home storage.

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HEXANAL AS SHELF-LIFE INDICATOR: ACCELERATED STORAGE TEST OF NUTS ANALYSED BY SPME

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ABSTRACT

Different packaging techniques such as modified atmosphere, oxygen-barrier packaging material and active packaging (oxygen scavengers) have been developed to delay food oxidation processes. The addition of natural or synthetic antioxidants to packaging materials has also been recently investigated.

The present work was carried out to evaluate the effect of different storage conditions of nuts. Nuts were chosen as a model food due to their particular sensitivity to oxidative processes. Grounded nuts were stored under controlled conditions of light and temperature with and without oxygen scavengers.

Hexanal was chosen as indicator of lipid oxidation and product shelf life. Oxygen and carbon dioxide were monitored during storage time as well as hexanal content in order to establish a correlation between shelf-life indicator and product oxidation.

Oxygen scavengers were shown to reduce the oxidation; however the analysis of pouch content revealed that volatile compounds from the package headspace were also absorbed.

Key words: shelf-life, accelerated oxidation tests, hexanal, SPME, oxygen scavengers

INTRODUCTION

Lipid oxidation is one of the main causes of quality deterioration of fatty food, since this process involves the formation of off-flavours, which could reduce the consumer acceptability (Jensen *et al.,* 2005). Light and temperature have been identified as factors that accelerate the oxidation. In order to evaluate lipid oxidation simple and rapid methods have been described: 2-thiobarbituric acid reactive substances test (TBARS) is one of the most common; others are based on a titration such as the determination of the peroxide value.

Hexanal is considered a good marker to monitor rancidity since is one of the major products formed during the oxidation of linoleic acid (Goodridge *et al.,* 2003). In order to delay the lipid oxidation several approaches have been investigated: addition of antioxidant (Stashenko *et al.,* 2002) and special packaging techniques including the use of oxygen scavengers (Lee *et al.,* 2002).

In the present study an accelerate oxidation test was conducted by storing the samples under UV radiation as well as at 40°C in order to evaluate the shelf life of hazelnuts. The effect of oxygen scavenger on rancidity was also evaluated. Oxidation process was monitored analyzing hexanal content by a SPME-GC method.

MATERIALS AND METHODS

Reagents and samples

Hexanal was supplied by Sigma-Aldrich (Steinheim, Germany) and had a purity of 98%. The stock standard solution was made in triacetin and stored at 4°C. Hazelnuts samples were purchased from a local supermarket. With the aim of studying the effectiveness of the oxygen scavengers, the samples were stored in bags of an oxygen barrier plastic film and in headspace vials hermetically closed.

Instrumentation and analytical procedure

SPME procedure was conducted according to a previous method developed in our laboratory (Pastorelli *et al.,* in press). A double phase SPME fibre of Carboxen/Polydimethylsiloxane (CAR/PDMS, 75 μm) was selected. The analyses were carried out on a 5890 Hewlett-Packard gas chromatograph equipped with a flame ionization detector (FID).

A 0.75 mm i.d. narrow-bore glass liner (Supelco, Bellefonte, USA) was installed in the injector in order to improve peaks resolution (Vas and Vébey, 2004).

The injection was made in splitless mode at a temperature of 250°C. Hexanal was separated on a Carbowax capillary column (30 m 0.25 mm ID, 0.5 μm). The GC temperature was initially 50°C, then raised to 100°C at the rate of 10°C/min, increased at 20°C/min until 200°C and finally held for 7 min. The carrier gas was helium with a flow rate of 1.2 mL/min.

Oxygen and carbon dioxide in pouches and vials headspace were monitored by a headspace analyser PBI Dansersor Checkmate 9900 (Dansensor Italia srl, Segrate, Milano).

Accelerated oxidation test

Hazel-nuts were stored under three different storage conditions: room temperature, 40°C and UV light. Sample stored at room temperature and 40°C were prepared packaging hazel-nuts, hazel-nuts + oxygen scavenger and oxygen scavenger both in plastic barrier pouches (10*10 cm) and in vials (20mL). For UV light samples were packed only in plastic barrier pouches.

Samples with hazel-nuts were prepared by weighting 1 g of food for each bag or vial. Headspace content of packages was controlled at 0, 2, 5, 16 and 31 days.

RESULTS AND CONCLUSION

During the accelerated oxidation study the content of hexanal changed indicating that UV radiation accelerate the reaction. A reduction of hexanal content in hazelnuts samples with oxygen scavengers was observed when comparing with the ones stored without the oxygen absorbing sachet in the same conditions. The efficiency of active packaging was also confirmed by the very low oxygen content (0.01%) of headspace package stored with oxygen scavenger.

Samples stored in pouches shows an increase of hexanal content only in the case of UV while in samples stored at room temperature and at 40°C the hexanal profile remained constant along the storage period. In order to understand better hazel-nuts oxidation process at room temperature and at 40°C further experiments were conducted storing the same amount of sample in a reduce headspace volume (20mL vials).

The experiments carried out with vials showed a similar behaviour comparing with the correspondent in pouches at room temperature (fig. 1) while a progressive increase of hexanal content at 40°C is shown in figure 2.

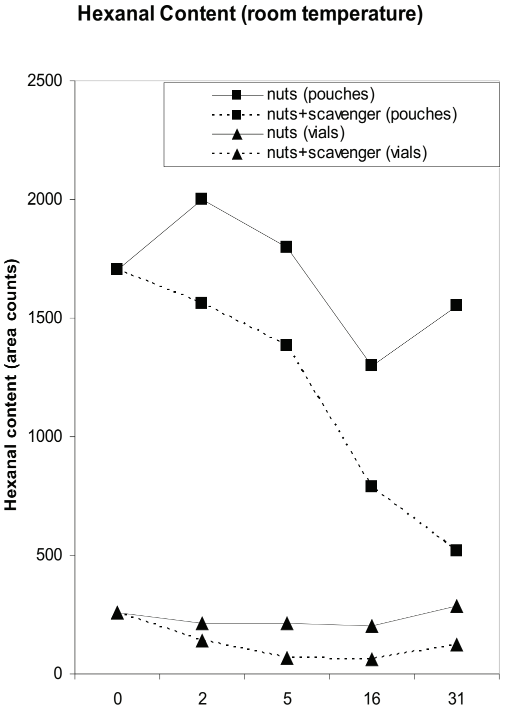

Fig. 1. Hexanal content of samples stored with and without oxygen scavenger at room temperature.

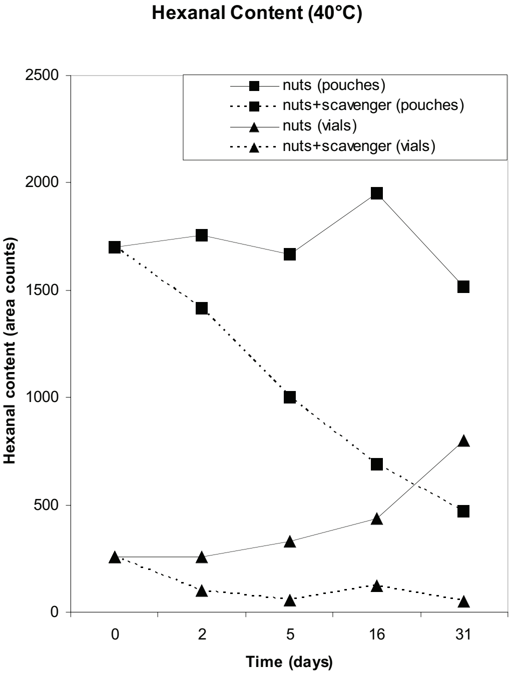

Fig. 2. Hexanal content of samples stored with and without oxygen scavenger at 40°C.

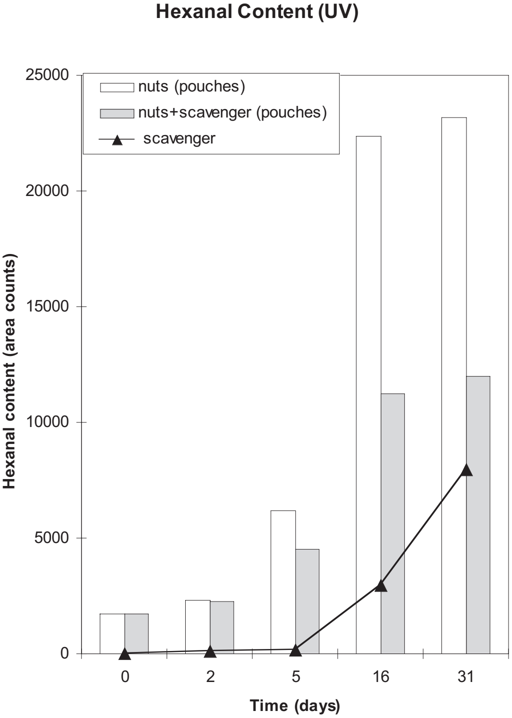

Fig. 3. Hexanal content of samples stored with and without oxygen scavenger stored under UV in correlation with their scavenger hexanal content.

This phenomenon could depend on the different starting oxidation state of food product.

In fact, due to the high initial hexanal content of hazel-nuts used for pouches experiments, only in case of very drastic storage conditions like UV light an increase of hexanal content is observed along the 31 days.

In the case of vials experiments even a slight increase of temperature (40°C) was enough to determine an increase of product oxidation comparing with the ones stored at room temperature.

The analysis by SPME/GC-FID of the powder contained in the oxygen scavenger revealed the presence of hexanal absorbed by the scavengers.

The content of hexanal absorbed by the active packaging increased along the time of storage as shown as example for UV in figure 3.

In order to preserve the product and extend the shelf-life light and temperature should be controlled during storage. Oxygen scavenger could be a good approach to extend fatty foods shelf-life. More detailed experiments should be done to evaluate the interaction between active packaging and food shelf-life indicators in order to avoid consumer misleading.

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SHELF LIFE OF READY TO USE *"CAPRESE"*

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ABSTRACT

The aim of this work was to evaluate the shelf life of ready to use "*caprese*". Caprese samples were packaged in high barrier plastic bags and stored at 4 and 15 °C. The growth kinetic of the lactic + bacteria and of spoiling microbial groups of tomato slices and mozzarella cheese were studied. The pH and the amount of O_2 and CO_2 in the head space were monitored, too. Shelf life values at 4 °C were 4.36 and 3.00 days for coliforms and *Pseudomonadaceae* of mozzarella cheese, respectively. The CO_2 of the head space increased in all the samples while the amount of O_2 decreased up to the undetectable level. Because of the microbial activity, pH of mozzarella cheese changed from 6.6 to 5.5 at 15 °C; in tomato slices, instead, it was observed a light increase of pH value.

Key words: caprese salad, coliforms, convenience foods, *Pseudomonadaceae*

INTRODUCTION

Convenience foods or "tertiary processed foods" are product which are designed to save consumers time in the kitchen and reduce costs due to microbial spoilage. These foods require minimum preparation, typically just heating, and are packaged for a long shelf-life, with little loss of flavour and nutrients over the time (en.wikipedia.org, 2006).

Caprese salad is a traditional Italian dish and, like 4th range products, it's a minimally processed, "ready to use" and convenience food. The ingredients of this dish are tomato and cow or buffalo mozzarella cheese slices, extra-virgin olive oil, basil and wall rocket.

Despite several benefits, as the simpleness and quickness of preparation, it has a very short shelf-life (1-2 days), that hampers its presence in canteens or in local markets.

The aim of this work was to evaluate the shelf life of ready to use "*caprese*", prepared with tomato slices (variety Pachino) and mozzarella cheese, purchased in cheese factory of Apulian area.

MATERIALS AND METHODS

Caprese preparation. Cow mozzarella cheeses of about 50 g and tomatoes, belonging to Pachino variety, were purchased from a local market of Apulia (a region of Southern Italy).

After removing the calyxes and washing tomatoes, the berries and mozzarella cheeses were cut in slices of about 10 g of weight.

Mozzarella cheese and tomato (five slices of cheese and six slices of tomato for each caprese sample) were packaged into high barrier plastic bags [Nylon/Polyethylene, 102 µm (Tecnovac, San Paolo D'Argon, Bergamo, Italy)] and stored for 12 days at 4 and 15 °C. The bags were 170 mm×250 mm long with properties specified by the manufacturer as follows: CO_2 and O_2 permeability of 3.26×10^{-19} mol m m^{-2} s^{-1} Pa^{-1} and 9.23×10^{-19} mol m m^{-2} s^{-1} Pa^{-1}, respectively, and water vapour transmission rate of 1.62×10^{-10} kg m^{-2} s^{-1}.

Microbiological analyses. 10 g of mozzarella cheese and 10 g of tomato slices were diluted separately with 90 mL of sterile saline solution (0.9% NaCl) and homogenized in a Stomacher bag for 1 min by a Stomacher Lab Blender 400 (PBI International, Milan, Italy). Decimal dilutions of cheese and tomato homogenates were performed and microbiological counts were carried out.

On tomato homogenates the cell load of lactic acid bacteria, yeasts and moulds was evaluated; on mozzarella homogenates, instead, the cell load of mesophilic and psychrotrophic bacteria, lactic acid bacteria, total coliforms, enterococci and *Pseudomonadaceae* was carried out.

The media and the conditions were as follows: Plate Count Agar (PCA), incubated at 5 °C for a week or at 32°C for 48 hr for psychrotrophic and mesophilic bacteria, respectively; MRS Agar, modified by adding 0.17 $g*L^{-1}$ of cycloheximide (Sigma-Aldrich, Milan, Italy) after autoclaving at 121°C for 15 min, incubated at 30°C for 4 days under anaerobic conditions for lactic acid bacteria; Violet Red Bile Agar (VRBA), incubated at 37°C for 18-24 hr for total coliforms; Slanetz Bartley Medium, incubated at 37°C for 48 hr for enterococci; Pseudomonas Agar Base, modified by adding Pseudomonas CFC selective supplement after autoclaving at 121°C for 15 min, incubated at 30°C for 48 hr, for *Pseudomonas* spp.; Sabouraud Dextrose Agar, added by chloramphenycol (0.1 $g*L^{-1}$) (C. Erba, Milan, Italy), incubated at 25°C for 2 days for yeasts; Malt Extract Agar, acidified at pH 4.5 with a citric acid solution (1:1 $w*w^{-1}$) (C. Erba), after autoclaving at 115°C for 10 min, incubated at 25°C for 4 days for moulds.

All the media were from Oxoid (Milan, Italy).

pH measurement. pH was evaluated on the first homogenate of mozzarella cheese and tomato slices by a pH-meter Crison mod. 2001 (Crison Instruments, Barcelona, Spain).

Evaluating the gas in the head space. Before opening the plastic bags of caprese samples, the amounts of oxygen and carbon dioxide in the head space was evaluated through a Gas Analyser Checkmate 9900 O_2/CO_2 (PBI). The data were expressed as $v*v^{-1}$.

Data modeling and shelf-life calculation. All the analyses were performed in triplicate; the experimental points are the average of three replicates and are accompanied by the standard deviation.

Cell load data were modeled according to the Gompertz equation, modified by Zwietering et al. (1990), by the option “Non linear Regression” of the software Statistic for Windows (Tulsa, USA).

By using the cell load of total coliforms and *Pseudomonadaceae* of mozzarella slices, the shelf-life of the samples was evaluated; the critical values were set to $1*10^5$ CFU*g^{-1} and $5*10^5$ CFU*g^{-1}, for coliforms and *Pseudomonadaceae* respectively, as reported by the Italian law (Anonymous, 1997) and Bishop and White (1986).

RESULTS AND CONCLUSION

Table 1 reports the Gompertz parameters of lactic acid bacteria of mozzarella cheese. The values of the regression coefficient (0.997) underlines the high adequacy

Table 1. Gompertz parameters regarding the lactic acid bacteria of mozzarella cheese and tomato slices (a). Kinetic parameters and shelf-life values of the spoilage microorganisms of mozzarella cheese (coliforms and *Pseudomonadaceae*), stored at 4 and 15 °C (b).

FUNCTIONAL MICROBIOTA (lactic acid bacteria)						
	k[a]	**A**	μ_{max}	λ	**R**	
Mozzarella cheese						
4 °C[b]	4.80±0.21	3.17±0.32	0.46±0.05	0.96±0.08	0.997	
15 °C	4.81±0.35	3.34±0.19	2.32±0.34	0.36±0.03	0.997	
Tomato slices						
4 °C	2.19±0.45	4.05±0.71	0.53±0.10	0.82±0.49	0.994	
15 °C	2.25±0.09	5.16±0.02	4.92±0.08	0.87±0.00	0.999	
SPOILAGE MICROORGANISMS						
	k[a]	**A**	μ_{max}	λ	**R**	**shelf-life**[c]
Total coliforms						
4 °C	3.30±0.21	2.91±0.33	0.54±0.11	1.15±0.07	0.994	4.36
15 °C	3.37±0.17	2.77±0.23	2.59±0.38	1.29±0.11	0.997	1.93
Pseudomonadaceae						
4 °C	4.11±0.26	3.60±0.35	0.76±0.16	0.91±0.07	0.993	3.00
15 °C	4.15±0.00	3.67±0.01	19.86[E]	0.91±0.00	0.999	0.98

[A] Gompertz parameters: k, initial cell load (Log CFU*g^{-1}); A, maximum increase of the cell load attained at the stationary phase (Log CFU*g^{-1}); maximal growth rate (ΔLog (CFU*g^{-1})*day^{-1}); λ, lag phase (days); R, regression coefficient. Gompertz parameters are accompanied by the standard error.

[b] Storage temperature.

[c] Time (days) to attain the critical value, set to $1*10^5$ Log CFU*g^{-1} and $5*10^5$ Log CFU*g^{-1}, for coliforms and *Pseudomonadaceae,* respectively.

[d] Not significant (P>0.05).

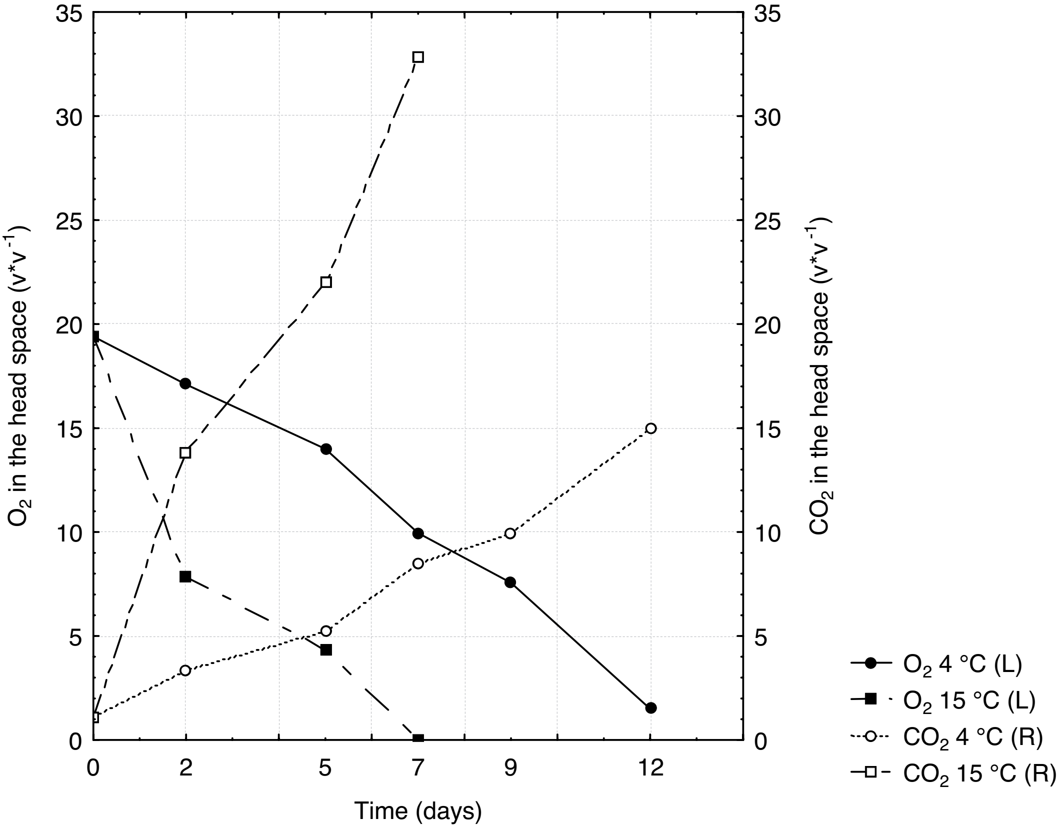

Fig. 1. Evolution of O_2 and CO_2 ($v*v^{-1}$) in the head space of caprese samples, stored at 4 and 15 °C.

of the proposed model to the experimental data. As expected, the cell load of lactic acid bacteria was moderately high (4.80 Log $CFU*g^{-1}$) on the raw material; within 12 days (time of storage), it increased of about 3 Log $CFU*g^{-1}$, without significant differences between the samples stored at 4 °C and those stored in thermal abuse (15 °C). The storage temperature influenced the parameter μ_{max}; moreover a light decrease of the lag phase was observed.

Tomato microbial population was mainly composed by lactic acid bacteria; yeasts and moulds were never detected during the storage. The initial load of lactic acid bacteria on tomato slices was very low (2 Log $CFU*g^{-1}$), but it increased up to 6.24 and 7.41 Log $CFU*g^{-1}$ at 4 and 15 °C, respectively.

According to the Italian law, coliforms are the test microorganism for evaluating the shelf-life of mozzarella cheese; the critical value was set to 5 Log $CFU*g^{-1}$ (Anonymous, 1997). The presence of coliforms in cheese, in fact, is an indication of poor sanitation; coliforms grow rapidly in cheese during the first days of storage and their metabolites include lactic acid, acetic acid, formic acid, succinic acid, ethanol, 2,3-butylenglycol, H_2 and CO_2. The production of the gas results in early blowing; moreover a cell load of coliforms of 10^7 $CFU*g^{-1}$ in retailed prepacked cheese cause gassy defects and the swelling of the plastic bags (Tamime, 2000).

Table 1 reports also the Gompertz parameters and the shelf-life values regarding the coliforms and *Pseudomonadaceae* of mozzarella cheese. The thermal abuse increased signficantly the growth rate of coliforms (μ_{max}), but it did not affect the others kinetic parameters; moreover the shelf-life of mozzarella slices decreased from 4.36 to 1.93 days.

Psychrotrophic bacteria can cause defects in fresh soft cheese, like mozzarella; the microorganisms responsible of the spoilage belong to the genus *Pseudomonas*. The most common defects are the surface discoloration, off-odours and off-flavours. Moreover thermostable lipolytic enzymes produced by this genus in raw milk survive cheese-making, causing rancidity; proteases from psychrotrophic bacteria are responsible of proteolysis, leading to bitterness in cheese (Tamime, 2000). In our experimental conditions, the critical value for *Pseudomonadaceae* was set to $5*10^5$ $CFU*g^{-1}$, according to Bishop and White (1986); shelf-life values, for these microorganims, were 3.00 and 0.98 days, at 4 and 15 °C, respectively.

The CO_2 of the head space increased in all the samples and reached the maximum value after 12 and 7 days, respectively at 4°C and 15°C, while the amount of O_2 decreased up to the undetectable level within the experimental time (figure 1).

Because of the microbial activity, pH of mozzarella cheese changed from 6.6 to 5.5 at 15 °C; in tomato slices, instead, it was observed a light increase of pH value (data not shown).

The data of this work can be considered as preliminary, but it allowed us to point out some interesting considerations: a) the shelf-life of caprese salad was of about 3.00 days, in refrigerated storage conditions; b) mozzarella cheese was the critical element for the shelf-life of this product; c) the shelf-life of caprese salad might be calculated by using both coliforms and *Pseudomonadaceae* cell load. The use of total coliforms as the only test microorganisms could lead to an upper estimation of the shelf-life, as in the experimental conditions of this work

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EFFECT OF DIFFERENT STORAGE CONDITIONS IN SEMI-INDUSTRIAL TANKS ON THE SHELF-LIFE OF EXTRA VIRGIN OLIVE OIL

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ABSTRACT

During one year the effect of the use of different temperatures and atmospheres during the storage of an extra-virgin olive oil in semi-industrial tanks was investigated. The oil was stored in three different tanks immediately after the extraction process. Each tank had a capacity of 250 l. Initially all the tanks were filled up completely. Two of the tanks were saturated with nitrogen. Only one of the three tanks was refrigerated. The oils remained in this condition for six months. After this period the tanks were partially emptied to half, in order to simulate the frequently storage condition in the industrial mill plant. The oils remained in this conditions for another six months. The oils were sampled every 30 days for analysis. The analytical parameters considered were: free acidity degree, peroxide value, ultra violet indices (K_{232}, k_{270}, ΔK), total phenol content and induction time. Our results show both temperature and the atmosphere that had influence on the extra virgin olive oil shelf life in a semi-industrial storage plant.

Key words: virgin olive oil, shelf life, total phenol content, olive oil stability

INTRODUCTION

Oxidative rancidity development has been recognised as the leading cause of oil deterioration during storage. This reaction happens between unsaturated fatty acids, and oxygen. The two compositional factors of oils that determine their susceptibility to oxidation are the fatty acid composition and inherent antioxidant compounds. Virgin olive oil holds a rich source of natural antioxidants including carotenoids, tocopherols and phenolic compounds. The phenolic compounds are considered to be the main antioxidant compounds in virgin olive oil, and they play an important role in oil stability. The presence of this compost associated with the absent of oxygen has already been evaluated that to extend the shelf life of olive oil. (Baldioli, Servili, Perretti and Montedoro 1996) According to the scientists , this is evidence that the antioxidant fighting power of extra virgin olive oil has a limited shelf life. Air, heat, light, and age affect the quality and the shelf life of olive oil, which deteriorates through oxidation (rancidity). The oxidation process is greatly enhanced when olive oil is stored in containers that are not air tight and in areas where it is exposed to heat and light. If improperly stored, olive oil can easily take on other flavors. An unpleasant smell or taste indicates that olive oil is no longer desirable for consumption. If olive oil has become rancid very quickly, it may prove that the oil was improperly stored and has oxidized (Frankel, 1998).

The aim of this current study was to evaluate the effect of different factorson the stability of olive oil.The factors were the different composition of headspeace of the storage tanks, storage time and temperature. The study investigated the olive oil in different kinds of storage condictions: with air, with modified atmosphere of 100 per cent of nitrogen gas and also with modified atmosphere with a control of the temperature. The parameters of the oil's quality were followed during 12 months of storage in stainless steel tanks. The tanks were completely filled according to the different storage conditions and then the quality of the oil stored in the tanks after they were partially emptied until half of the capacity of containers, were evaluated. A good quality was found in all oil after a six month storage period. After 12 months of storage we could observe that the atmosphere and temperature control produced a loss of all parameters of quality, in particular a significantly increased of total phenol content and the stability of oil.

Stainless steel is much better and currently is considered one of the best storage methods to use frequently in industry of oil but is important to control the storage conditions.

MATERIALS AND METHODS

Samples. Olive fruits (*Olea europaea* L.) of two varieties: Leccino and Cellina di Nardò, grown in the Apulia Region (Italy) and harvested during the crop 2004-2005. The olives randomly picked at industrial optimum ripening stage, according to their skin colour. Harvesting was done by hand, using rakes. The olives were put into 30 kg boxes and immediately taken to an industrial oil mill. The oil samples were extracted using a industrial plant equipment with a hammer crusher a mixer and a triple-phase decanter. After crushing, the olive paste obtained was malaxed for 45 min at 30°C.

Storage experiments. The virgin olive oils immediately after the extraction process were stored in three industrial tanks (capacity of 250 litres). The stainless tanks were almost completely filled (3% headspace). In the first phase, of the experiment the oils were stored in three different conditions: (*A*) saturating the headspace of the tank with air at ambient temperature; (*B*) saturating the headspace of the tank with nitrogen at ambient temperature; (*C*) saturating the headspace of the tank with nitrogen and maintaining the temperature under 20°C. The oils were sampled after 30, 60, 90, 120, 150 and 180 days of storage. After six months of storage, the tanks were emplied until half of their capacity. The tanks were followed for anther six months. The oil samples were analyzed for free acidity, peroxide value (PV), specific extinction coefficients K_{232} e K_{270}, total phenol content. The stability was measured using a Rancimat apparatus at 120 °C (20 l/h). The samples were run in duplicate and analyses were done in triplicate. The flux diagram of the experimental procedures are shown in figure 1.

Acidity value, peroxidase index and specific extinction coefficient at 232 and nm (K_{232} and K_{270}). Acidity value, peroxide index and ultra-violet light absorption (K_{232}, K_{270}) were carried out according to the European Official Method of Analysis (EEC/1989/2003 of the Commission Regulation).

Oxidative stability. The oxidative stability was evaluated according to an accelerated automatic test using the Rancimat apparatus, (Metrohm AG, Herison, Switzerland) using an oil sample of 2,5 g, warmed to 120°C and a purified air flow rate of 20 l/h. Stability was expressed as the oxidation induction time (h). In this process, the volatile oxidation products were stripped from the oil and dissolved in the water, increasing the water conductivity. The time taken until there is a sharp increase of conducibility is termed the induction time, and it is expressed in hours (Halbault, Barbé, Aroztegui, & De La Torre, 1997).

Total phenol content. Phenolic compounds were isolated from a solution of oil in hexane by triple-extraction with water–methanol (60:40 v/v). Total phenols, expressed as gallic acid equivalents (ppm), were determined with a UV visible spectrophotometer (Beckman) at 765 nm using the Folin-Ciocalteu reagent (Swain & Hillis, 1969).

Statistical analysis. Experiments to determine the different olive oil parameters were performed in five times, the result are

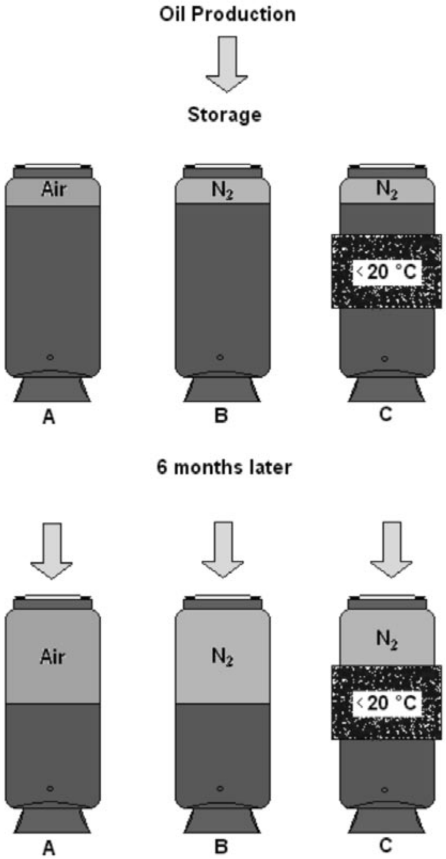

Fig. 1. Flux diagram of the processing procedures.

expressed as means ± standard deviations. One-way analysis of variance (Anova) were used. Differences between groups were considered statistically significant at $p<0,05$.

RESULT AND DISCUSSION

During the initial six months of storage, when the tanks were completely filled, the acidity values of the oils from all conditions storage were lower than 0,5%. No substantial difference was noticed. The increase in acidity value in the oil during storage was positively with the tanks partial empty either in the condition where temperature control or the atmosphere control was used. Therefore remained below the limits reported in EEC regulation 1989/2003, which prescribes a value inferior to 0.8 for a virgin olive oil. Similar beneficial effects of low oxygen atmosphere on acidity value were reported by (Garcia and Streif, 1991, Guttierez *et al.*, 1992).

K_{232} value is an indication of conjugation of polyunsaturated fatty acids whereas K_{270} is an indication of carbonylic compounds (aldehydes and ketones) in olive oil (Garcia *et al.*, 1996b) All K_{232} values of the oils from all increased already after two months of storage and remained approximately the same for another 60 days (Figure 2). Lower K_{232} were obtained in oils kept in tanks completely filled. A K_{232} value of 2.50, which is the limit for extra virgin olive oil, was not found during all the experiments. Our results on the content of carbonylic compounds indicated by K_{270} values show that there was a significant effect on the storage temperature and atmosphere. In fact 0,22 (the limit for extra virgin olive oil) was not surpassed in any

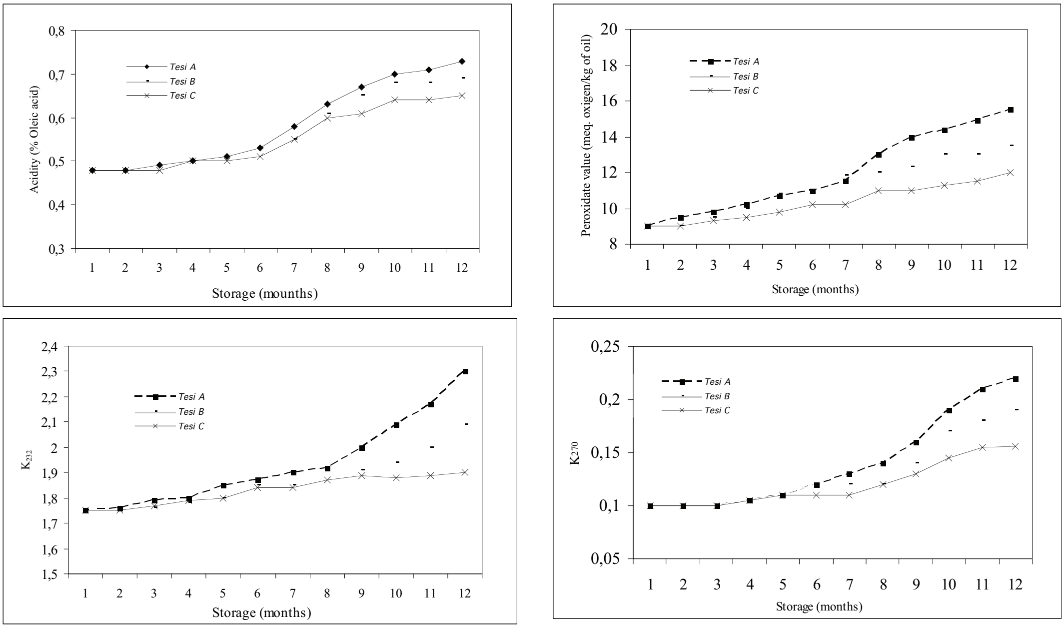

Fig. 2. Changes in the free acidity, peroxidase value and specific extinction coefficient at 232 nm and 270 nm of oils stored in dark bottles for 12 months. Each data point represent mean of three replicates ± SD.

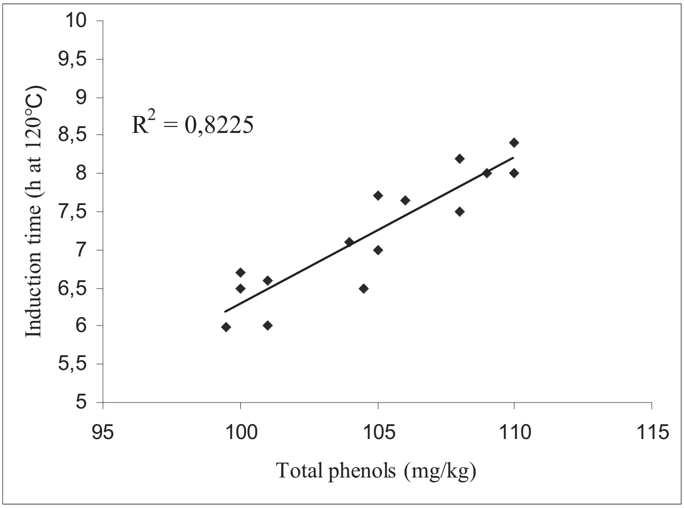

Fig. 3. Correlation between total phenols and induction time in tanks completely full.

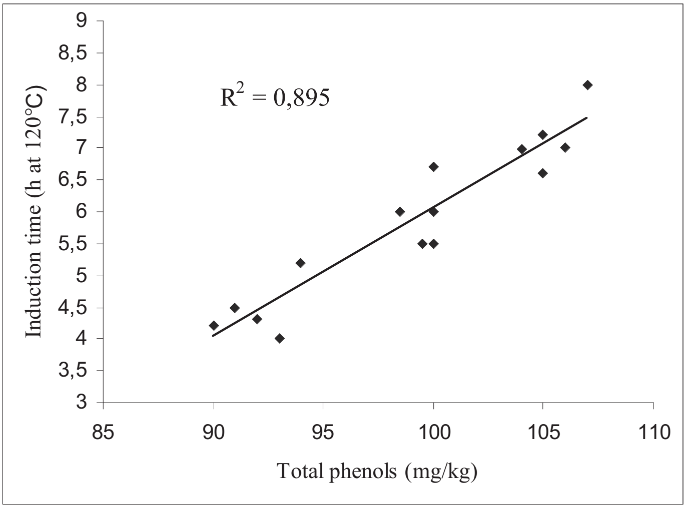

Fig. 4. Correlation between total phenols and induction time in half filled tanks.

experiment except in the oil stored without the control of the headspace filled with nitrogen and the control of the temperature.

Peroxide values followed a similar trend to that of the specific extinction coefficient. Lower peroxide values for the oil stored in tanks completely filled may be a result of the reduction of O_2. Therefore in the condition A, after six months of storage in presence of air, the peroxidate level was 15,5 mEq of oxygen per Kg of oil and surpassed the limit for extra virgin olive oil (20mEq of oxygen per Kg). All the experiments on the half filled tanks, showed an increase of peroxidate values after two months of storage.

The antioxidant activity of hydrophilic phenols of virgin olive oil has been well studied In fact, as reported by different authors, the concentration of phenolic compounds, evaluated colorimetrically and expressed as total phenols, was highly correlated to the shelf life of virgin olive oil, tested using accelerated methods such as Rancimat (Ranalli *et al.*, 2003). Phenolic compounds constantly decreased during storage; samples stored in the tanks completely full revealed a significantly higher value than those stored in the tanks partially empty. Oxidative stability of the samples analyzed showed a decrease after the sixth month period (Figure 3). The overall values show that a direct correlation exists between the two parameters. The values changed significantly from those at the beginning of the storage. Samples kept in the tanks completely filled showed a significantly longer induction time than those stored in tanks partially emply. Figure 4 relates to the total phenols data to the corresponding induction times for the oils in the three different conditions after 12 months of storage in tanks partially emply. The overall values show that a direct correlation exists between the two parameters.

CONCLUSION

The storage of oil in stainless tanks is possible without controlling of the temperature and without the modification of the atmosphere. These conditions offer a good quality of oil only in tanks completely filled and for short periods. When the tanks are partially empty, because of the normal procedures of the bottling in the oil mill, it may be useful to use nitrogen in the headspace atmosphere (to reduce the oxygen pressure in the tank headspace) and temperature control, to control the oxidation during the storage. Frequently these practices are not used in the oil industry. This often determines a decay of the conserved oil.

ACKNOWLEDGEMENT

The authors have contributed to the same extent to the present study.

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DIFFERENTIATION OF FRESH SEAFOOD PRODUCTS AND STORAGE TIME USING AN ELECTRONIC NOSE: FEATURES SELECTION FOR DATA ANALYSIS

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ABSTRACT

Electronic nose devices are becoming a practical tool for the identification of differences among food products based on their origin, intrinsinc quality and process conditions. The difficulty in using such instruments is to obtain, from the sensors response patterns, suitable parameters to identify the differences among samples. In this work electronic nose analyses, trimethylamine determination and panel test were performed on samples of three species of farmed fishes (*Dicentrachus labrax*, *Sparatus aurata* and *Salmo salar*) stored for 8 days both under ice and abuse temperature of 6°C. Data from electronic nose were analysed choosing a group of features i.e average of the last 5 points, maximum point, average of the whole points and average of the signal response characterized by the highest variation. A polynomial regression was performed on the different features in order to identify the contribution of the sensors to the sample response patterns. The data from the best sensors were then used to create an optimum feature (mixed feature). A principal component analysis (PCA) was performed on the data of all the features and the degree of separation of the tridimentional scatterplots used as a selective tool. Based on the results obtained, the features "average of the last 5 points" and "mixed feature" gave a good discrimination of the samples, "mixed feature" being the best. The scatterplot from "mixed feature" data allowed the separation of the fish samples based on species, freshness and storage time. The evolution over time of the scores of the PCA, accounting for the highest variation of the best two features, was correlated both with the TMA content and the overall evaluation of the product given by panelists.

Key words: electronic nose, feature selection, seafood, storage time

INTRODUCTION

The shelf-life of seafood products has been traditionally assessed by microbial counting, physico-chemical measurements (pH, colour) and instrumental determinations such as FTIR, NIR, HPLC and GC analyses for TMA. The release of volatile molecules of a seafood product is the result of process conditions, biochemical reactions and microbial activity. Instruments such as GC-FID and GC-MS are generally used to identify and qualify the individual volatile chemical compounds. However, the relationship between these analyses and the perception of global aroma of a product is not easily established and understood (Giese, 2000). There is a growing interest in the exploitation of electronic noses in the food industry because sensor array devices are qualitative, low-cost, real-time and portable instruments which allow an objective and reproducible determination of volatile molecules (aroma and off-flavor compounds) as a global fingerprint. The adsorption of volatiles on the sensor surface induces an electrical or physical change which is recorded overtime. The resulting curve represents various phases of the instrumental determination (i.e. adsorption, de-sorption and equilibrium between the adsorption and the de-sorption phases). Generally, data from the steady-state and/or from the maximum response of the sensors are retained and put into a classification system. However, the non selective use of these data often results in a reduction of the discriminating power of this technique. Therefore, the aim of the present study was to evaluate an alternative way to extract and analyse the most suitable information from selected signals of the sensors in order to discriminate three seafood products according to the fish species, their freshness and storage time.

MATERIALS AND METHODS

Samples of three fish species (*Dicentrachus labrax*, *Sparatus aurata* and *Salmo salar*), imported in Italy from the same production farm in Greece, were gutted and sliced into 100g portions. The sliced portions obtained were immediately stored under ice and under abuse temperature of 6°C. Chemico-physical and sensory analyses were performed during storage on three different slices of each fish from the same temperature condition at 0, 5 and 8 days.

Determination of trimethylamine

The determination of TMA was carried out according to the method of Miller *et al.* (1972) modified as follows. 10g of sample were homogenized with 15ml of perchloric acid 6% (w/v) (Sigma-Aldrich) and 2ml of the homogenate introduced in a 10ml vial together with a magnetic stirrer, then closed hermetically. 1 ml of NaOH 40% (w/v) (Carlo Erba Reagenti) was then added into the closed vial. The sample was successively equilibrated at 65°C for 20 min and then cooled to 35°C before introducing an SPME fiber (PDMS, 100 μm, Supelco) for 30 min at 35°C. The fiber was then introduced for 5 min into a GC Varian 3800 equipped with a flame ionization detector and a capillary column (Supelcowax™-10, 60m x 0.32 mm x 1.0 μm). The injector and the detector were held at 200°C. The temperature was programmed from 40°C to 75°C at a rate of 3°C/min and 75°C to 200°C at a rate of 5°C/min and then held at 200°C for 4 min. The carrier gas was helium with a flow rate of 1.0ml/min. The analysis was performed in splitless modality. The calibration curve was obtained by diluting the appropriate concentration of TMA hydrochloride (Sigma-Aldrich) into 2ml of perchloric acid (6%) and then following the same procedure as above reported.

Sensory evaluation

Sensory quality was determined with the same frequencies as those of electronic nose analyses. 20 inexperienced panelists were trained to evaluate the following descriptors: rancid, ammoniac and sulphur odours, acidulous and fresh flavours and overall evaluation. The scores were progressive from 0 to 10 and proportional to the intensity of each descriptor. Samples were considered acceptable when the scores were higher or equal to 6.

Electronic nose and analysis setup

A portable electronic nose type Pen 2 Airsense analytics was used. It was composed of ten tin oxide conductivity devices with the following characteristics; WMA_CCTO1 (sensor 1, toluene); WMA_US5 (sensor 2, ozone); WMA_CCTO2 (sensor 3, ammonia); WMA_US6 (sensor 4, hydrogen); WMA_CCTO3 (sensor 5, propane); WMA_US1 (sensor 6, methane); WMA_CW1 (sensor 7, chlorine); WMA_US2 (sensor 8, CO); WMA_CW3 (sensor 9, chlorine warm); WMA_U3 (sensor 10, methane). 20g of product were introduced into a 40ml vial closed with polytetrafluoroethylene caps and left to equilibrate at 25°C for 30 min. After the adsorption phase, the injection occurred at 180°C. Three repetitions of 3 different slices were analysed for each sample. The sensor response data was recorded at interval of 1 sec for 95 sec and expressed as ratio between sensor signal and minimum sensor signal recorded.

Features selection

In order to determine the most suitable sensor signals to discriminate the various samples, four features, i.e. average of the last 5 points (A5s), maximum point (Mp), average of the whole points (Ahp) and average of the signal response characterized by the highest variation (interval 5-15s, Amv), were extracted as previously described by Xiaobo *et al.* (2003) and Skov and Bro (2005) (Fig. 1).

An analysis of covariance (ANCOVA) was performed to determine the sensors that mostly contributed to the discrimination of the samples within each feature. The first nine most significant sensors of all the features were then used to create a new one named “mixed feature” (Mf). Correlations between the selected features and the TMA production and the panel test data were determined by canonical correlation analysis. All basic computations and statical analyses were carried out using Statistica, vers.6 (StatSoft, inc. USA).

RESULTS AND DISCUSSION

A PCA was performed on the data obtained from the different features, and the first three highest PC’s used to draw a scatterplot. The tridimentional representation obtained from the features Mp, Amv and Ahp did not show any separation of the different samples. In figure 2 the scatterplot representation of the first PC’s from

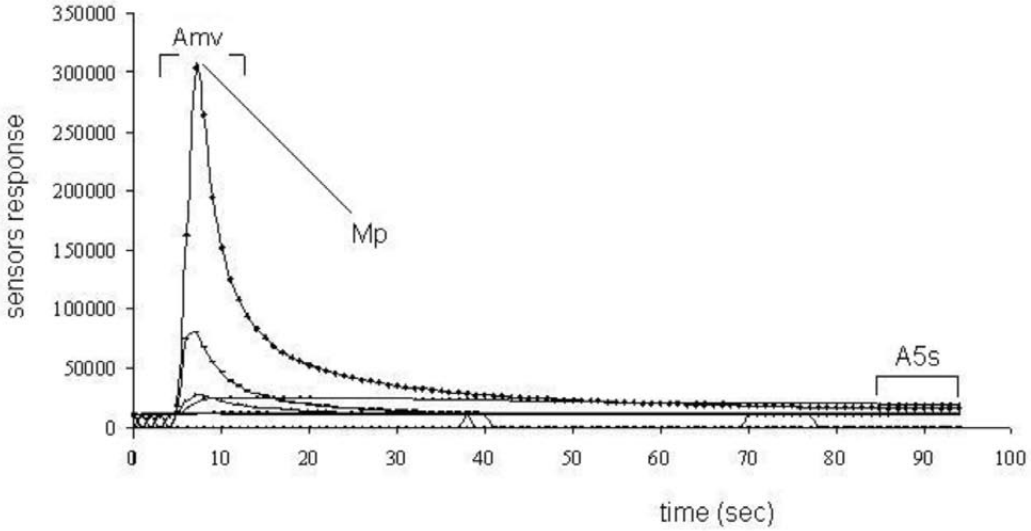

Fig. 1. Typical response of the ten sensors of an electronic nose and features selected for the statistical analyses.

the feature Mp is represented as an example. The first three PC's accounted for the 90.9% of the variability of the data. Despite this high percentage, no separation of the samples was obtained thus indicating that the feature used was not appropriate for this type of sample. The same results were obtained with Amv and Ahp (data not shown). Computing the same analyses on data from A5s, the first three PC's accounted for the 88.7% of the data variability (Fig. 3). A good separation between the three fish species was observed when considering A5s feature. However, no agreement with storage time and freshness within the same species was obtained if compared to the sensory test results (data not shown).

In order to create an optimum feature with a higher discriminating power, the contribution of each sensor was determined by ANCOVA (table 1). Data from the first nine most significant sensors of all the features were then used to create a new one named "Mixed feature". Sensor 3 was the most significant one for three out of the

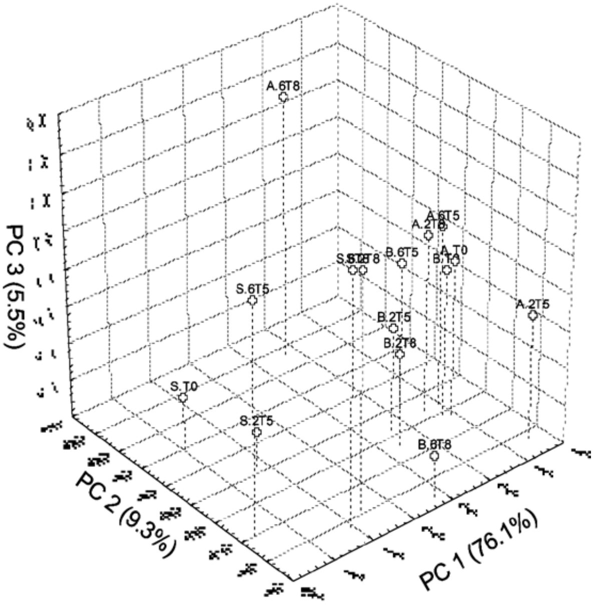

Fig. 2. 3D scatterplot obtained from the PCA of the feature Mp. Fish species: S: , A: *Sparatus aurata*; B: *Dicentrachus labrax*. Storage temperature: 2: under ice; 6: 6°C. Time of storage: To, T5, T8: 0, 5 and 8 days respectively.

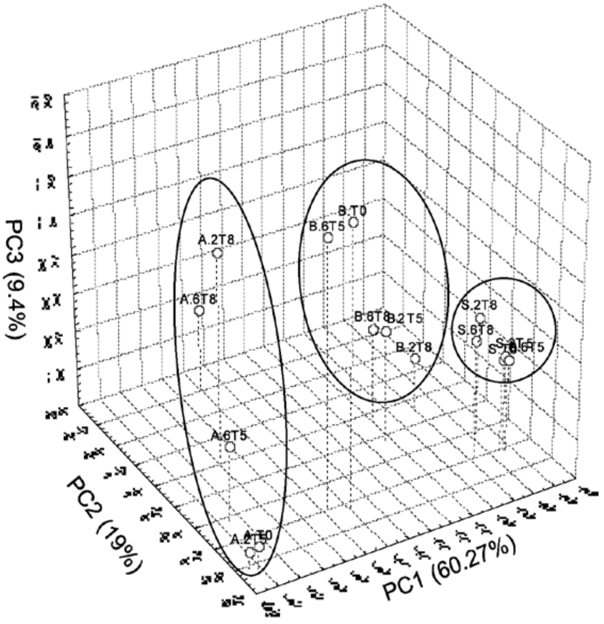

Fig. 3. 3D scatterplot obtained from the PCA of the feature A5s. Fish species: S: *Salmo salar*, A: *Sparatus aurata*; B: *Dicentrachus labrax*. Storage temperature: 2: under ice; 6: 6°C. Time of storage: To, T5, T8: 0, 5 and 8 days respectively.

four features. In fact this sensor, whose volatile molecule target is ammonia according to the manufacturer, was characterized by the highest coefficients. On the other hand the accumulation of ammonia as well as of other nitrogenous compounds generally increases during storage of fish products. In figure 4 the result of a PCA performed on the Mf obtained combining data from the selected sensors is rappresented. The first three PCs accounted for the 89.6% of the total variability. A good separation among samples based on fish species and their degree of freshness was observed, the latter being negatively correlated with temperature and time of storage. This result is in agreement with the observations of sensory tests were *Salmo salar* showed a slow spoilage rate during storage with respect to the other species. Samples of *Dicentrachus labrax* and *Sparatus aurata* analysed on the 8th day of storage are clearly separated from all the others of the same fish species, while those of *Salmo salar* are closely gouped. Scores obtained from the highest PCs of both the Mf and A5s were

Table 1. Significant coefficients of the different sensors on the different selected features.

	Features			
Sensor signals	Mean total signals	Mean of last 5s From 5-15s	Mean signals signal	Maximum
S1	781.834	-425.1	3445784	ns*
S2	-319.647	-68.8	ns	-0.00012
S3	1594.867	4934571.1	-6602979	ns
S4	ns	ns	ns	ns
S5	278.33	376.4	7592026	ns
S6	324.211	14.8	998	0.001
S7	1453.776	Ns	-599	ns
S8	147.019	11.1	Ns	0.0033
S9	882.034	18.0	-50	ns
S10	-197.688	-4.3	ns	0.0065
corrected model R^2	0.97	0.999	0.999	0.999
MODEL P-LEVEL <	0.000	0.000	0.000	0.000

* = sensor non significant for the chosen feature at p<0.05

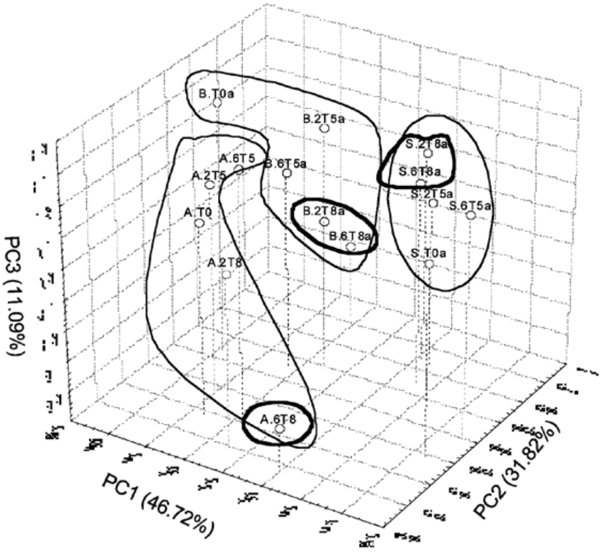

Fig. 4. 3D scatterplot obtained from the PCA of the feature Mf. Fish species: S: *Salmo salar*, A: *Sparatus aurata*; B: *Dicentrachus labrax*. Storage temperature: 2: under ice; 6: 6°C. Time of storage: To, T5, T8: 0, 5 and 8 days respectively.

correlated with the TMA production and the overall evaluation obtained from the panel test (table 2). Scores from A5s gave lower correlation coefficients compared to those obtained from Mf, regardless storage temperature. Scores from Mf did not correlate with TMA and panel data from *Salmo salar* stored under abuse temperature, while they were well correlated under ice storage.

CONCLUSION

The optimum feature selected allowed the discrimination of the three species of fish, *Dicentrachus labrax*, *Sparatus aurata* and *Salmo salar*, and the assessment of their storage time better than the features generally used. The approach used in this experiment showed to give appreciable results, underlying the importance of the selection of the suitable features when analysing data from sensor array devices. The identi-

Table 2. Correlation between the scores obtained from the highest PCs resulting from the PCA based on Mf and A5s features, TMA production and panel test scores over time for the products stored under ice and in abuse temperature.

Feature	Storage condition											
	6°C						ice					
	S. aurata		*D. labrax*		*S. salar*		*S. aurata*		*D. labrax*		*S. salar*	
	TMA[1]	Pt[2]	TMA	Pt	TMA	Pt	TMA	Pt	TMA	Pt	TMA	Pt
S. aurata												
Mf [3]	0.93	0.94					0.79	0.97				
D. labrax												
Mf			0.99	0.94					0.89	-0.5		
S. salar												
Mf					-0.53	0.26					-1	0.98
S. aurata												
A5s[4]	0.72	0.73					0.58	0.85				
D. labrax												
A5s			0.95	0.56					0.99	0.88		
S. salar												
A5s					-0.83	0.63					-0.66	0.48

[1]: TMA = Trimethylamine accumulation; [2]: Pt = Overall evaluation of the panel test; [3]: Scores from “mixed feature” obtained from the highest PC; [4]: Average of the last 5 points.

fication of the most significant sensors in the separation of a food product can be helpful in the construction of new sensors array devices, specific for a group of food products.

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DETERMINATION OF OIL COLOUR DURING STORAGE: A TOOL FOR A RAPID QUALITY ASSESSMENT

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ABSTRACT

The oxidation of virgin olive oil leads to the degradation of pigments, which are responsible for oil colour, and to the formation of new compounds absorbing in the UV-Vis spectral range and thus contributing to the colour changes. Therefore, colour can be a critical tool for oil quality assessment and for monitoring the changes occurring during storage. The aim of this study was to evaluate the correlations between the colour parameters, measured as ΔE (colour difference between fresh oil sample and the samples stored in different conditions and for a certain period of time), and some important analytical indexes measured during a storage period of 12 months in different conditions. The obtained results showed significant regression coefficients for the correlations between the colour parameters and the concentrations of chlorophylls, carotenoids and triglyceride oligopolymers, as well as with the K_{270} values.

Key words: extra virgin olive oil, colour, quality, storage, triglyceride oligopolymers

INTRODUCTION

An increasing effort has been devoted in recent years to the development of methods that enable direct rapid analysis of products without additional sample pre-treatment. Spectroscopic techniques are widely employed in such methods, and one of the simplest and widely available spectroscopic techniques is absorption spectroscopy in the UV-Vis spectral range. This technique enables the monitoring of colour of food, which, together with flavour and texture, is one of the key factors of food acceptability.

Virgin olive oil, obtained from the fruits of *Olea europaea* L. by mechanical means without any other treatment, is one of the few vegetable oils consumed in its natural state (Rahmani and Csallany, 1998). It has a high resistance to oxidative deterioration mainly due to its fatty acid composition, characterized by a high monounsaturated-to-polyunsaturated fatty acid ratio, and to a pool of minor compounds of powerful antioxidant activity (VELASCO and Dobarganes, 2002). Despite its antioxidant properties, during storage extra virgin olive oil may undergo oxidative and hydrolytic degradations, which are the predominant cause of oil quality deterioration (Morelló *et al.*, 2004). Furthermore, it can be affected by a partial loss of the minor constituents, considered primarily responsible for the beneficial effects on the consumers' health (Visoli and Galli, 1998). In particular, the oxidation of olive oils leads to the degradation of pigments, namely carotenoids and pheophytins, which are responsible for oil colour (Morelló *et al.*, 2004), and causes the formation of new compounds absorbing in the UV-Vis spectral range and thus contributing to the colour changes. Therefore, colour can be a critical tool for oil quality assessment.

The aim of this study was to verify if it is possible to evaluate the quality of an oil by a simple instrumental measurement. At this purpose the correlations between the colour parameters, measured as ΔE (colour difference between fresh oil sample and the samples stored in different conditions and for a certain period of time), and some important analytical indexes measured during storage were evaluated.

MATERIALS AND METHODS

Extra virgin olive oil from the *Coratina* cultivar stored in different ways – in the dark and under diffuse lighting in clear and green glass bottles, simulating the conditions of a supermarket shelf – was used for experimental tests. UV spectrophotomeric characteristics, chlorophylls, carotenoids, and triglyceride oligopolymers were determined for each sample at fixed times. Colour measurement were made on a spectrophotometer (Cary 5E, Varian) in a glass cell with 0.5 cm optical path length. The transmittance measurements were made over the 380-780 nm range, 120 nm/min scan speed was used. Colour parameters were calculated from the transmittance spectrum of each sample using illuminant C (daylight) and 2° observation angle. Final value for each sample were obtained by averaging over the three replicates. Colour parameters in the CIELAB system were calculated, where L* indicates lightness, a* indicates hue on a green (-) to red (+) axis, b* indicates hue on a blue (-) to yellow (+) axis, hue angle is defined as $h° = \arctan(b^*/a^*)$, and chroma as $C = [(a^*)^2 + (b^*)^2]^{1/2}$. Colour difference (ΔE) was calculated as $\Delta E = [(L_0-L)^2 + (a_0^*-a^*)^2 + (b_0^*-b^*)^2]^{1/2}$, where L_0, a_0^* and b_0^* are colour parameters of fresh oil samples (Calvo, 2004). The determination of K_{270} was carried out according to EC regulation no. 2568/91 (OJEC, 1991). The chlorophyll fraction was quantified from the absorption spectrum of each oil sample dissolved in hexane, according to the AOCS method (1996). The chlorophyll content was expressed as mg of pheophytin "a" per kg of oil. The concentration of total carotenoids was evaluated by measuring the absorption at 449 nm of 0.25 g of oil dissolved in 5 ml of UV-hexane, using a calibration curve previously obtained with solutions of β-carotene having a known concentration. Triacylglycerol oligopolymers were determined by HPSEC analysis of polar compounds separated from oils by silica gel column chromatography, according to the AOAC method (2003). The description of the HPSEC system and the quantification of triacylglycerol oligopolymers are reported in a previous paper (Gomes and Caponio, 1999).

RESULTS AND CONCLUSIONS

The colour of oils is a matter of transmission of light, being determined by two groups of pigments, carotenoids and chlorophylls. The degradation of these pigments during storage affects oil colour. The aim of this work was to determine the correlations between ΔE and some analytical indexes during storage and thus to verify if it is possible to evaluate the quality of an oil by a simple instrumental measurement.

The ΔE values (data not shown) were very low for the oils stored in the darkness, thus indicating colour stability in this storage condition. On the other hand, the samples exposed to light exhibited pronounced changes in their colour, which were greater during the first phases of the storage period and tended to achieve a constant value after 10 months. The rapid initial colour changes during storage were mainly due to the fast photo-degradation of chlorophylls. The samples bottled in green glass and stored under light showed an intermediate behaviour with respect to the other samples. Regarding colour parameters (data not shown) the value of a* increased and that of b* decreased monotonously, which corresponds to a shift from green to red, and from yellow to blue, respectively. The value of lightness L* increased during storage as an effect of degradation of the oil pigments.

The correlations between ΔE and chlorophyll concentrations, carotenoids, K_{270} and the amount of triglycerides oligopolymers are shown in figs. 1 and 2. Relatively high regression coefficients were obtained for the correlation between ΔE and the chlorophyll concentrations, while lower regression coefficients were obtained for the carotenoids concentration, due to their larger stability and considerably lower degradation rates. Moreover, the correlations between colour changes and K_{270} and triacylglycerol oligopolymers content yielded a high regression coefficient. The K_{270} value is correlated to the presence of secondary oxidation compounds containing a carbonyl functional group (Di Giovacchino *et al.*, 2002), while triacylglycerol oligopolymers, a class of substances of oxidation of triglycerides which constitutes a reliable parameter for a proper evaluation of the level of degradation of a lipid matrix, are considered to be good indices of the level of oxidation of edible oils and fats owning to their high stability and low volatility (Paradis and Nawar, 1981; Stevenson *et al.*, 1984; White and Wang, 1986).

These results prove that the measurement of oil colour can provide valuable information regarding some important analytical parameters correlated to its quality; therefore, this technique could be a much cheaper and faster alternative to quantitative analytical determinations involving chromatographic separation techniques. A limit of this practice is that each olive cultivar has its own pool of pigments which influences the oil colour during storage. Further investigations about other cultivars are therefore necessary, in order to verify the applicability of this technique to all kinds of oil.

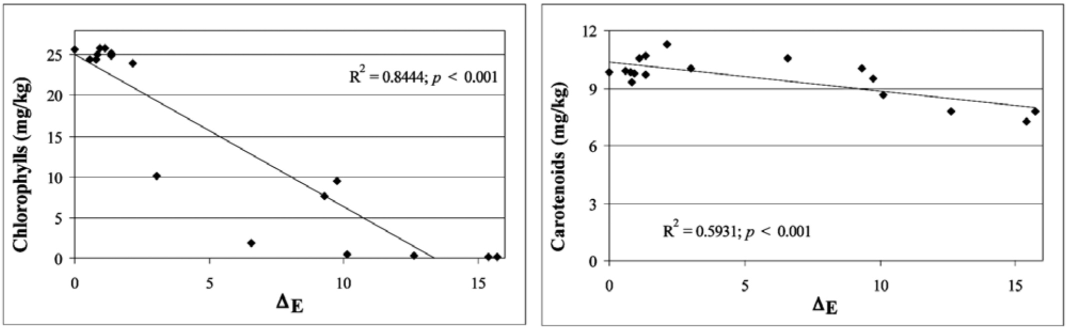

Fig. 1. Correlations between ΔE and concentrations of chlorophylls and carotenoids (*n* = 17).

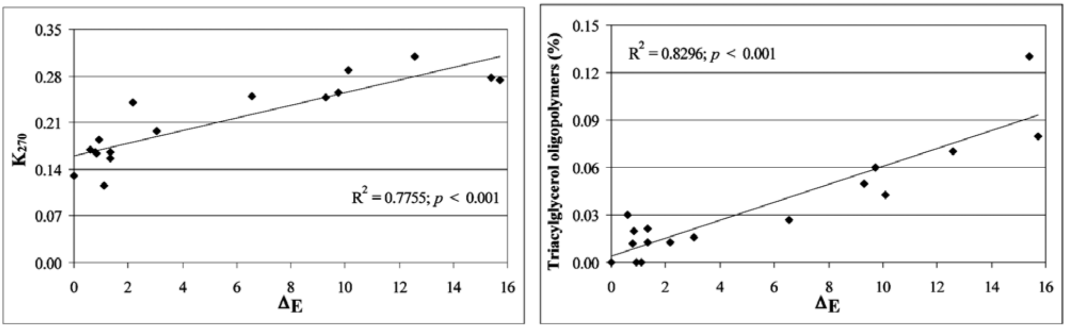

Fig. 2. Correlations between ΔE and values of K_{270} and triacylglycerol oligopolymers (*n* = 17).

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SHELF LIFE EVALUATION OF TOMATOES BY THE HS-SPME METHOD

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ABSTRACT

Flavour, comprised of aroma and taste, is an important food quality attribute. The aroma or odour of a food product is detected when its volatiles enter the nasal passages at the back of the throat and are perceived by receptors of the olfactory system (retro nasal).

The flavour of tomatoes can be characterized by nearly the entire set of their constituents. Indeed, the flavour is not only directly reflected by the sum of the volatile and non-volatile components, but it also depends on their interactions.

Solid phase micro-extraction (SPME) headspace sampling combined with gas chromatography (GC), can be used as a reference method for flavour analysis.

Another significant factor for buyers and consumers quality is tomato's colour. After harvesting, ripening continues and tomatoes can become overriped very quickly. This can result in a loss of quality and a reduced shelf life.

Tomatoes (*Lycopersicon esculentum* Mill.) "loose tomatoes" and "cluster tomatoes" at red ripeness stage were used. During the experiment the shelf life conditions (atmosphere, temperature and relative humidity) were controlled.

Attributes such as colour, size, shape and external defects of fruit and vegetables were investigated in our study because they influence the choice made by consumers.

Changes in tomato aroma profiles, dry matter, colour, tytrable acidity, sum of sugars and pH have been monitored at different times and a short-term anoxia treatment of 24 h was applied.

Many compounds undergo important concentration changes during shelf life of tomatoes. The HS-SMPE method is useful to evaluate the above mentioned tomato's changes.

Key words: headspace, shelf life, SPME, tomato, *Lycopersicon esculentum*

INTRODUCTION

For fresh tomatoes, the two quality attributes that are most important to buyers, are texture and skin colour. Texture is influenced by flesh firmness and skin strength (Kader *et al.*, 1978). Changes in firmness were highly correlated with surface appearance characteristics of tomatoes (Yang *et al.*, 1988) which was related to colour, shape and sense of feel for firmness at the time of purchasing.

Tomato colour is another important quality factor for the consumer. Colour is the most visible ripening indicator hence prediction of colour development appears to be a logical factor in investigating modified atmosphere storage of tomatoes. It has been noted that one of the significant factors affecting colour development in tomatoes is the gas composition of the environment. In addition to their external appearance, flavour is becoming more important to vegetable purchasers. Along with sugars and acids, aroma volatiles contribute to tomato flavour. Although over 400 compounds have been identified as volatile constituents of tomato (Petrò-Turza *et al.*, 1986), only a limited number are essential for tomato flavour.

Volatile compounds present in fresh and ripened tomatoes are included in various chemical classes such as ketones, aldehydes, alcohols, esters, phenols, free acids (Baldwin *et al.*, 1991; Buttery *et al.*, 1990).Some of these compounds are formed from amino acids and carotenoids during fruit ripening while others are produced by the breakdown of lipids, when the tissue is damaged, forming predominantly C6 compounds through an enzyme "cascade" (lypase, lypoxigenase, hydroperoxideliase, isomerase and alcohol dehydrogenase). Although the activities of some enzymes in the cascade change during ripening. Hexanal and (Z)-3-hexenal are formed from 13-hydroperoxides of linoleic and linolenic acid, respectively while (E)-2-hexenal is generated by the action of an isomerase on (Z)-3-hexenal. The aldehydes can be further transformed into alcohols by the action of the alcohol dehydrogenase. With this sequential formation of volatiles, it might be expected that the relative amounts of the C6 compounds change with time as the tomatoes are macerated and the enzyme cascade is activated (Prestage *et al.*, 1999).

Several flavours have been associated to different storage procedure (Maul, 2000): it appears that both temperature and atmospheric conditions have a direct effect on fruit metabolism, leading to changes in the formation of flavour compounds. There are a few studies on the effects of modified atmosphere storage on the postharvest metabolism of tomato and on its volatile compounds (Kliever *et al.*, 1996).

Headspace analysis by solid phase micro-extraction (SPME) has been proposed to analyze volatiles compounds in foods. Previous reports on SPME have demonstrated its suitability to extract volatiles from the headspace of tomatoes (Song *et al.*, 1998; Berna *et al.*, 2004).

MATERIALS AND METHODS

Batches of locally grown tomatoes (*Lycopersicon esculentum* Mill) were obtained from a greenhouse located in Condofuri (Reggio Calabria, South Italy).

Daily harvested tomatoes (*cv Harabat*) at pink maturity stage were used (USDA 1976). Tomatoes were homogenized for 30 s at room temperature using a commercial mixer; the homogenized juice was stirred for 15 min at room temperature to activate endogenous enzymes.

Skin colour values and juice colour values were measured by using a Minolta Chromameter Model CR 300: redness value of tomatoes were recorded as *a/b* value (Yang & Chinnan, 1988). Penetration force (g) into tomato fruit was also recorded (Batu *et al.*, 2004). Tomato reducing sugars were determined in accordance with EU Reg. n.2676/90. pH was measured by a Crison pHmeter Basic 20. The total acidity is expressed by g/L citric acid. For solid phase microextraction 1,5 g of tomato juice were put in a 12 mL vial with 0,5 mL of a saturated solution of $CaCl_2$ and thermo stated for five minutes at 29 °C. The saturated $CaCl_2$ solution was used to stop the enzymatic reactions and to promote the release of aroma volatiles into the sample headspace (Buttery *et al.*, 1987).

A SPME fibre (2cm-50/30um DVB/Carboxen/PDMS) was exposed to the sample for 30 min at the 29 °C and desorbed in 3 min in a split-splitless injection port. Volatile compounds were verified by comparison of the mass spectral data and the retention times with those of the authentic reference compounds. GC analysis were performed by using a thermoelectron GC 8000 series gas chromatograph equipped with an FID detector.

GC-MS analysis were performed by using a Hewlett Packard (Agilent) 5890 gaschromatograph coupled to a Hewlett Packard (Agilent) 5973 quadrupole mass selective spectrometer. Helium was used as carrier gas, at linear velocities of 23 and 17 cm^3 /s for GC-FID and GC-MS respectively. Separation was performed on column Supelcowax-10 (30m x 0,25 mm i.d., 0,25 μm film thickness) for GC-MS and Supelcowax-10 (30m x 0,25 mm i.d., 0,5 μm film thickness), both purchased from Supelco Ltd.

Column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C/min. FID temperature was set at 280 °C and temperature of the ion source was set at 280 °C.

Electron impact mass spectra were recorded at 70 eV ionization energy in the 15-250 amu mass range, 2 scan/s.

Quantitative analyses of each of them were repeated three times by a GC-FID in accordance with internal standard (4-methyl-2-pentanol) method and the relative response factors were calculated.

RESULTS AND DISCUSSION

The aim of this work is to determine the effect of anoxia treatment on the release of volatile compounds during a “home ripening” (table 1).

Data in table 2 show how the pH of control tomatoes, at first, has a value higher than the treated tomatoes: the reason could be a change in the metabolism of anoxia treated tomatoes. Ripening is so influenced by anoxia treatment that control doesn’t arrive at 35’ day because of an over-ripeness that doesn’t allow to inquire further. The anoxia treatment therefore has produced an increase of shelf life. The reason could be a reduction of the respiration rate as demonstrated by the delayed development of volatile compounds and by the a/b maturation ripening parameter behaviour in treated tomatoes as to control tomatoes.

Total sugar levels showed a general increase followed by no change or a slight decrease during ripening. This trend is in agreement with trends in other studies.

The short anoxia treatment doesn’t influence the hexanal percentage during the storage (figure 1), in opposition to the literature (Boukobza *et al.*, 2001).

Table 1. Effect of modified atmosphere storage on release of volatile compounds from macerated tomato fuit.

	Immediately		After 7 days		After 14 days		After 21 days		After 28 days		After 35 days		After 42 days	
	Control	N_2 treated	Control	N_2 treated	Control	N_2 treated	Control	N_2 treated	Control	N_2 treated	Control	N_2 treated	Control	N_2 treated
	µg/Kg		µg/Kg		µg/Kg		µg/Kg		µg/Kg		µg/Kg		µg/Kg	
Pentanal	297	106	149	115	78	109	189	103	88	64	//	126	//	121
Hexanal	296	532	1490	1650	1959	1789	13959	13462	197	15598	//	8264	//	9974
Hexenoic acid methyl ester	30	46	95	112	117	121	24	81	24	438	//	109	//	106
1-Butanol-3-methyl	13	143	271	380	582	642	305	270	773	59	//	67	//	124
(E)-2-Hexenal	6	19	45	54	85	90	798	523	24	198	//	169	//	233
1-Pentanol	47	35	51	71	63	89	93	94	93	101	//	122	//	182
(Z)-3-Hexenoic acid methyl ester	59	18	56	20	13	22	12	11	194	24	//	12	//	16
Hexyl acetate	134	99	45	135	37	157	0	0	220	0	//	0	//	11
(E)-2-Heptenal	6	29	3	58	4	63	7	8	14	6	//	15	//	42
(Z)-2-Penten-1-ol	44	10	33	54	55	73	32	9	63	103	//	116	//	110
6-Methyl-5-hepten 2-one	36	462	350	511	462	695	1013	922	4969	977	//	4518	//	2878
1-Hexanol	457	0	890	0	1090	0	723	1491	93	0	//	0	//	0
(Z)-3-Hexenol	868	443	670	334	344	741	520	735	2212	318	//	691	//	1020
Nonanal	90	74	35	22	39	24	24	14	144	56	//	33	//	31
Isobutylthiazole	0	1	7	8	9	11	33	21	4	28	//	7	//	6
2,4-Hexadienal	9	62	10	41	14	8	72	60	10	24	//	11	//	9
(E)-2Hexen-1-ol	6	17	21	39	35	51	34	16	130	31	//	138	//	99

Table 2. The effect of anoxia treatment on several quality traits of tomato fruit.

	Dry matter (%)	Total sugars	pH	Acidity	L	a/b	Texture
Immediately	5,15	2,38	4,70	0,11	31,03	0,11	1287
After 24h anoxia treatment	5,26	1,94	4,60	0,18	34,81	-0,79	1235
After 7days harvested							
Control	5,89	2,36	4,51	0,12	45,31	0,56	1112
N2 treated	5,92	2,01	4,56	0,13	39,1	0,51	860
After 14days harvested							
Control	6,45	2,35	4,40	0,12	47,46	1,03	1096
N2 treated	6,53	2,20	4,47	0,15	37,61	0,90	783
After 21days harvested							
Control	6,52	2,31	4,14	0,13	37,06	1,37	570
N2 treated	6,25	1,99	4,53	0,12	42,17	1,50	479
After 28days harvested							
Control	6,51	2,25	4,42	0,18	37,22	1,65	348
N2 treated	6,42	2,08	4,52	0,11	36,28	1,22	450
After 35days harvested							
Control	//	//	//	//	//	//	//
N2 treated	6,58	2,09	4,62	0,14	37,67	1,41	421
After 42days harvested							
Control	//	//	//	//	//	//	//
N2 treated	6,93	2,22	4,73	0,15	37,18	1,45	461

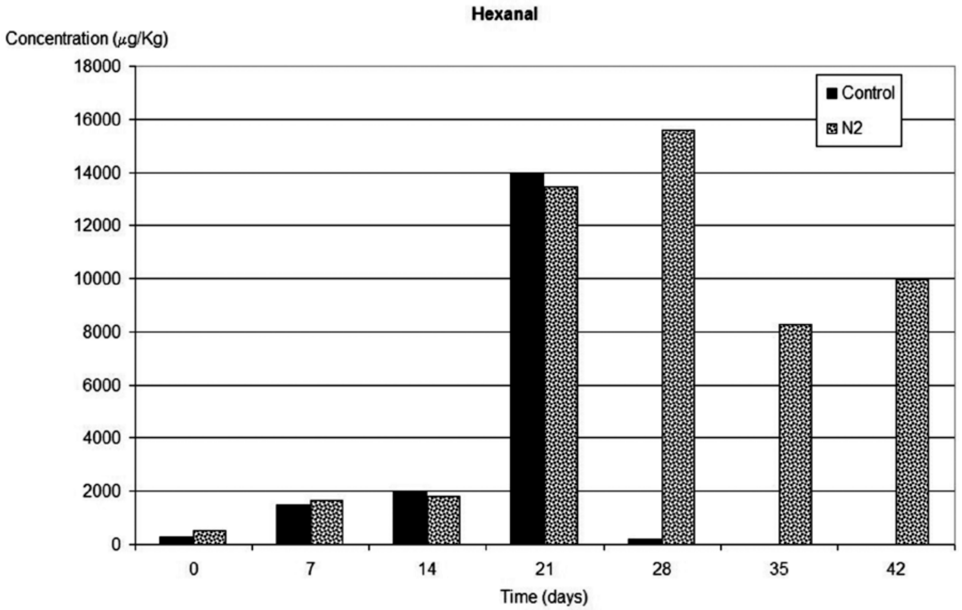

Fig. 1. Effect of storage on the release of hexanal.

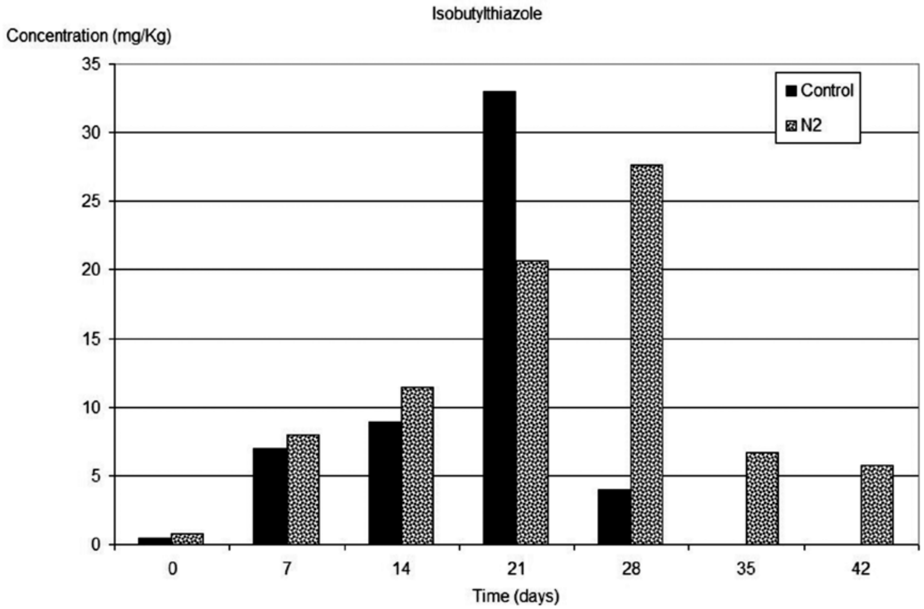

Fig. 2. Effect of storage on the release of isobutylthiazole.

The isobutylthiazole and 6-methyl-5-hepten-2one amounts are maxima at 21 days in the control tomatoes and at 28 days in the treated tomatoes (figures 2 and 3): the reason could be a slackening of ripening due to anoxia treatment. Therefore the percentage of these compounds could be an indicator of decay state. Instead, the amyl alcohol value could be suggesting the ripeness state (figure 4).

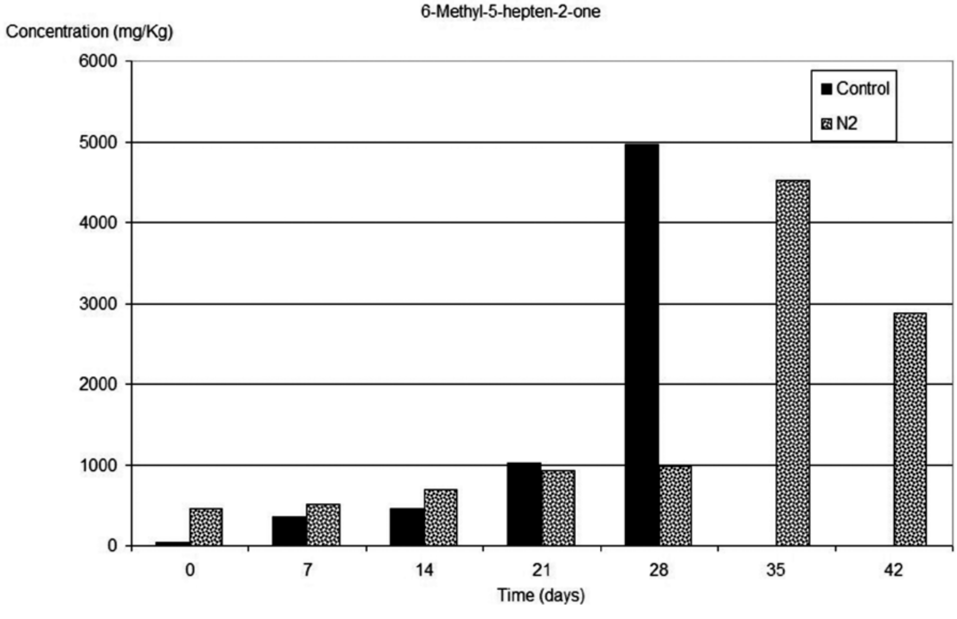

Fig. 3. Effect of storage on the release of 6-methyl-5-hepten-2-one.

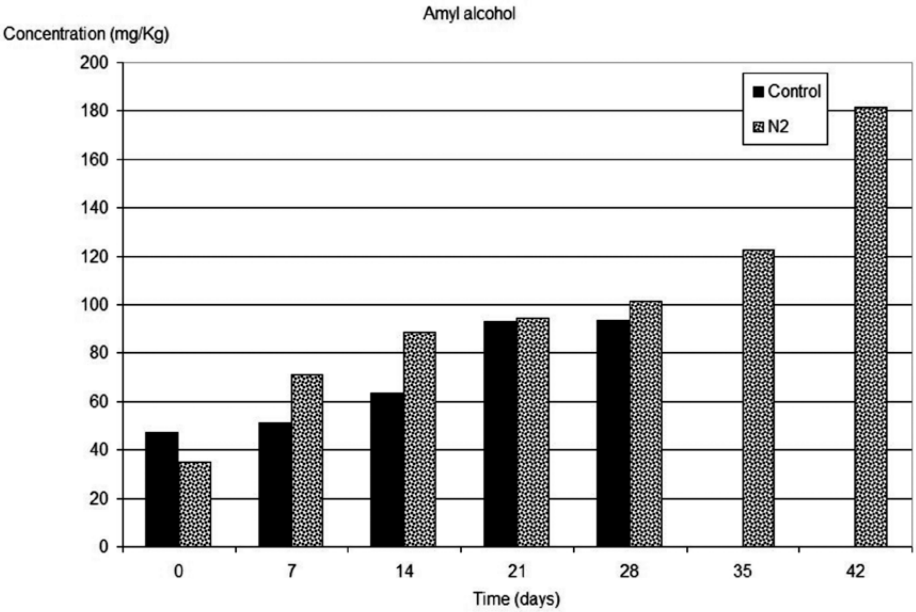

Fig. 4. Effect of storage on the release of amyl alcohol.

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SHELF-LIFE OF CHINESE CABBAGE (*BRASSICA CAMPESTRIS* L. SSP. *PEKINENSIS* LOUR) PACKAGED IN ORDINARY ATMOSPHERE

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ABSTRACT

The suitability of four different cultivars of Chinese cabbage ('Bilko', 'Kasumi, 'Manoko' and 'Nikko') to be transformed as ready-to-use product was evaluated. To the purpose, immediately after harvesting, cabbage samples were cutted, washed with clorinated water, packaged under ordinary atmospheric conditions and stored at 4 °C for 8 days. Samples were analyzed at the production day, at the 4th and at the 8th day of storage to determine variations of some physical (color), chemical (dry matter, sugars, phenolic content and titratable acidity), physiologic (CO_2 and O_2 production), microbiologic (mesophilic viable counts, yeast and moulds, *Enterobacteriaceae*) and enzymatic (poliphenoloxidase activity) parameters. Results of this study provided information to evaluate, for each cultivar, quality of cut Chinese cabbage during storage.

Key words: Chinese cabbage, minimally processed, cultivar, quality

INTRODUCTION

Packaged ready to use (RTV) product offers several advantages over traditionally packaged food: it is perceived by consumer to be more nutritious, convenient, efficient in terms of meal preparation and creates less of waste disposal problem. Producing RTV involves cleaning, trimming, peeling, coring, slicing, shredding, washing, etc. However the processing causes physiological and biochemical reactions that shorten storage life of the product. Enzymatic browning is caused by the activity of Polyphenol oxidase [PPO (E.C. 1.14.18.1)] present in plant tissues. The activity of PPO can lead to a significantly diminished quality of foodstuffs by the appearance of melanins which change both sensory properties (colour, aroma, texture, etc.) and nutritional quality of products (Whitaker, 1995). In addition processing of vegetables promotes a faster microbial (Brackett, 1987) degradation of the product in comparison with the raw commodities, and increases the risk of foodborne disease outbreaks (Alzamora *et al.*, 2000).

Although lettuce is predominantly used for prepared salads, other types of vegetable are now used in salad mixtures. Information on the postharvest behavior of these vegetables is larking, especially with regard Chinese cabbage. Aim of these work is to evaluate suitability of various cultivars of Chinese cabbage prepared as fresh-cut product.

MATERIALS AND METHOD

Plant material Chinese cabbage cultivars ('Bilko', 'Kasumi', 'Manoko' and 'Nikko') were field-grown in Southern of Sicily under the Mediterreanean climate. The plants were transplanted in open air in April and harvested in June 2005. Crop management was similar to a commercial one. Chinese cabbage heads were selected for uniformity and were stored at 5°C until they were processed at the factory.

Product preparation Outer leaves were discarded and then Chinese cabbage leaves were put into the regular processing line. The leaves were cut into segments approximated the size of piece used in 'salad packs'. The pieces were washed with chlorinated water (0.01%) and then centrifuged to remove excess water. About 200g of cut tissue was packaged with a anti-fog film for condensation control and stored at 4°C for 8 days. Chinese cabbage samples were taken for analysis at 0, 4 and 8 days. For biochemical characteristics the samples were analyzed also at 15 days. The analyses were performed in triplicate.

Quality evalutation Soluble solids were determined with a digital refractometer (DBX-55A ATAGO CO., LTD., Japan) and were defined as brix value. Titratable acidity was measured by titrating 10 ml of the extract with a 0.1 N NaOH solution to pH 8.3 and converting as predominant acid content (meq/dl).

Gas analysis O_2 and CO_2 percentage within the package during storage were monitored with CheckPoint O_2/CO_2 (PBI Dansensor - Denmark).

PPO Extraction and Assay PPO extraction and assay was made according to Pifferi, *et al.,* (1973) and Rodriguez-Lopez (1994).

Browning *(ΔL^*)* The variation of L* parameter (ΔL^*) was considered as browning index. It was determined by Adobe Photoshop. L* parameter was analyzed in complete and cut surface of vegetable, because in this area it was more stressed and so more exposed to the biochemical alterations.

Every analysis has been done in both portions of the vegetable: leaf fabric and vascular tissue.

Total polyphenol analysis Total polyphenol analysis was made according to Singleton *et al.*, (1965).

Microbiological analyses The microbiological analyses of each trial were performed in triplicate at 0, 4 and 8 days of storage at 4°C. Ten grams of each sample were aseptically weighed, homogenised using a Stomacher (Brinkmann, Westbury, NY, USA) for 2 minutes and serially diluted in sterile physiological solution (0.9 % NaCl) up to 10^{-9}. Yeast and mould counts were carried out in Sabouraud Dextrose Agar supplemented with Chloramphenicol Selective Supplement (Oxoid, Basingstoke, UK) after incubation at 25°C for 4 days; Mesophilic Viable Counts (MVC) were performed in Plate Count Agar (PCA) after incubation at 32°C for 48 h; *Enterobacteriaceae* counts were performed on Violet Red Bile Glucose Agar (Oxoid) incubated for 48 h at 37°C. Colony forming units (cfu) were manually counted.

RESULTS AND DISCUSSION

Quality evalutation. Brix index varied significantly among cultivars from 3.5 for 'Kasumi' to 4.1 for 'Bilko'. Average brix index increased, during storage, from 3.8 to 4.1. Significant changes of titratable acidity were not detected among cultivars, during storage. With regard weight loss, the effect of storage was not significant according with Watada et al. (1999) because relative humidity generally is very high in packages and dehydration typically is not a problem.

Gas analysis. There were significant differences in the O_2 and CO_2 percentage in the packages between the Chinese cabbage cultivars. A pronounced decrease and increase of O_2 and CO_2 respectively were found during the first 4 days of storage and were relatively stable thereafter (fig. 1). All cultivars, except 'Bilko', exhibited a similar profile. At end of the storage period the O_2 percentage in the packages ranges from 3.6 % for 'Manoko' to 9% for 'Kasumi', whereas the CO_2 percentage ranges from 10,9% for 'Kasumi' and 13% for 'Nikko'.

PPO activity In order to consider initial PPO activity and its temporal evolution during storage, it was taken into consideration the integral curves of PPO activity.

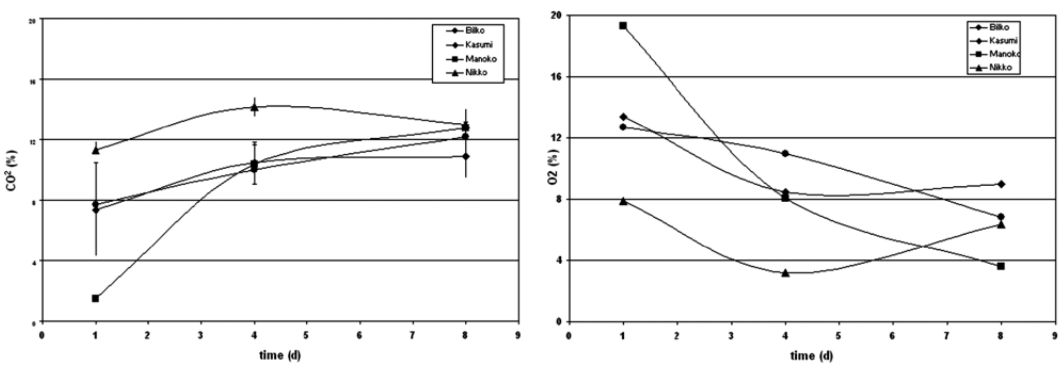

Fig. 1. CO_2 (picture a) and O_2 (picture b) variation into packages of 'Bilko', 'Kasumi', 'Manoko' and 'Nikko' cultivars during storage.

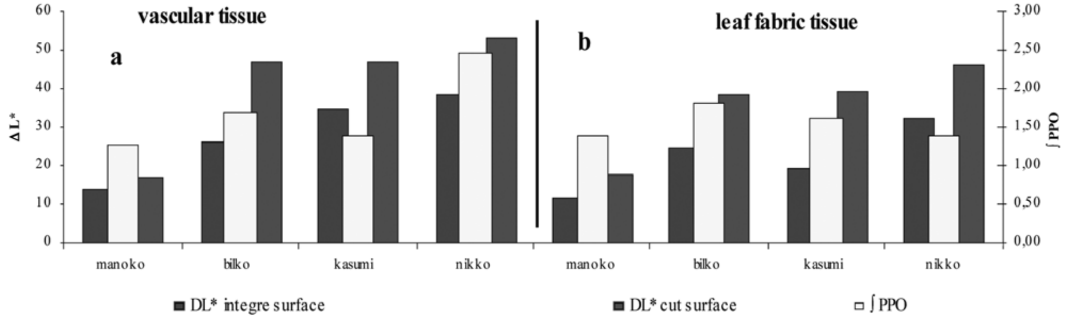

Fig. 2. Total PPO activity expressed as integral (∫)and ΔL* variation in complete and cut surface of vegetable in vascular (picture a) and leaf fabric (picture b) tissue of 'Manoko', 'Bilko', 'Kasumi' and 'Nikko' cultivars during storage.

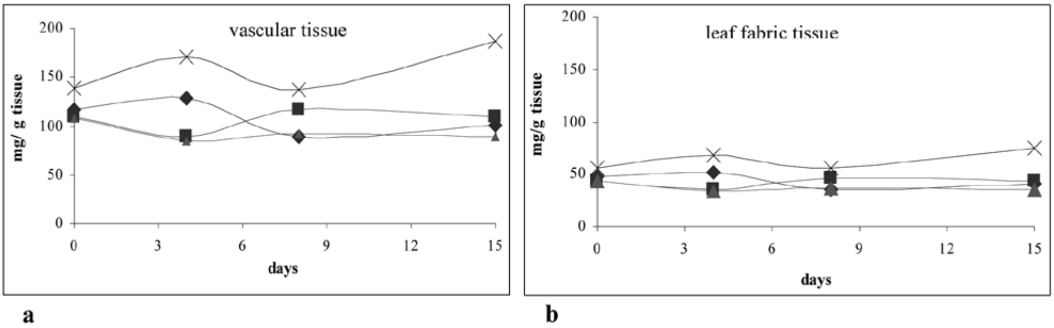

Fig. 3. Total polyphenol variation in vascular (picture a) and leaf fabric (picture b) tissue of 'Manoko', 'Bilko', 'Kasumi' and 'Nikko' cultivars during storage.

Figure 2 shows total PPO activity during storage in both portions of samples: 'Manoko', 'Kasumi' 'Nikko' and 'Bilko'. It was noticed that:

• total PPO activity was quite similar in all samples except for 'Nikko' cultivar that had the greatest enzymatic activity.

• 'Manoko' cultivar had the lowest PPO enzymatic activity.

• PPO activity was more presented in the leaf fabric tissue rather than in the vascular ones except for the cultivar 'Nikko'.

Browning

Figure 2 shows colour variation (ΔL*) during storage, it was noticed that:

- browning was harder in cut surface rather than in complete ones.
- cultivar 'Nikko' showed the greater browning in both portions of vegetable.
- cultivar 'Manoko' showed lowest browning in both portions of vegetable.
- cultivar 'Bilko' e 'Kasumi' showed middle browning in both portions of vegetable.

Figure 2 showed also correlation between total PPO enzymatic activity and browning in both portions of vegetables.

It was noticed a good correlation between PPO activity and browning according to other authors reported (Severini et al. 2003), in fact cultivar with high enzymatic activity had high browning except for vascular tissue of 'Nikko'.

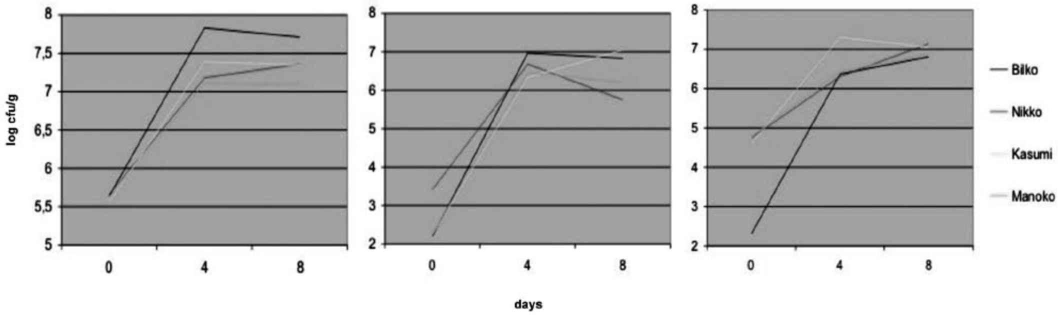

Fig. 4. MVC (a), yeast and moulds (b) and *Enterobacteriaceae* (c) counts of Chinese cabbage samples during storage at 4°C.

Polyphenol content

As shown in figure 3:

- polyphenol content didn't present generally hard variations during storage except for 'Nikko' cultivar that presented a light irregular curve.

Microbiological analyses

Almost all samples of vegetable samples showed an increasing microbial population during storage period at 4°C, of about two log units.

In Chinese cabbage samples MVC count dramatically increased during the first 4 days of storage, keeping then constant from the 4th to the 8th day (Fig. 4a). A similar trend was showed by yeast and mould counts on Sabouraud Dextrose Agar (Fig. 4b) and by *Enterobacteriaceae* on VRBGA (Fig. 4c).

CONCLUSION

The results indicate that Chinese cabbages are an interesting vegetable to use as ready-to-use product. In fact there were little changes of titratable acidity, Brix° index and weight loss for 'Manoko'; CO_2 and O_2 percentage changes were less for 'Bilko'.

The total level of PPO resulted varying in different cultivars during storage in the order of 'Manoko' < 'Kasumi' < 'Bilko' < 'Nikko', with notable variations according to the tissue analyzed, in fact leaf fabric tissue had generally higher activity than vascular ones, except for 'Nikko' cultivar.

There were found variations in polyphenol content according to the tissue analyzed, in fact leaf fabric tissue had generally higher content than vascular ones.

A good correlation between PPO activity and browning was found according to other authors (Severini *et al.*, 2003).

These results indicate the suitability for 'Manoko' as ready-to-use product.

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CHANGES IN SICILIAN VIRGIN OLIVE OIL DURING STORAGE WITH PARTICULARLY HIGHLIGHTED BIOPHENOL COMPONENTS

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ABSTRACT

In order to study the changes during storage of virgin olive oils extracted from *Biancolilla*, *Cerasuola* and *Nocellara del Belice* cultivars, homogeneous batches of olive fruits were processed by using different malaxing temperatures. Also, traditional three-phases decanter with different water temperatures was used for oil separation from the solid phase. Oil samples were shared out amber glass bottles and stored in the dark at cellar temperature.

The changes in turbidity and in lipid substrate were monitored within the year of olive fruit processing. For each sample the results showed changes of conventional chemical parameters (free acidity, peroxide and UV absorption values), of total phenolic content and some of hydrophilic phenols contents. Oil turbidity decreased with the lower rate for the olive oil by processing at colder temperature. The evolution of total phenols, α-tocopherol and hydrophilic phenols contents showed that the conditions of storage play an important role for oxidative stability of olive oils.

Key words: biophenol components, Sicilian virgin olive oil, storage

INTRODUCTION

Virgin Olive Oil (VOO) is one of the few alimentary fats which undergoes no chemical treatment. It is resistant to oxidative deterioration because it has a high monounsaturated-to-polyunsatured fatty acid ratio and minor components with antioxidant activity. VOO is a basic part of the Mediterranean diet, it helps in the prevention of certain diseases and also of ageing. α-tocopherol, carotenoids and phenolic compounds are all antioxidants whose activity have been demonstrated in vitro and recently in vivo by several researches among which Carrasco-Pancorbo *et al.* (2005), Fogliano *et al.* (1999), Gutierrez *et al.* (2001), Visioli *et al.*, (1998), M. Maiuri (2005), Manna *et al.* (1999), Martinez-Dominguez *et al.* (2001), De La Puerta Vázquez *et al.* (2004), Owen *et al.* (2000).

During storage its nutritional and health properties may be spoiled by hydrolytic biochemical and oxidative phenomena affected by compositive and environmental factors .

This research studies the influence of storage at cellar temperature on monovarietal Sicilian virgin olive oils. Preliminary analysis and qualitative comparison of the examined oils were effected.

MATERIALS AND METHODS

Oil samples

Virgin olive oil (VOO) from cv. *Biancolilla*, *Cerasuola* and *Nocellara del Belice* were produced during the 2004/2005 olive-oil year in a small area of Jato valley, in Palermo province. Each sample of olives harvested in November at an optimal stage of ripeness was split up into two lots which were processed in two different mills. Both the mills had identical continuous-cycle plant a three phases centrifugal decanter but different processing conditions during malaxation and centrifugal extraction were used (table 1). A common defect of both mills was the absence of protective cover during malaxation.

Each oil sample collected at the end of the production cycle was poured into five amber bottles which were all filled up, closed immediately and stored in a dark cellar at temperatures ranging from 7 °C in winter to 22 °C in summer. Periodically each sample was analysed, each test was repeated three times. The first sample was tested on 15/12/04 (I), the other four samples were tested on 03/05/05 (II), 04/07/05 (III), 15/09/2005 (IV), 12/12/05 (V). Assuming that the winter temperatures would cause negligible alterations, a longer interval of time between the first and second analysis was taken.

Table 1. Different technical parameters of the mills.

Mill	**Cultivar**		**Malaxation**		**Centrifugal Extraction**
			Time (min)	Temperature (°C)	Temperature (°C)
1	Biancolilla	**B1**	28	25.5	27.0
	Cerasuola	**C1**	42	25.0	27.3
	Nocellara del Belice	**N1**	30	26.0	28.3
2	Biancolilla	**B2**	40	31.0	30.0
	Cerasuola	**C2**	40	28.7	28.5
	Nocellara del Belice	**N2**	40	30.3	31.7

Instruments

Carlo Erba Lovibond DRT-15CE turbiditymeter, Beckman DU 620 spectrophotomer, Perkin-Elmer AutoSystem XL gas-chromatograph equipped with FID detector and Perkin-Elmer series 200 HPLC equipped with diode array detector.

Oil analyses

Each sample was examined for free acidity, peroxide index and UV absorption values according to the methods set out by the Reg. EEC n.2568/91.

Turbidity

The results expressed in NTU were obtained at 20 °C, preliminary standardization with formazin was effected.

Fatty acidic composition

The methylic esters were obtained according to the official method set out by the above Reg. EEC and the composition was carried out by gas-chromatography. The conditions for the analysis were as follows: capillary column in fused silica, *DB*WAX, l. 30 m, i.d. 0.25 mm, f. 0.25 μm; transport gas He; T injector: 250 °C; T detector: 300 °C; T oven: 150 °C for 2 min increasing by 10 °C/min until 250 °C, isotherm for 18 min.

Total phenols

The extraction and quantitative analysis followed the procedure indicated by Montedoro *et al.* (1992) with the extracting mixture methanol:chloroform (95:5). Determination of the phenols was carried out on the extract with Folin-Ciocalteu reagent. The concentration was expressed as gallic acid equivalents (GAE, mg/kg). Calibration curve was obtained using standard solutions of gallic acid (Fluka).

HPLC determination of hydrophilic phenols

The extraction and HPLC analysis followed the procedure indicated by Fogliano *et al.* (1999). Analysis conditions were as follows: Varian column S5 ODS-3 (250x4.6 mm); T 25 °C; λ 279 nm; flow rate 1 ml/min; volume injected 20 μl. Tyrosol (Fluka), was used as external standards for quantitative analysis (Tsimidou *et al.*, 1992).

HPLC determination of α-tocopherol

The α-tocopherol was determined by above HPLC according to method Rovel-

Table 2. Initial chemical and physical-chemical parameters.

Sample	Turbidity (NTU)	Acidity (% oleic acid)	Peroxide Index (meq O_2/kg)	Total Phenols (ppm, GAE)	α-Tocoferol (ppm)	Chlorophylls (ppm)	Carotenoids (ppm)	K_{232}	K_{270}	$\Delta K*10^3$
B1	1050	0.58	5.5	181	221	11.8	5.6	1.54	0.09	5
B2	14	0.88	11.7	123	286	7.6	4.0	1.69	0.14	-5
C1	400	0.84	10.9	114	220	14.6	6.7	1.71	0.15	5
C2	350	1.00	10.1	109	230	14.7	6.1	1.64	0.15	-5
N1	670	0.57	7.1	202	213	10.1	5.0	1.52	0.09	-5
N2	13	1.35	13.3	167	220	13.1	5.9	1.84	0.18	-5

lini *et al.* (1997). The calibration curve was obtained using standard solutions of α-tocopherol (Fluka) in acetone.

Pigments

Carotenoids and chlorophylls contents were determined by measuring the absorption of solution oil at 20% in hexane. The absorbances at 435 nm and 670 nm for carotenoids and chlorophylls respectively were measured. The coefficients of extinction in hexane reported in literature (Modi *et al.*, 1992) were used for quantitative analysis.

Statistical analysis

Statistix 7.0 1985-2000, Analytical Software was used for to evaluate significative differences of considerate parameters after one year of storage.

Table 3. Initial fatty acids composition (%).

Fatty Acids	Sample					
	B1	B2	C1	C2	N1	N2
Palmitic	12.28	11.47	9.33	9.34	10.24	10.25
Palmitoleic	1.15	0.57	0.32	0.26	0.52	0.52
Eptadecanoic	0.11	0.18	0.06	0.05	0.04	0.06
Eptadecenoic	0.21	0.32	0.08	0.06	0.06	0.10
Stearic	2.00	2.14	2.44	2.14	2.20	2.08
Oleic	76.57	75.51	77.06	77.69	77.78	78.14
Linoleic	6.36	7.68	8.21	8.19	7.13	7.21
Arachidic	0.35	0.38	0.43	0.39	0.36	0.35
Eicosenoic	0.21	0.26	0.36	0.34	0.26	0.27
Linolenic	0.57	0.75	0.69	0.63	0.65	0.74

RESULTS AND CONCLUSIONS

The data showed that the work temperature in Mill 1 was more suitable for quality of the oils. In fact during malaxation the paste reached a maximum temperature of 26.0°C and during centrifugation the oil must reach 28.3°C max. The time taken for malaxation was similar only for the cultivar *Cerasuola* (42 min.), this was due to the rheologic properties of the paste which made the extraction of oil more difficult than the other two cultivars.

The initial quality of the oil reflected the different technological parameters used in the two Mills (tables 2, 3, 4 and 5). The oils coming from *Biancolilla* and *Nocellara del Belice* of Mill 2 were into the virgin oil merceological class whereas the oils from Mill 1 were extravirgin for considered parameters. The difference in quality also was revealed from the lower total phenolic content in oils from Mill 2. The

Table 4. Parameters variation during storage.

Sample	α-Tocopherol (ppm)		Chlorophylls (ppm)		Carotenoids (ppm)		Turbidity (NTU)	
	I	V	I	V	I	V	I	V
B1	221	248	11.8	9.5	5.6	4.4	1050	80
B2	286	270	7.6	7.0	4.0	3.7	14	9
C1	220	249	14.6	12.9	6.7	5.9	400	12
C2	230	220	14.7	14.4	6.1	6.0	350	22
N1	213	173	10.1	9.2	5.0	4.4	670	37
N2	220	186	13.1	11.6	5.9	4.7	13	11

I = Initial value – V = Value after one year of storage

Table 5. Varations of hydrophylic phenols during storage (ppm tyrosol).

Sample	OHTy		Ty		OHTy-EDA		Ty-EDA		OHTy-EA		Ty-EA	
	B1	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**
I	0.85	0.27	3.65	0.67	16.96	5.48	25.19	11.73	5.85	0.85	7.44	6.83
II	10.05	0.81	13.62	1.38	7.32	3.86	18.49	10.56	6.11	1.13	7.51	8.24
III	11.02	0.63	17.25	1.46	5.21	4.04	14.25	10.63	6.06	0.81	7.63	8.90
IV	16.52	2.56	18.67	1.56	3.19	1.83	13.08	8.06	6.52	0.92	7.52	7.94
V	15.51	1.03	18.46	1.59	2.77	3.77	13.47	10.46	4.50	0.40	7.31	8.67
	C1	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
I	0.40	0.92	4.81	5.64	2.02	1.50	20.30	17.88	1.39	0.63	3.18	10.32
II	3.15	1.71	11.85	11.40	2.44	1.33	18.08	15.88	0.50	0.60	3.46	11.46
III	2.27	18.13	10.60	10.92	1.62	0.85	16.67	12.79	0.46	0.65	3.37	10.08
IV	4.41	3.35	13.91	12.46	1.39	1.33	8.33	10.79	0.87	0.52	3.50	10.73
V	2.41	1.60	13.93	11.33	1.42	0.81	14.40	13.40	0.44	0.33	3.41	10.79
	N1	**N2**	**N1**	**N2**	**N1**	**N2**	**N1**	**N2**	**N1**	**N2**	**N1**	**N2**
I	0.69	0.37	2.33	2.23	13.25	13.07	29.94	24.46	0.40	1.27	12.35	11.89
II	6.20	1.43	10.40	5.57	7.84	10.47	26.44	22.42	2.43	0.75	13.27	12.47
III	3.43	2.27	4.43	5.66	12.76	10.62	33.85	23.06	3.39	1.98	13.82	12.72
IV	9.23	4.90	9.69	5.35	4.94	9.13	20.23	22.27	2.58	2.19	13.00	13.79
V	8.03	3.29	10.79	5.81	6.32	9.56	25.83	23.52	2.48	0.58	13.17	13.71

oils from cv. *Cerasuola* showed similar analytical characteristics, both the samples belong to the virgin oil category, their peroxide index (PI) and their phenolic content were almost the same. Probably, the low phenolic contents were correlated to the long time that both oils required for malaxation (Servili *et al.*, 2004). All the oils had good acidic composition with a linoleic acid/oleic acid ratio over 9.4 and oleic acid content was always over 75%. The chromatographic profile for hydrophilic phenolic composition showed a different pattern for the oils from the two mills. Dialdeidic form of derivate ligstroside was the main compound in all the oils except one. Total phenols and hydrophilic phenols contents are dependent on oxidative reactions catalysed by endogenous oxidoreductases and on hydrolytic reaction during processing. The data were a consequence of different conditions of processing, there were negative relationships between these phenolic contents and temperature-time of processing as several papers report also (Angerosa *et al.*, 2001, Capella *et al.*, 1997). The highest differences were found in the oils from cv. *Biancolilla*.

α-Tocopherol, important as antioxidant protecting the oil by photooxidation (Rahmani *et al.*, 1998), was well represented in all samples. Carotenoid compounds, also important as antioxidant, were ranged from 4.0 and 6.7 ppm, the highest contents were found in the oils from cv. *Cerasuola*.

Variation of chemical and chemical-physical parameters during one year of storage showed increase in acidity, in the UV parameters and in peroxide index. The increased acidity for a sample of *Biancolilla* (0.97%) reduced its merceological class at the end of storage. In summer two samples of oil with an initial value of K_{270} reached the maximum allowed by law but never exceeded. Decrement in total phenols, in pigments content and in turbidity was also carried out. Graphs 1, 2 and 3 show the variation of free acidity, peroxide index and total phenols during storage. The variations of the other parameters are reported in tables 3 and 5.

Tyrosol (Ty) and hydroxityrosol (OHTy) variations depend on their activity as antioxidants and on the hydrolysis reaction rate of oleuropein and ligustroside derivates. In the first step of storage they were less involved like antioxidant compounds and their concentration increased for hydrolysis secoiroides derivates. The variations of dialdeidic form of decarboxymethyl elenolic acid linked to hydroxityrosol (OHTy-EDA), one of the phenolic compounds with high antioxidant activity (Gòmez-Alonso *et al.*, 2003) showed a low decrement during storage (graphs 4, 5 and 6). The isomers of oleuropein and ligstroside aglycon (OHTy-EA and Ty-EA) were the more stable phenolic compounds. In order to evaluate significative differences on chemical and physical-chemical parameters data were analyzed by Student's Paried t test. It was applied on the first and the last sample for each oil (December 2004 and December 2005) and for all the considered parameters. Significative differences at 99% ($p<0.01$) for acidity, $K_{232,}$ $K_{270,}$ total phenols, carotenoids and chlorophylls were found. Peroxide index, tyrosol (Ty),

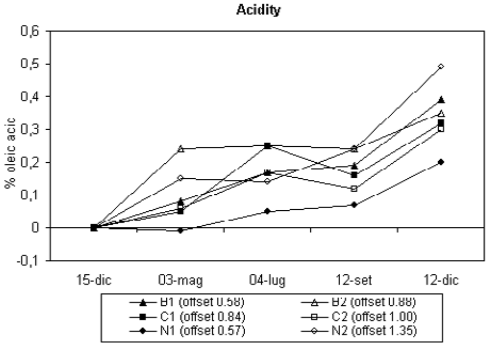

Fig. 1. Trend of free acidity during storage.

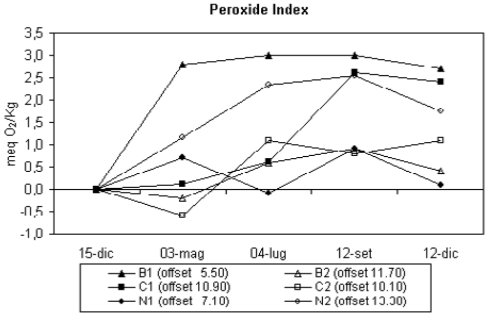

Fig. 2. Trend of peroxide index during storage.

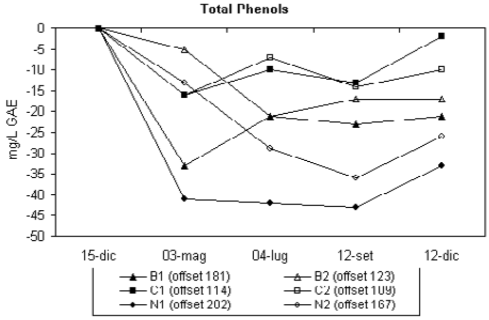

Fig. 3. Trend of total phenols during storage.

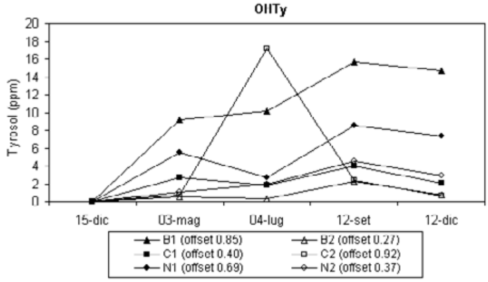

Fig. 4. Trend of OHTy during storage.

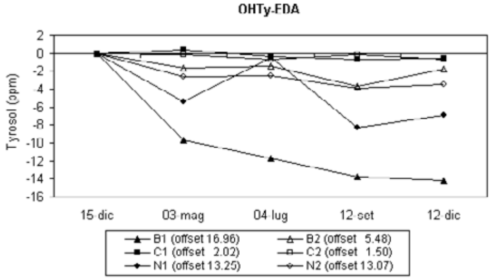

Fig. 5. Trend of OHTy-EDA during storage.

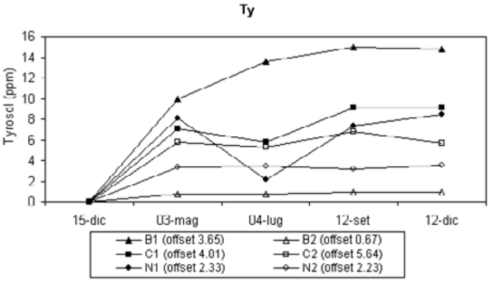

Fig. 6. Trend of Ty during storage.

dialdeidic form of decarboxymethyl elenolic acid linked to tyrosol (Ty-EDA) and turbidity showed differences probably significative($0.01<p<0.05$). No significative difference were carried out for α-tocopherol, hydroxytyrosol (OHTy), dialdeidic form of decarboxymethyl elenolic acid linked to hydroxityrosol (OHTy-EDA) and isomers of oleuropein and ligustroside aglycon.

Our research shows once more the importance of processing on quality of the VOO. Although the considered oils aren't of exceptional quality, their shelf-life is satisfactory when rightly stored. After one year of storage the oils were still protected from oxidative processes as show by their final composition.

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POSTER

EFFECT OF VARIOUS PARAMETERS ON VIRGIN OLIVE OIL STABILITY MEASURED BY RANCIMAT

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ABSTRACT

The quality of virgin olive oil decreases over the course of time as a consequence of oxidative and hydrolytic degradations.

The present study was carried out to determine the oxidative stability of nine Sicilian virgin olive oils from three cultivars (*Biancolilla*, *Cerasuola* and *Nocellara del Belice*). Free acidity, peroxide index, fatty acidic composition, UV absorption values, pigments, total phenolic and tocopherol contents were carried out. The oxidative stability measured by the Rancimat method was correlated with the total phenolic content, peroxide value and oleic acid/ linoleic acid ratio.

Besides, an investigation on each samples was carried out in order to study the influence that exposure to light, to air and to heat, has on the quality of virgin olive oils during five weeks of storage. Oils exposed to light and to air were stored in transparent and amber glass bottles at room temperature. Samples exposed to heat were kept in the dark at 50 °C. Also, each sample was kept in the dark at room temperature. The observed variations were analysed with statistical method; correlations with initial oxidative stability, measured by Rancimat and by the needed time (days) to reach IP=20, were investigated. The results suggested that the shelf life of oils depends on chemical composition and storage conditions.

Key words: Rancimat test, storage, virgin olive oil

INTRODUCTION

Rancidity is an indication of the deterioration of lipids, the reaction rate depends on the insaturation grade of the fatty acids, on the presence of antioxidant and pro-oxidant compounds, and on the storage conditions. The limiting factor in autoxidation is the low reaction rate of initiation stage that gives rise to free radicals, then in propagation stage they give origin to hydroperoxides. The limit of peroxide index by law for “fresh” VOO is 20 meq O_2 /kg. The natural antioxidants of VOO protect it, as they interrupt propagation of the free radical chain reaction and lengthen the induction period. The shelf-life expectation of oil is difficult because many hexogen and endogen factors are involved in the process (Capella *et al.*, 1997; Coultate, 1996).

The Rancimat test is a quick way to obtain comparative data on oxidative stability and, indirectly, on the different grades of protection that the antioxidants offer.

This work studies the influence of composition and of some storage conditions on the oxidative stability measured by Rancimat test. Temperature, light, air and type of bottle were considered. The observed variations were analysed with statistical method. Correlations with initial oxidative stability measured by Rancimat and by the needed time (days) to reach IP=20, were investigated.

MATERIAL AND METHODS

Oil samples

The study was carried out on nine samples of Sicilian virgin oils from three main cultivars in Western Sicily: *Biancolilla* (B1, B2, B3), *Cerasuola* (C1, C2, C3) and *Nocellara del Belice* (N1, N2, N3). Oils came from continuous cycle plant with a three phases centrifugal decanter. The samples, in filled and sealed amber bottles, were kept in a freezer at 18 °C until analysis.

In order to study several hexogen factors on the oxidative stability, samples were divided into four groups. Each group was stored for 5 weeks in the following conditions:

- ❖ room temperature, diffused light, air, transparent glass bottles (A)
- ❖ room temperature, diffused light, air, amber glass bottles (B)
- ❖ temperature 50 °C, darkness, air (C)
- ❖ room temperature, darkness, air (D)

The oil was put into glass bottles Simax (250 ml) with a spread of 3 cm and an internal diameter of 6 cm. The ratio between the surface exposed to air and volume of oil was 0.15.

Instruments

Beckman DU 620 spectrophotomer, Perkin-Elmer AutoSystem XL gas-chromatograph equipped with FID detector, Perkin-Elmer series 200 HPLC equipped with diode array detector, Metrohm Rancimat mod. 679.

Oil analyses

In order to study the influence of oil composition on the oxidative stability all the following analytical parameters were determined.

During storage under conditions A, B, C e D the peroxides index, UV parameters and pigments were determined. The analyses were carried out daily during the first week, then weekly until the end of the experiment.

Free acidity, peroxide index and UV absorption values were determined according to the methods set out by the Reg. EEC n.2568/91.

Fatty acid composition

The analysis was carried out on the methylic esters by gas-chromatography. The preparation of the methylic esters was done according to the official method set out by the above Reg. EEC. The conditions of analysis were as follows: capillary column in fused silica DBWAX, l. 30 m, i.d. 0.25 mm, f. 0.25 μm; transport gas He, 17 psi; T injector 250 °C; T detector 300 °C; T oven 150 °C for 2 min, increasing by 10 °C/ min until 250 °C, isotherm for 18 min.

Total phenols

The extraction and quantitative analysis followed the procedure indicated by Montedoro *et al.* (1992) with the extracting mixture Methanol:Chloroform (95:5). Determination of the phenols was carried out on the extract by Folin-Ciocalteu reagent. The concentration was expressed as gallic acid equivalents (GAE, mg/kg). Calibration curve was obtained using standard solutions of gallic acid (Fluka).

Tocopherols

The analysis was followed according to G. Tonolo *et al.* (1989) using The calibration curves were obtained using standard solution of α, β and γ-tocopherol (Fluka). This method does not allow the separation of β and γ-tocopherol.

Pigments

Carotenoids and chlorophylls contents were determined by measuring the absorption of solution oil at 20% in hexane. The absorbances at 435 nm and 670 nm for carotenoids and chlorophylls respectively were measured. The coefficients of extinction in hexane reported in literature (Modi *et al.*, 1992) were used for quantitative analysis.

Oxidative Stability

The induction time was measured by Rancimat test. The air flow was 20 L/h and temperature 120 °C.

Statistical analysis

The software Statgraphics Plus, 5.0 version from Statistical Graphics Corp. (Rockville, USA) was used to calculate the linear regressions, analysis of the variance (ANOVA), the significant differences and the comparisons among multiple samples by the Duncan test.

RESULTS AND CONCLUSIONS

The examined oils were into the extravirgin merceological class (tables 1 and 2). Statistical analysis of data showed that the most important parameter, in order to establish the possible shelf-life of oil, is the total phenolic content which has a significant correlation (at 99%) with induction time measured by Rancimat (Fig. 1).

Probably significative correlations (level of confidence 95%) were found between the time of induction and: peroxide index (p= 0,0382; r = -0,69) and oleic/linoleic ratio (p= 0,0351; r = 0,70). The correlations between induction time and the other parameters examined were not statistically significant. The equation drawn up from the correlations linked the result of the Rancimat test (RT) to total phenols (TP), to peroxide index (PI) and to ac. oleic/ac. linoleic ratio (O/L).

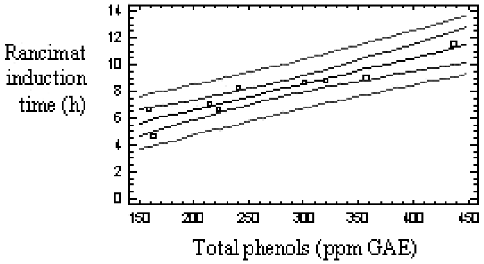
$$RT = 4.2219+0.0166*TP-0.3687* P\ I +0.2081*O/L\ (R^2 = 93\%,\ p = 0{,}0021)$$

Table 1. Initial parameters of oils.

Sample	Acidity (%)	Peroxide Index (meq O_2/kg)	K_{232}	K_{270}	ΔK $*10^3$	Total Phenols (ppm GAE)	α-tocopherol (ppm)	(β+γ) tocopherols (ppm)	Induction time (h)	Chlorophylls (ppm)	Carotenoids (ppm)
B1	0.26	7.2	2.28	0.08	-2.5	163	189	8	4.7	4.3	2.8
B2	0.27	5.2	2.02	0.08	-1.7	215	181	5	7.1	10.0	5.3
B3	0.18	4.6	2.16	0.09	-0.8	301	184	5	8.7	9.3	5.0
C1	0.34	4.2	1.84	0.08	-2.5	159	171	7	6.7	9.5	7.5
C2	0.71	5.9	2.15	0.09	-0.3	240	164	5	8.3	6.3	5.8
C3	0.28	6.3	2.23	0.10	-2.3	320	164	4	8.8	6.8	7.8
N1	0.44	6.3	1.92	0.10	-1.0	223	124	6	6.7	19.5	9.5
N2	0.21	5.3	1.86	0.07	-1.3	357	131	5	9.0	9.0	5.3
N3	0.26	4.3	1.88	0.10	-2.0	437	135	5	11.5	17.0	9.5

Table 2. Initial fatty acid composition (%).

Sample	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C18:1/C18:2
B1	19.55	1.70	2.65	60.82	14.39	0.48	0.14	4.23
B2	15.90	2.01	2.65	67.63	10.26	0.86	0.28	6.59
B3	17.84	1.25	2.82	66.07	11.03	0.60	0.15	5.99
C1	9.91	0.33	3.65	72.86	11.10	0.76	0.39	6.56
C2	12.23	0.66	3.06	70.69	12.07	0.67	0.43	5.86
C3	18.40	1.25	2.81	64.95	11.48	0.70	0.19	5.66
N1	16.04	2.02	3.88	66.41	10.27	0.55	0.26	6.47
N2	14.32	1.40	3.64	69.11	10.55	0.66	0.23	6.55
N3	14.48	1.17	3.79	70.16	9.21	0.74	0.34	7.62



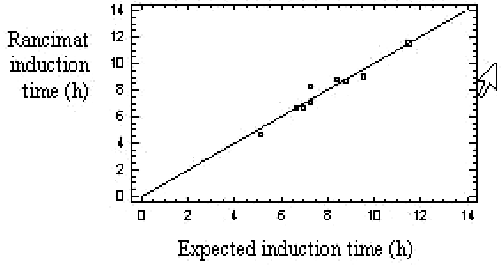
```
Analysis of Variance
-----------------------------------------------------------------------------------------------
Source          Sum of Square          Df        Average²        F-Ratio          P-Value
-----------------------------------------------------------------------------------------------
Model           25,7644                1         25,7644          47,99            0,0002
Residual        3,75785                7         0,53697
-----------------------------------------------------------------------------------------------
Total           29,5222                8         RT = 2,75171 + 0,0193518 · TP

Correlation Coefficient = 0,934190          R² = 87,2711 %
Standard Error of Estimated Values = 0,73269              Absolute Error of  Average = 0,52840
```

Fig. 1. Linear regression analysis for total phenols and induction time.

Fig. 2 shows the results of the Rancimat test versus expected values of the proposed model.

The influence of the hexogen parameters on oils quality was studied and the multiple test on the range of Duncan was adopted for the comparisons. It was applied on all samples and all storage condition in order to determine which type of storage was significantly different under each parameter examined (IP, UV parameters and chlorophylls). Results showed that the peroxide index in the samples stored in the light (A and B) were different at 90% from the samples stored in the dark, except for two samples whose level of confidence was <90%.

The comparison of the absorbance in UV showed that storage at 50 °C gave significant differences to test in



```
Analysis of Variance
---------------------------------------------------------------------------------------------
Source        Sum of Squares        Df      Average²        F –Ratio        P-Value
---------------------------------------------------------------------------------------------
Model         27,6173               3       9,20576         24,16           0,0021
Residual      1,90495               5       0,38099
---------------------------------------------------------------------------------------------
Total         29,5222               8

R² = 93,5474 %
Standard Error of Estimated Values = 0,61724              Absolute Error of  Average = 0,329573
```

Fig. 2. Linear regression analysis for expected induction time and induction time by Rancimat test.

room temperatures (A,B and D) with levels of significativity ranging between 85%-95% for all except one. Significative difference at 95% for chlorophylls to tests in room temperatures were found, too. In order to find which kind of storage could give information on oil oxidative stability, the needed time (days) to reach IP= 20 (T20) was determined. PI analyses were carried out daily for each storage conditions. T20 was not determined on the oils stored in the dark at room temperature because only one sample has reached the limit value by law at the end of the experiment. Statistical analysis showed that data obtained for group D were at 90% statistically different compared to storage in the light which form a group of homogeneous results according to Duncan test. The comparison between Rancimat induction times and the calculated values of T20 showed that C assay (dark, air and 50 °C) was well correlated with Rancimat data (Table 3). The equation drawn up from the correlations linked T20 to total phenols (TP), to peroxide index (PI) and to ac. oleic/ac. linoleic ratio (O/L).

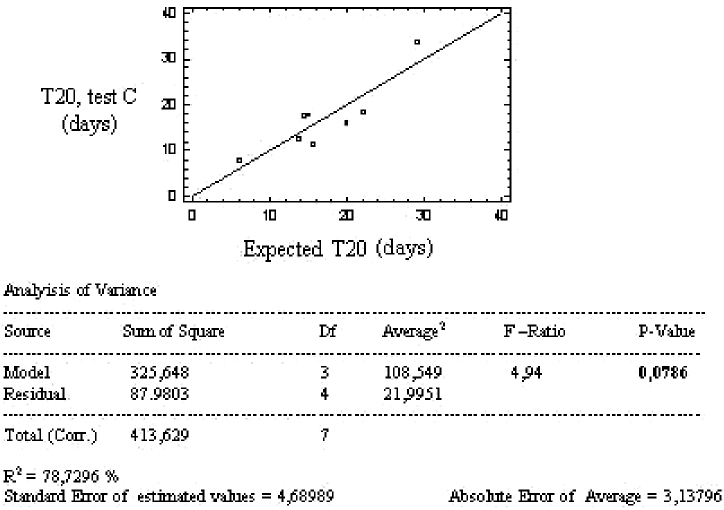
$$T20 = 6{,}6036+0{,}0457*TP-1{,}9499*IP+1{,}4282*O/L \quad (R^2=79\%,\ p=0{,}0786)$$

This expression showed at 90% significant correlation between observed and estimated values (fig. 3), lower than that obtained with Rancimat test (95%).

Table 3. T20 and induction time by Rancimat.

Sample	T 20 (days)			Rancimat
	A	B	C	(h)
B1	7	9	8	4,7
B2	7	8	11	7,1
B3	8	15	16	8,7
C1	8	15	18	6,7
C2	8	10	18	8,3
C3	10	11	6	8,8
N1	9	10	13	6,7
N2	12	15	19	9,0
N3	11	14	34	11,5

Results show that Rancimat test can be used for forecasting the oxidative stability of the oils as the compositive parameters affecting the oxidative reaction rate are correlated with measured induction time. Storage assays show that light does effect the appearance of the first signs of oxidation and that there is no difference between clear and amber bottles, an aspect probably linked to the continuous availability of oxygen. Heat affects UV parameters and chlorophylls content rather than the peroxide index. These useful observations maybe used to pick out the cause of oxidation of the oils. The correlation found between T20 and the compositive parameters



```
Analysis of Variance
--------------------------------------------------------------------------------------------
Source           Sum of Square        Df      Average²        F-Ratio         P-Value
--------------------------------------------------------------------------------------------
Model            325,648              3       108,549         4,94            0,0786
Residual         87.9803              4       21,9951
--------------------------------------------------------------------------------------------
Total (Corr.)    413,629              7

R² = 78,7296 %
Standard Error of  estimated values = 4,68989          Absolute Error of  Average = 3,13796
```

Fig. 3. Linear regression analysis for expected T20 and determined T20 by test C.

can be useful to make a comparison between the oxidative stability of several oils when the Rancimat test cannot be used.

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PACKAGING EFFECTS ON ESSENTIAL OIL CONTENT AND COLOR OF MEDITERRANEAN ORIGIN *ORIGANUM VULGARE* ECOTYPES

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ABSTRACT

Volatile compound fraction of essential oil belonging to *Origanum* spp. ecotypes of Mediterranean origin packaged under various storage conditions was assessed. Essential oil composition was evaluated under vacuum and nitrogen modified atmosphere packaging and at different storage temperatures for medium to long term storage. Also, chromatic evolution during storage was performed in order to assess visual changes from fresh product. Ecotypes under trial were characterized by two main chemotypes, performing in a similar way under same storage conditions. Oil content showed a significant decrease for all storage conditions, and remained almost constant between medium and long term storage. Color characteristic changes were instead not very significant, with the exception of luminance values. From the results obtained it appears that the nitrogen modified atmosphere condition can be effectively used for long term preservation of key volatile compounds in *Origanum* spp.

Key words: oregano, shelf life, MAP, essential oil content

MINIMALLY PROCESSED ORANGE FRUITS: EVALUATION OF PRODUCTS FROM LATE TAROCCO GENOTYPE FRUITS STORED AT LOW TEMPERATURE

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ABSTRACT

The success in the Italian market of Tarocco oranges, due to their excellent sensory and nutritional characteristics, has prompted breeders to select genotypes with high qualitative standards and with early or late maturity period. A clonal selection program, carried out by CRA-Istituto Sperimentale per l'Agrumicoltura, allowed to obtain a late Tarocco genotype named 'Messina', whose fruits can be marketed toward late spring. Recent studies on Tarocco fresh-cut oranges have demonstrated that these fruits are suitable to be minimally processed. In particular, some minimally processed Tarocco clones did not show marked decay phenomena during 14 storage days.

The aim of our research was to study the possibility of extending the marketing period of these products. Thus, fruits of a late Tarocco genotype ('Messina') were minimally processed at the harvest time and after 20, 40 and 60 storage days at 6°C, RH 85-90%. Fresh and stored fruits were sliced along the equatorial axis, packaged under normal atmosphere with a film PET+PP by a heat-sealing vacuum/gas machine and stored at 4 ± 1 °C, for about 14 days. Physicochemical, sensory and microbiological parameters were determined during storage.

Physicochemical results showed no important change during slices storage. However, there was a slight reduction in the ascorbic acid content in slices obtained from fruits stored for 40 and 60 days.

A parameter, named 'Sensory Acceptability' was determined for the sensory analyses of fresh-cut Tarocco oranges. Similar behavior for fresh and stored fruits was found. Only a decrease of this parameter of about 20% at the last storage day in all packaged fresh-cut oranges was observed. Microbiological analysis showed that until the 14th storage day, products obtained from fresh fruits and from oranges stored for 60 days, had CFU/g values of about $2x10^2$ both for mesophilic viable count and for moulds and yeasts count. Products from Tarocco 'Messina' fruits stored for 20 and 40 days, instead, had higher CFU/g values than the above cited value, but always lower than those established by microbiological criteria for minimally processed fruits and vegetables (CNERNA-CNRS, 1996).

Key words: fresh-cut Tarocco oranges, packaging, post-harvest storage

INTRODUCTION

Blood oranges (Tarocco, Moro, Sanguinello) are distinguished among the sweet oranges for their unusual flavour the balanced sugar/acid ratio and the presence of water soluble pigments belonging to the anthocyanins class. Other peculiar aspects of these varieties are the high content of vitamin C, flavanones and hydroxycinnamic acids (Rapisarda, 2001).

One of the most promising late genotypes selected in the CRA- Istituto Sperimentale per l'Agrumicoltura is Tarocco 'Messina'. The peculiarity of this clone is the late maturation of the fruits that allows to extend the harvest time up to late May (Starrantino, 1999).

Recent researches carried out in order to evaluate the shelf-life of minimally processed blood orange fruits, showed that this product does not present substantial nutritional and sensory decay during 14 days of cold storage (Caggia *et al.*, 2004). This work aimed to evaluate physicochemical, sensory and microbiological changes of a late Tarocco genotype ('Messina') that was minimally processed at harvest time and after 20, 40 and 60 storage days at 6°C, RH 85-90% in order to extend the market period of this products.

MATERIALS AND METHODS

Sample preparation

Fruits of Tarocco 'Messina' grown in the experimental field of the CRA-Istituto Sperimentale per l'Agrumicoltura (Acireale, Italy), were harvested at commercial maturity (May 2005). The fruits were treated with a fungicide, (Imazalil, 1 g/l), with the aim of extending their preservation. Then they were cold stored at 6±1°C and 85-90% relative humidity. Fruits were sampled every 20 days up to 60 days, washed with a solution containing 200 ppm of Cl_2, rinsed with distilled water, dried and peeled manually. The oranges were then sliced (about 1cm thickness) along the equatorial diameter using an electric slicer and packaged, under normal atmospheric conditions, in CPET plastic boxes (117x227x35 mm) that were heat sealed with PET-PP film (Table 1). A TecnoFoodpack, mod. Easypack (Pavia, Italy) sealing machine was used for packaging. All the boxes, containing four orange slices (about 180g), were stored for 14 days at the temperature of 4 ±1 °C. Every three days the boxes were withdrawn in order to perform physicochemical, sensory and microbiological analyses.

Table 1. Technical characteristics of film used to package orange slices.

Property	PLASTIC FILM
Stratigraphy	Polyester (PET) 41.7 g/m^2
Permeability to oxygen	56 cc/m^2/24h
Permeability to water vapour	13 g/m^2/24h
Permeability to CO_2	217 cc/m^2/24h
Sealing point	140/220 °C

Physicochemical analyses

The physicochemical parameters determined in slices and juice samples obtained squeezing orange slices from each box, were firmness, pH, total acidity, total soluble solids, ascorbic acid, anthocyanins and sugars (Rapisarda *et al.*, 2006). Five boxes were sampled randomly to perform the analysis.

Statistical differences of physicochemical parameters during the storage days were evaluated by variance analysis (ANOVA) and the means were separated by Tukey's test.

Sensory analyses

The profile method (ISO 13299, 2002) was used for the sensory evaluation of the slices during storage. This method involves the quali-quantitative description of the sample sensory attributes made by a trained panel (ISO 8586-1, 1993) in a laboratory organized in the Institute. Twenty panelists, male and female, aged between 28 and 45 years old, were selected among the staff of CRA-Istituto Sperimentale per l'Agrumicoltura (Italy). In a preliminary session, the judges selected the attributes to describe the product (freshness, aroma, acidity, juiciness, sweetness, firmness, off-flavor, intensity of the taste and acceptability).

The descriptors were grouped in order to obtain a single parameter, named 'Sensory Acceptability' (SA) that allowed to reduce the influence of the single parameters and evaluate the product shelf-life. The parameter 'Sensory Acceptability' was obtained as follows:

$$\text{Sensory acceptability (SA)} = \frac{(F_n + Ar_n + Ac_n + J_n + S_n + Fi_n + It_n)\text{-}O_n}{(F_0 + Ar_0 + Ac_0 + J_0 + S_0 + Fi_0 + It_0)\text{-}O_0}$$

where: **F** (freshness), **Ar** (typical citrus aroma), **Ac** (acidity), **J** (juiciness), **S** (sweetness), **Fi** (firmness), **It** (intensity of the taste), **O** (off-flavor), **n** (0, 3, 6, 10, 14 storage days), **0** (slice evaluation at 0 storage day). The obtained data were submitted to variance analysis (ANOVA) to verify significant differences of the SA during slices storage. The means separation was evaluated by Tukey's test. Factorial ANOVA analysis of 'Sensory Acceptability' data was carried out in order to evaluate the influence of the different periods of fruit storage (Factor 1) without considering the interaction of the slices storage period (Factor 2) and vice versa.

Microbiological analyses

The microbiological analyses were performed on three independent samples every three days.

All the slices of each box were aseptically weighed and put in a sterile plastic bag to be blended in a Stomacher (Lab-Blender 400, Steward Medical, London, UK) for 3 minutes; subsequently serial diluting and plating, using spread plate technique, were done. Mesophilic viable counts (MVC) were performed on Plate Count Agar (Oxoid, CM325), after 48 h incubation at 32 °C. Yeast and mould counts were determined with Sabouraud Dextrose Agar (Oxoid, CM41) after incubation at 25 °C for 4 days. Lactic acid bacteria (LAB) counts were determined on Man-Rogosa-Sharp (Oxoid, CM359) Petri plates incubated at 37 °C for 48 h in anaerobic conditions.

RESULTS AND CONCLUSIONS

Physicochemical analyses

During storage period, a statistically significant decrease of total acidity in all the slices obtained from fruits stored for 0, 20, 40 and 60 days, was observed (Table 2), and this trend was reflected in increasing pH values in some packaging (fresh fruits and 20 days stored fruits) (Table 3).

Anthocyanin content of slices showed a high variability. Therefore, no univocal behavior was observed. Only for the fruits packaged after 60 storage days there was an evident increase in pigment content, probably due to the activation of the anthocyanin biosynthesis during cold storage of slices (Table 4). Regarding ascorbic acid content, statistically significant differences were observed in the slices obtained from fruits stored for 40 and 60 days (Table 5). With regard to the head-space gases of the boxes, a decrease of oxygen and a sharp increase of carbon dioxide content, due to the high respiration activity of fruits, were observed (Tables 6 and 7). No statistically significant changes of firmness, total soluble solid (TSS), and total sugars, in all the samples analyzed, were noted during storage (results not reported).

Table 2. Change in Total Acidity (%).

FRUIT STORAGE DAYS	SLICE STORAGE DAYS				
	0	**3**	**6**	**10**	**14**
0	0,58A	0,52AB	0,48AB	0,43B	0,42B
20	0,57A	0,46AB	0,50AB	0,43B	0,41B
40	0,52A	0,42B	0,41B	0,40B	0,42B
60	0,48 A	0,43AB	0,41B	0,40B	0,40B

significant differences among slice storage days values: $p<0.01$ - capital letters; $p<0.05$ - small letters; n.s. - not significant.

Table 3. Change in pH.

FRUIT STORAGE DAYS	SLICE STORAGE DAYS				
	0	**3**	**6**	**10**	**14**
0	3,98C	4,07BC	4,10ABC	4,18AB	4,26B
20	3,88C	4,12BC	4,02C	4,34AB	4,38A
40	4,22ns	4,20ns	4,24ns	4,30ns	4, 18ns
60	4,08ns	4,20ns	4,22ns	4,24ns	4,24ns

significant differences among slice storage days values: $p<0.01$ - capital letters; $p<0.05$ - small letters; n.s. - not significant.

Table 4. Change in anthocyanins (mg/L).

FRUIT STORAGE DAYS	SLICE STORAGE DAYS				
	0	**3**	**6**	**10**	**14**
0	1,53B	2,11A	1,75AB	1,71AB	2,03AB
20	1,7300	2,21ab	2,57a	2,12ab	1,68b
40	2,20AB	1,90B	2,66A	1,95B	2,65A
60	2,63B	2,70B	2,78AB	3,32AB	3,52A

significant differences among slice storage days values: $p<0.01$ - capital letters; $p<0.05$ - small letters; n.s. - not significant.

Table 5. Change in Vitamin C (mg/100ml).

FRUIT STORAGE DAYS	SLICE STORAGE DAYS				
	0	**3**	**6**	**10**	**14**
0	48,14ns	50,38ns	49,70ns	49,49ns	47,65ns
20	52,49ns	50,74ns	50,80ns	51,56ns	47,97ns
40	49,60A	44,56AB	41,42B	41,77B	41,80B
60	48,79A	45,69AB	45,76AB	43,67B	43,23B

significant differences among slice storage days values: $p<0.01$ - capital letters; $p<0.05$ - small letters; n.s. - not significant.

Table 6. Change in O_2 (%).

FRUIT STORAGE DAYS	SLICE STORAGE DAYS				
	0	**3**	**6**	**10**	**14**
0	21,00A	16,00B	13,72C	10,11D	7,91E
20	21,00A	16,10B	13,44C	9,44D	6,91E
40	21,00A	16,92B	14,00C	10,37D	8,55E
60	21,00A	16,30B	11,68C	9,00D	6,14E

significant differences among slice storage days values: p<0.01 - capital letters; p<0.05 - small letters; n.s. - not significant.

Table 7. Change in CO_2 (%).

FRUIT STORAGE DAYS	SLICE STORAGE DAYS				
	0	**3**	**6**	**10**	**14**
0	0,03E	4,98D	8,08C	11,10B	13,36A
20	0,03E	5,95D	8,96C	12,14B	15,06A
40	0,03E	5,86D	8,96C	12,92B	14,84A
60	0,03E	6,34D	8,70C	12,80B	16,84A

significant differences among slice storage days values: p<0.01 - capital letters; p<0.05 - small letters; n.s. - not significant.

Table 8. Sensory acceptability change of slices from fresh and stored fruits.

FRUIT STORAGE DAYS	SLICE STORAGE DAYS				
	0	**3**	**6**	**10**	**14**
0	100,00a	89,98ab	92,50ab	87,93ab	81,39b
20	100,00A	89,04AB	86,12AB	85,39AB	78,45B
40	100,00a	93,22ab	86,88ab	85,81ab	82,67b
60	100,00A	88,89AB	86,27AB	85,69AB	72,84B

significant differences among slice storage days values: p<0.01 - capital letters; p<0.05 - small letters; n.s. - not significant.

Table 9. Factorial ANOVA analysis of 'Sensory Acceptability' data.

FRUIT STORAGE DAYS	FACTOR 1	SLICE STORAGE DAYS	FACTOR2
0	90,36ns	**0**	100A
20	87,80ns	**3**	90,28B
40	89,71ns	**6**	87,94B
60	86,74ns	**10**	86,20BC
		14	78,84C

significant differences: p<0.01 - capital letters; p<0.05 - small letters; n.s. - not significant.

Sensory analyses

The values of the 'Sensory Acceptability' of orange slices obtained from fresh fruits and from the cold stored ones for 20, 40 and 60 days, showed a slight decay during the 14 days of storage with statistically significant differences between the first and the last day of storage (Table 8). The results of factorial analysis demonstrated that no statistically significant differences among fruit stored for 0, 20, 40 and 60 days were observed (Table 9). In addition, slices decay during the 14 days of storage was confirmed.

Microbiological analyses

The trend of the microbiological evolution (expressed in CFU/g), of the orange slices obtained both from the fresh fruits and from fruits after 20, 40 and 60 days of cold storage, remained, for the whole storage period at 4°C, below the levels established by microbiological criteria for minimally processed fruits and vegetables (DGCCRF, 1992; CNERNA-CNRS, 1996) (Figures 1, 2, 3 and 4). The microbiological results concerning the mesophilic viable count (PCA) values at the last storage day ranged between of 2,3-4,3 logCFU/g. With regard to acid bacteria, these did not develop in any packaging of both fresh and stored fruits. Also yeasts and moulds (SAB) number was below the CNERNA-CNRS (1996) values.

The results of the physicochemical analyses showed no important differences among the four packaging. The high

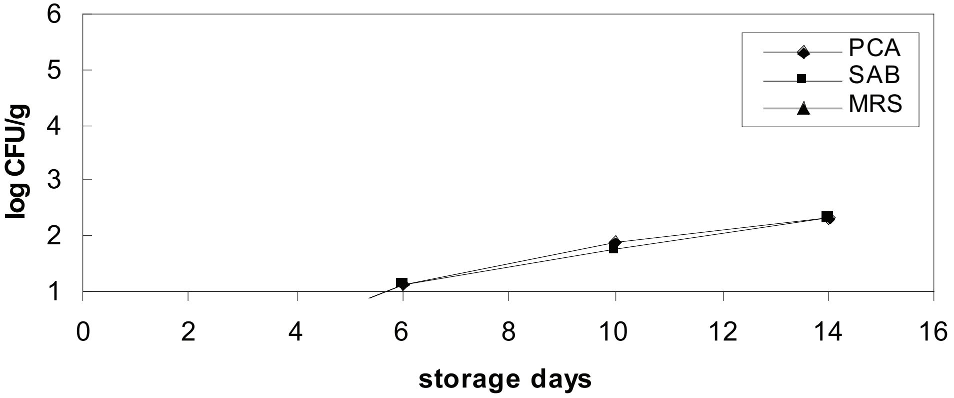

Fig. 1. Microbiological evolution of slices obtained from fresh fruits.

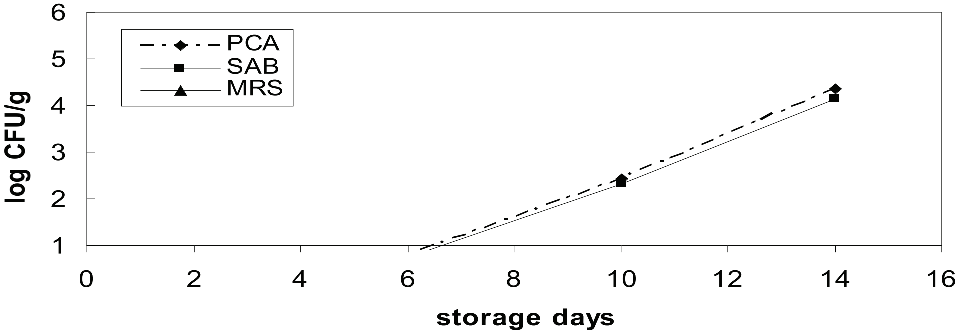

Fig. 2. Microbiological evolution of slices obtained from fruits stored for 20 days.

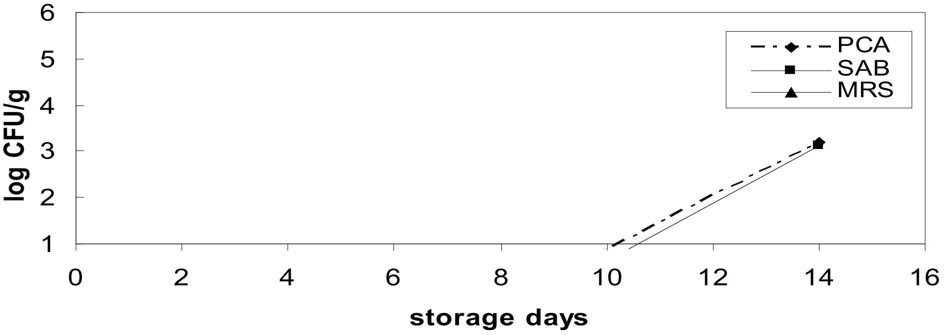

Fig. 3. Microbiological evolution of slices obtained from fruits stored for 40 days.

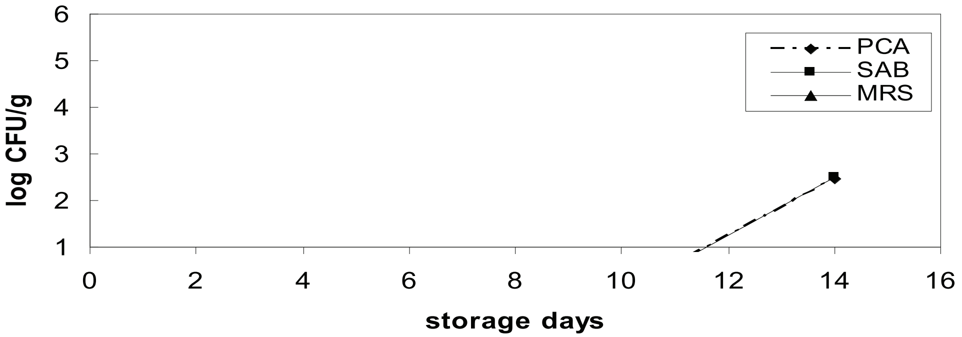

Fig. 4. Microbiological evolution of slices obtained from fruits stored for 60 days.

acidity, a typical characteristic of Tarocco cultivar, and the persistence of high vitamin C levels, contributed to the inhibition of enzymatic browning processes and thus no marked changes in the color of the slices was observed.

The microbiological and sensory results showed a high stability both for fresh and post-harvest treated Tarocco 'Messina' fruits. Thus, it is possible to affirm that these fruits are suitable for minimal processing.

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CHANGES IN POLYPHENOL, VITAMIN C CONTENT AND ANTIOXIDANT CAPACITY IN MINIMALLY PROCESSED TAROCCO FRUITS

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ABSTRACT

Changes in polyphenol, vitamin C content and antioxidant activity of minimally processed Tarocco 'TDV' fruits were investigated. The fruits were sliced, packaged in normal air with three different permeability films and stored at 4±1°C for 15 days in order to find the best packaging conditions to preserve bioactive compounds and maintain antioxidant efficiency of slices. During storage, anthocyanins, hydroxycinnamic acids, flavanones and ascorbic acid were determined and antioxidant capacity was evaluated by means of two different *in vitro* tests (DPPH radical scavenging capacity and inhibition of induced linoleic acid oxidation). The results of chemical analysis showed that, during the 14-15 days of storage, Tarocco 'TDV' slices did not show marked decay phenomena. The slices showed an evident antioxidant capacity during storage period. Moreover, the less permeable films used for packaging resulted, in both tests employed, the most suitable to maintain the antioxidant efficiency and nutritional characteristics of minimally processed Tarocco 'TDV' oranges.

Key words: antioxidant activity, anthocyanins, minimal processing, Tarocco, storage

INTRODUCTION

In Italy, about 40% of the sweet orange production is represented by Tarocco cultivar, whose growing success in domestic and international markets is a result of its exceptional sensorial characteristics, such as the brilliant red flesh colour and the distinctive and agreeable fragrance, as well as the large size and balanced levels of sugar and acids (Rapisarda 2000; Rapisarda *et al.,* 2001). In addition, these fruits contain high levels of antioxidant compounds including vitamin C (Rapisarda and Intelisano 1996; Rapisarda and Russo 2003),

flavanones (Postorino and Gionfriddo 1999), hydroxycinnamic acids (Rapisarda *et al.* 1998) and anthocyanins (Maccarone *et al.,* 1998; Rapisarda *et al.,* 1999). The greater health benefits of blood oranges with respect to the blonde ones and the commercial success of fresh-cut fruits and vegetables have prompted research into the production of ready-to-eat sliced oranges in order to realize a new market segment for Sicilian blood oranges as an alternative to fresh fruit consumption. However, operations such as peeling and cutting significantly reduce the shelf life of these products, since various metabolic processes are accelerated. Mechanical damage causes increased respiratory activity with a consequent rise in some bio-chemical effects, such as enzymatic discolouration and loss of aroma, firmness and nutritional qualities (Rolle and Chism 1987; Watada *et al.,* 1990). Low temperature, during storage, considerably slow bio-chemical activity and microbial proliferation (Rolle and Chism 1987). To maintain good quality, therefore, the product must be stored at temperatures no higher than 5°C (Yildiz 1994).

The following work aimed at evaluating polyphenol, vitamin C content and antioxidant activity changes occurring during storage of a fresh-cut Tarocco clone ('TDV') with high anthocyanin content packaged with three different permeability films and stored at 4±1°C for 15 days.

MATERIALS AND METHODS

Sample Preparation

The study was carried out on fruits of Tarocco 'TDV' [*Citrus sinensis* (L.) Osbeck], harvested in March 2005 in the Palazzelli experimental farm of the CRA - Istituto Sperimentale per l'Agrumicoltura (Acireale, Italy).

The samples were processed by a pilot-plant (PND mod. BABY, Salerno, Italy) located in a suitably equipped and hygienically controlled environment. To reduce microbial contamination, the operators wore gloves and masks during all the sample preparation stages and packaging operations were done under a laminar flow hood.

Fruits were washed with a solution containing 200 ppm Cl_2, rinsed with distilled water, dried and peeled. The oranges were then sliced using an electric slicer and packaged in plastic boxes under normal air with three different permeability films (Table 1). A TecnoFoodpack mod. Easypack (Pavia, Italy) sealing machine was used for packaging. Each box, containing four orange slices, was kept at 4±1°C for 15 days. During storage the boxes were sampled, in triplicate, every three days and the antioxidant components and antioxidant activity were determined on juice extracted by a domestic squeezer.

Chemical Analyses

Total soluble solids (TSS) and total acidity (TA) were determined according to standard methods (MAF, 1989; Kimball, 1999). The ascorbic acid concentration was evaluated by liquid chromatography using a Waters Alliance 2695 HPLC equipped with a Waters 2996 photodiode array detector and Empower Waters software (Rap-

Table 1. Technical characteristics of films[a] used for packaging the different Tarocco clone slices.

Property	PLASTIC FILM		
	Film A	Film B	Film C
Name	NPL Mailar Triplex ®	NPL Mailar Monostrato ®	NPL Accoppiato (PET12+PP50) ®
Stratigraphy	Oriented Nylon (OPA) 17 g/m^2	Polyester (PET) 41.7 g/m^2	Polyester (PET) 16.8 g/m^2
	Adhesive 2 g/m^2	- -	Adhesive 2 g/m^2
	Polyester (PET) 42 g/m^2	- -	Polypropylene (PP) 45.5 g/m^2
Permeability to oxygen	35 cc/m^2/24h	56 cc/m^2/24h	110 cc/m^2/24h
Permeability to water vapour	15 g/m^2/24h	13 g/m^2/24h	45 g/m^2/24h
Permeability to CO_2	-	217 cc/m^2/24h	-
Sealing point	140/220°C	140/220°C	135/150°C

[a] Supplied by TecnoFood-pack (Parma, Italy).

isarda and Intelisano 1996). Total anthocyanin content was determined spectrophotometrically (Varian UV/Vis spectrophotometer mod. Cary 100 Scan) by the pH differential method (Rapisarda *et al.*, 1994), and expressed as cyanidin-3-glucoside concentration. Flavanone glycosides, expressed as hesperidin, were determined by an HPLC procedure (Rouseff *et al.*, 1987) using the HPLC-PDA equipment described above. Hydroxycinnamic acids (*p*-coumaric, ferulic, caffeic and sinapic acid) were extracted from juice by solid-phase extraction (SPE) after alkaline hydrolysis of hydroxycinnamic esters and analyzed by HPLC (Rapisarda *et al.*, 2001).

Antioxidant activity

The free-radical scavenging capacity of juices was tested as bleaching of the stable 1,1-diphenil-2-picrylhydrazyl radical (DPPH$^\bullet$) (Brand-Williams *et al.*, 1995). In addition, antioxidant activity was determined through the lipoxygenase-linoleic acid assay. The enzymatic oxidation of linoleic acid was obtained by addition of lipoxygenase modifying the method of Grossman and Zakut (1979). The inhibition percentage of conjugated diene peroxides from linoleic acid oxidation in the presence of orange juice was considered as the antioxidant activity (Lo Scalzo *et al.*, 2004).

Statistical analysis

One-way ANOVA was performed for each different packaging to obtain a statistical assessment of the influence of storage period on chemical and antioxidant capacity evolution. Means were separated by Tukey's test at 5 or 1% levels of significance. Factorial ANOVA analysis of antioxidant activity data was carried out in order to evaluate the film influence on the slice antioxidant capacity without considering the interaction of storage days.

Table 2. Chemical parameters evolution during storage of Tarocco 'TDV' slices packaged with different films.

	FILM A						
Storage days	0	3	5	7	10	12	15
pH	3.95	3.97	3.88	4.14	4.07	4.02	4.02
	AB	AB	B	A	AB	AB	AB
TA (%)	0.94	0.85	0.89	0.66	0.72	0.76	0.88
	ns	ns	ns	ns	ns	ns	ns
TSS (%)	11.84	11.06	11.34	10.57	11.37	10.61	10.61
	a	ab	ab	b	ab	b	b
Ascorbic Acid (mg/100mL)	76.76	66.30	55.85	57.07	55.70	61.68	63.11
	A	AB	B	B	B	B	B
Anthocyanins (ppm)	102.68	119.12	130.89	99.77	101.09	101.18	80.61
	AB	AB	A	AB	AB	AB	B
Flavanones (ppm)	92.83	100.94	106.02	105.86	97.78	97.60	91.81
	ns	ns	ns	ns	ns	ns	ns
Hydroxycinnamic Acids (ppm)	74.12	98.92	100.00	99.16	90.54	91.86	74.34
	b	ab	a	a	ab	ab	b
DPPH (IC_{50})	22.12	19.97	18.73	21.27	21.30	19.40	19.61
	a	ab	b	ab	ab	ab	ab
Lipoxygenase Test (%)	64.15	54.84	58.52	51.10	52.20	49.65	52.09
	A	AB	AB	B	AB	B	AB

	FILM B						
Storage days	0	3	5	7	10	12	15
pH	3.91	3.87	3.94	3.95	4.08	3.92	3.91
	AB	B	AB	AB	A	AB	AB
TA (%)	0.92	0.94	0.86	0.83	0.74	0.74	0.77
	a	a	ab	ab	b	b	ab
TSS (%)	11.70	11.32	11.12	10.93	11.24	10.49	10.88
	A	AB	AB	AB	AB	B	AB
Ascorbic Acid (mg/100mL)	70.41	71.79	61.58	60.71	56.36	55.56	53.05
	A	A	BC	BC	BC	BC	C
Anthocyanins (ppm)	101.67	129.30	133.75	147.76	96.65	96.29	94.83
	b	ab	ab	a	b	b	b
Flavanones (ppm)	102.63	125.66	124.03	112.22	105.39	89.32	84.80
	ABC	A	AB	ABC	ABC	BC	C
Hydroxycinnamic Acids (ppm)	82.34	103.31	108.04	99.64	85.00	81.60	75.45
	AB	A	A	AB	AB	AB	B
DPPH (IC_{50})	21.50	20.16	19.07	19.39	21.99	21.61	22.77
	ABC	ABC	C	BC	AB	ABC	A
Lipoxygenase Test (%)	65.49	52.75	60.39	46.22	50.27	51.49	53.59
	A	BC	AB	C	BC	BC	BC

	FILM C						
Storage days	0	3	5	7	10	12	15
pH	3.96	3.89	4.06	4.05	4.14	4.10	4.15
	AB	B	AB	AB	A	AB	A
TA (%)	0.86	0.86	0.72	0.71	0.65	0.70	0.66
	A	A	AB	AB	B	AB	AB
TSS (%)	12.11	11.27	10.62	10.94	11.58	11.81	11.61
	A	ABC	C	BC	ABC	AB	ABC
Ascorbic Acid (mg/100mL)	67.38	57.78	54.18	53.96	56.85	54.76	52.40
	a	ab	ab	ab	ab	ab	b
Anthocyanins (ppm)	80.41	77.76	95.27	100.46	105.84	122.38	97.38
	b	b	ab	ab	ab	a	ab
Flavanones (ppm)	90.37	87.78	93.78	101.02	97.44	99.39	90.66
	ns	ns	ns	ns	ns	ns	ns
Hydroxycinnamic Acids (ppm)	50.63	62.08	74.95	83.67	82.77	85.57	68.38
	B	AB	AB	A	A	A	AB
DPPH (IC_{50})	22.73	20.68	22.44	23.96	22.35	20.95	22.61
	ns	ns	ns	ns	ns	ns	ns
Lipoxygenase Test (%)	65.74	50.73	58.80	48.18	48.98	42.75	47.98
	A	BC	AB	BC	BC	C	BC

$p<0.01$ capital letters, $p<0.05$ small letters; ns: not statistically significant.

RESULTS AND CONCLUSION

Evolution of chemical composition and antioxidant activity of Tarocco 'TDV' slices packaged with three different permeability films and stored at 4±1°C for 15 days is shown in Table 2. During the storage period, a slight decrease in ascorbic acid content was observed in all Tarocco slices packaged with the three films; however, vitamin C levels at the end of treatment (52- 63 mg/100 ml) were not such to lead to an excessive reduction in the antioxidant protection of fruits. Anthocyanin content in Tarocco 'TDV' slices showed a high variability probably due to different distribution of these pigments in the slices. However, a decrease at the end of treatment, in film A and B packaging, was observed.

Total concentration of the hydroxycinnamic acids and flavanones increased with

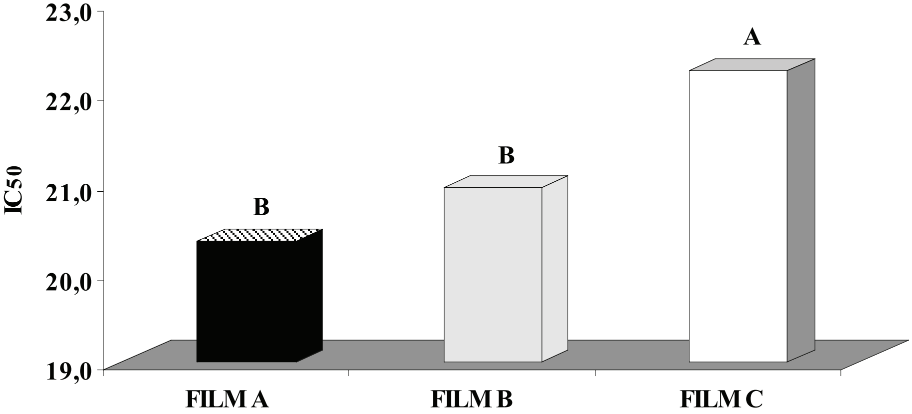

Fig. 1. Factorial ANOVA analysis of DPPH free radical scavenging capacity of Tarocco 'TDV' slices packaged with three different films ($p<0.01$).
(A stronger radical quenching capacity results in a lower IC_{50} value)

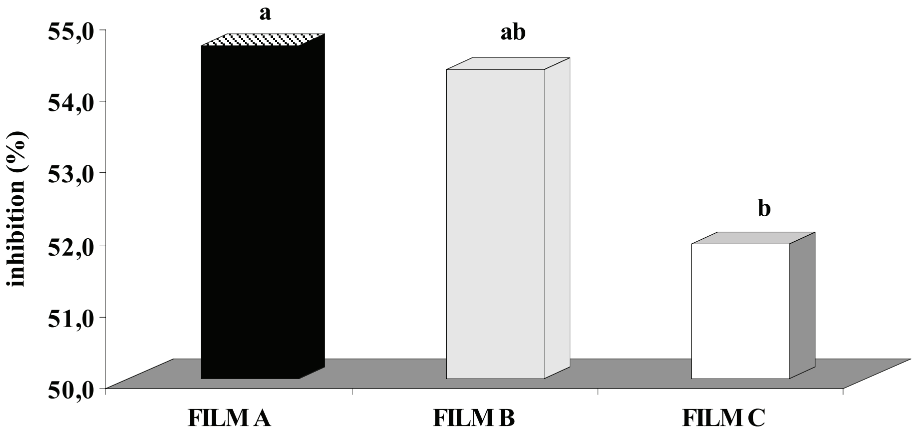

Fig. 2. Factorial ANOVA analysis of inhibition of induced linoleic acid oxidation of Tarocco 'TDV' slices packaged with three different films ($p<0.05$).

storage and that confirms the positive effect of low temperatures on phenylpropanoid metabolism but, at the end of storage, a slight decrease of these compounds was noted.

Factorial ANOVA analysis of antioxidant activity data (Fig. 1 and 2) showed that film C resulted, in both tests used, the least suitable for minimally processing of Tarocco 'TDV'. Film A and B, with lowest permeability to oxygen, better maintained antioxidant efficiency and, therefore, the health functionality of orange slices.

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EVALUATION OF THE STALING RATE OF ALTAMURA PDO BREAD

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ABSTRACT

In this paper the organoleptic characteristics and the mechanic properties of unpackaged Altamura bread were evaluated to assess its staling rate. The obtained results indicated that during the whole storage period the intensity of the sensory characters *crumb elasticity*, *crumb colour*, *fresh bread aroma*, *sour aroma and taste* decreased, while *stale aroma and taste* and *crumb consistence* increased. At the same time, mechanic measures indicated that crumb firmness increased significantly. The extent of this increase was minor during the first four days after baking, while at the end of the whole period the increase of firmness was markedly higher. Until four days from the production, the examined bread maintained acceptable values for both the sensory characteristics and the resistance to compression.

Key words: Altamura bread, mechanic properties, sensory properties, shelf-life, staling

INTRODUCTION

Altamura PDO (protected denomination of origin) bread is obtained from 100%

durum wheat re-milled semolina by a prolonged sponge-dough method based on the use of natural yeast. This confers it both a long shelf-life (BOYACIOGLU and D'APPOLONIA, 1994; CORSETTI *et al.*, 1998, 2000; RAFFO *et al.*, 2003) and typical organoleptic characteristics so that this kind of bread obtained a PDO mark (EC Official Journal, 2003). Usually, the staling rate of bread is monitored by determining the increase of firmness during time (AACC, 2000), although the whole sensory properties are affected by staling. In previous papers (RAFFO *et al.*, 2003; PASQUALONE *et al.*, 2005) the evaluation of the sensory properties was applied to durum wheat bread, but without checking the changes of these characteristics during time.

The aim of this work was thus to evaluate the staling rate of Altamura bread by monitoring both its mechanic properties (resistance to compression) and sensory characteristics (quantitative descriptive analysis).

MATERIALS AND METHODS

A set of 6 Altamura PDO breads was collected at bread-maker "Digesù" in Altamura (Bari, Italy) and stored unpackaged for 6 days at 20°C and 45% relative humidity. Firmness of the bread samples was measured on a Universal Testing Machine (Galdabini, Varese, Italia), according to the AACC approved method 74-09 (2000). Quantitative descriptive analysis of the sensory properties was carried out according to ISO 13299 (1998) by ten trained panelists. The list of sensory terms included 10 descriptors (Fig. 2) that were rated on an anchored line scale providing a 0-9 score range.

RESULTS AND DISCUSSION

A set of 6 Altamura breads was stored for a period of time of 6 days and examined at different times during the considered period. Fig. 1 reports the variations of the force needed to compress the crumb during the considered period of time. Plotting compression force versus decrease of bread slice thickness a visco-elastic behaviour was detected, with the slope of the curves progressively increasing at stronger compressions. The compression force measurements were taken in the area past the point of inflection of the curve, where the slope is less steeper than at the beginning of the test, and the force values did not change much with small changes in compression. The obtained results indicated that during the whole storage period the resistance to compression increased. The extent of this increase was minor during the first four days after baking, with a Δ/day of 2.11 N, while at the end of the whole period the increase of firmness was markedly higher, with an overall Δ/day of 3.37 N.

Regarding the sensory evaluation, descriptors of appearance (*crumb colour* and *crust thickness*), visual-tactile characteristics (*crumb elasticity*), aroma (*fresh bread, sour, stale*), taste (*sour* and *stale*) and texture attributes perceived by chewing (*crumb consistence*), were considered. The "overall acceptability" was also evaluated. Part of these parameters was chosen merely to describe the product, and were already used in similar samples in a previous work (RAFFO *et al.*, 2003) but other descriptors, such as *fresh* and *stale aroma*, were especially chosen to monitor the changes of bread during shelf-life.

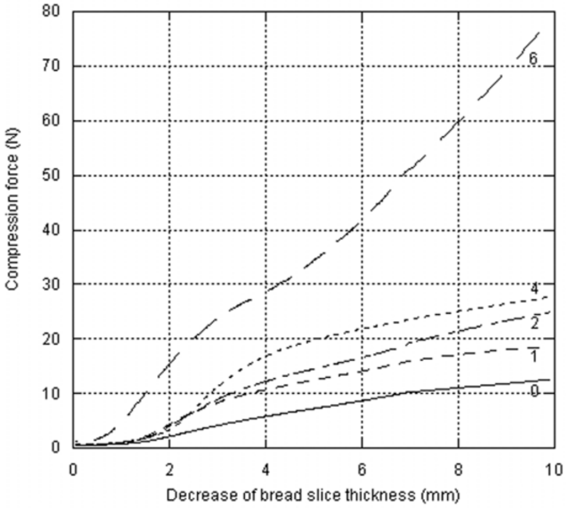

Fig. 1. Variations of the compression curves of the examined bread over a period of 6 days (0-6 = elapsed time after baking, expressed in days).

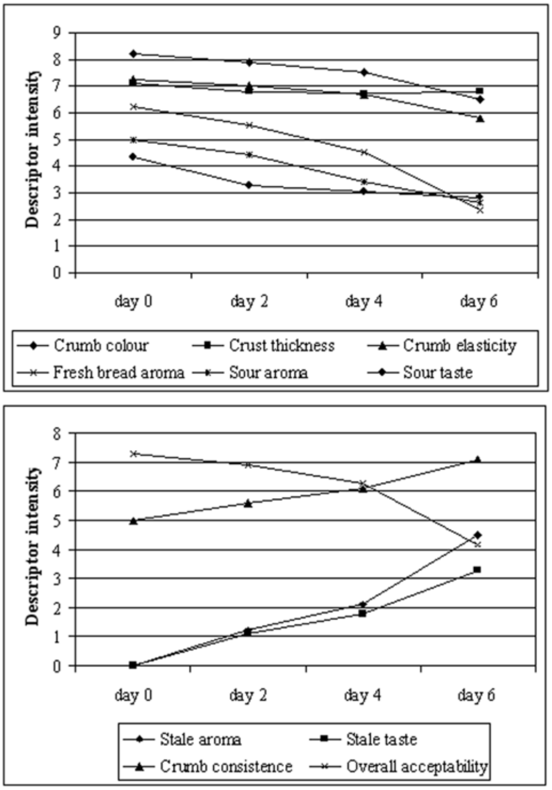

Fig. 2. Variations affecting the sensory properties of the examined bread over a period of 6 days.

Fig. 2 reports the variations affecting the sensory descriptors during 6 days from baking. It can be observed that, especially at the beginning of the considered period, some parameters were evaluated with high scores such as *sour aroma and taste*, *crumb colour and consistence*. *Sour aroma and taste* are typically related to the prolonged method of leavening (MARTINEZ-ANAYA *et al.*, 1990) adopted to obtain this kind of bread, while high scores for *crumb colour* and *compactness* were due to the use of durum wheat re-milled semolina, rich in carotenoid pigments and with a tenacious gluten. However, during the examined period of time all the palatable characters such as *crumb elasticity*, *fresh bread aroma* and *sour aroma and taste* decreased while the unpleasant *stale aroma*, *stale taste* and *crumb consistence* increased. In any case, the examined bread maintained good values of overall acceptability until four days from the production.

In conclusion, Altamura PDO bread showed a considerably slow staling rate in spite of the total absence of improvers in its formulation.

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VACUUM-PACKAGING OF RIPENED SAUSAGES: ASSESSMENT OF THE SENSORIAL AND CHEMICAL CHARACTERISTICS AND OF THE LIPID FRACTION DEGRADATION

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ABSTRACT

In order to determine the influence of vacuum-packaging on the sensorial characteristics and on the degradation of the sausages' lipid fraction, vacuum-packaged ripened sausages were stored for forty days and compared with the same sausages before the packaging at the end of their ripening period. As regards the sensory properties, vacuum-packaged sausages showed, after the storage period, significantly lower mean scores of *red intensity* ($p < 0.01$), *global flavour* ($p < 0.05$) and *ripened flavour* ($p < 0.001$), thus indicating a minor overall appreciation by the panellists, while no significant differences were found in relation to the descriptors that are considered defects for this category of products. Moreover, the obtained data showed a significant increase of the oxidative and hydrolytic degradation during the storage in vacuum packaging.

Key words: lipid fraction degradation, ripened sausages, sensory evaluation, vacuum-packaging

INTRODUCTION

The vacuum-packaging is commonly used for the storage of ripened sausages.

The product, not sliced, is introduced into the pack, and the air is removed without replacement by any other gas, creating a pressure differential between the inside and the outside of the pack. The pack, a flexible plastic, impermeable to gases and water vapour, adheres closely to the product. This modality of storage is also useful to avoid the further water loss of the ripened sausages and to reduce the oxidation of the lipid fraction, that involves modifications both of organoleptic characteristics and of nutritional value of the product (Alexander, 1998; Berra *et al.*, 2005). Lastly, it is noteworthy that some intermediate and final products of the oxidation reaction are potentially toxic to human health (Billek, 2000; Newburg and Concon, 1980). However, the anaerobic conditions of sausage under vacuum-packaging may affect the quality features of the product itself. Many studies have revealed a change in the prevailing microflore in vacuum-packed charcuterie products as compared to the microflore prevailing before storage (Sameslis *et al.*, 2000). This microbiologic change could determine some modifications of the sensory properties and it could affect the nutritional value and the chemical composition of products themselves.

The purpose of the present work was, then, to assess the sensorial and chemical characteristics of the lipid fraction in vacuum-packed ripened sausages as compared to the same samples before packaging.

MATERIALS AND METHODS

Samples were prepared at an industrial establishment sited near Potenza (Italy) following the current industrial processing technology. Before the stuffing two

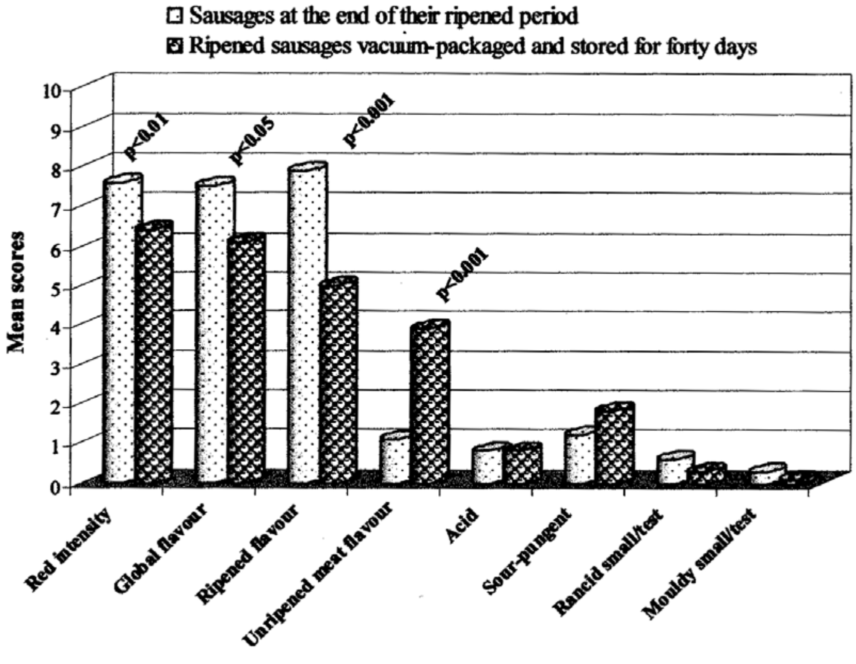

Fig. 1. Mean scores and results of the statistical analysis of the sensorial analysis of the examined sausages.

samples of the mix were tested in order to assess the lipid fraction quality on the raw matter. At the end of the ripening period (28 days at 12-14°C and 85% RH), four sausages were sampled: two of them were brought to the laboratory and tested immediately; the others were vacuum-packed and stored for forty days. Two different samplings were performed.

The vacuum packaged ripened sausages were stored in plastic films (Alpak, Taurisano, Italy) made of polyethylene and polyester (thickness 95 µ) having an O_2 permeability < 15 cm^3/m^2 in 24h (at 20°C and 75% RH), and H_2O permeability < 1.5 g/m^2 in 24h (at 23°C and 85% RH). The sensorial analysis was performed as described in a previous paper (Caponio *et al.*, 2006). The evaluation of the degradation of the lipid fraction of sausages was conducted by HPSEC analysis of the polar compounds separated by silica gel column chromatography according to the AOAC methods (2003), in order to separate and quantify the triacylglycerol oligopolymers, the oxidised triacylglycerols, and the diacylglycerols (GOMES and Caponio, 1999).

The data obtained were analysed by one-way analysis of variance (ANOVA) to detect any significant difference between the products tested.

RESULTS AND DISCUSSION

Fig. 1 shows the mean scores and the results of the statistical analysis of the sensorial analysis of the sausages at the end of their ripening period and of the same sausages vacuum-packaged and stored for forty days.

The vacuum-packaged samples showed a significantly lower ($p < 0.01$) mean score

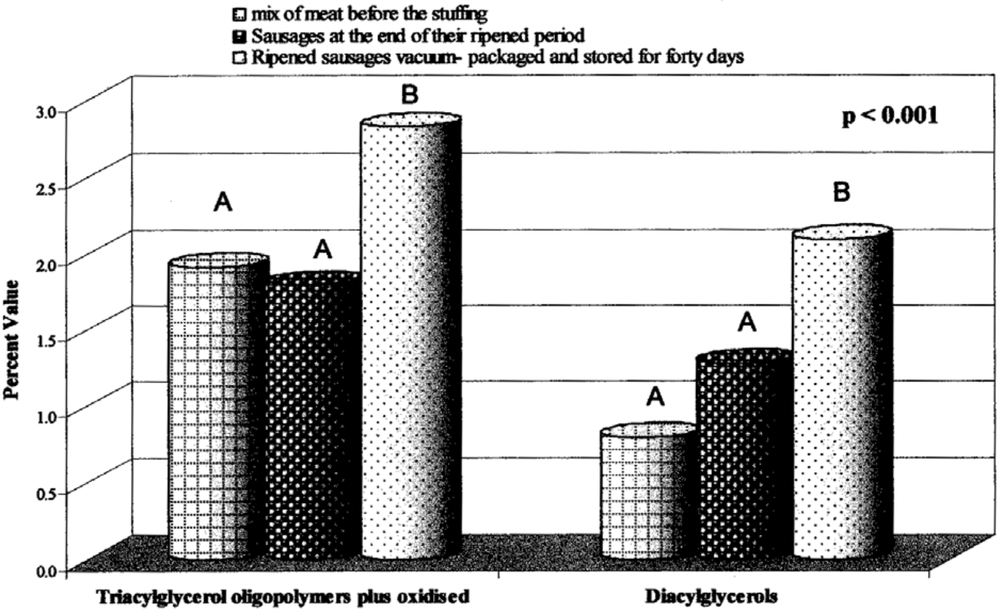

Fig. 2. Mean values and results of the statistical analysis of the analytical determinations performed to assess the hydrolytic and oxidative degradation of the sausages' lipid fraction.

of the *red intensity* as compared to samples at the end of their ripening period. The vacuum packing of sausages involves a decrease of the intensity of meat colour, which is a characteristic highly appreciated by consumers in sausages (Brewer *et al.*, 1998). Of special interest is the result of the *global flavour*, a parameter explaining the organoleptic quality of the product: mean scores significantly decreased ($p < 0.05$) during the vacuum-packing of tested sausages. Vacuum-packaging also caused a significant reduction ($p < 0.001$) of the *ripened flavour* together with a significant increase ($p < 0.001$) of *unripened meat flavour*. In relation to the other descriptors analysed, no significant difference was observed between the tested sausages at the end of their ripening period and after forty days of vacuum packaging.

Fig. 2 shows the mean values and the results of the statistical analysis of the analytical determinations performed to assess the hydrolytic and oxidative degradation of the sausages' lipid fraction. In relation to the hydrolytic degradation, the diacylglycerol content increased significantly ($p < 0.001$) during the vacuum-packing storage; this might be correlated to the lipolytic activities of lactic acid bacteria and *Microccocaceae* that, it is known, are the prevailing microrganisms during ripening and in the early phases of storage (Caponio *et al.*, 2006). The data showed in Fig. 2 evidenced a significant increase ($p < 0.001$) of the oxidative degradation, indicated by the sum of triacylglycerol oligopolymers and oxidised triacylglycerols, during the forty days of vacuum-packing storage. This increase, even removing oxygen by vacuum procedures and using a low-permeability packaging, might be due to the fact that this alteration is determined in greater extent by an oxygen presence in the internal part of the product, than by atmospheric oxygen (Fernàndez-Fernandez *et al.*, 1997).

In conclusion the vacuum-packaging of ripened sausages induced, after forty days of storage, a significant decrease of the main descriptors related to consumers' acceptance, such as the *global flavour* and *red intensity*. As to the degradation of the lipid fraction, a significant increase of the hydrolytic and oxidative degradation was observed during the vacuum-packaging storage.

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DEPENDANCE OF THE SHELF-LIFE OF CARBONATED SOFT DRINKS FROM PET PERFORMANCES AS REQUIRED BY "THE COCA-COLA COMPANY"

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ABSTRACT

PET (Polyethyleneterephthalate) is one of the most important resins used for the package of carbonated water and soft drinks. Preforms are intermediate products which are manufactured in a wide range of designs and colours by injection moulding of PET. The cost of the resin covers a high percentage in the overall cost of the produce, especially due to the recent increments on the price of petroleum, from which PET is derived. As a consequence, according to the numerous standards fixed by “The Coca-Cola Company®”, Sibeg S.r.l. decided to test a new cheaper PET preform which was suggested by a possible new supplier.

The experiment consisted in the comparison of the main performance characteristics of the old PET bottles in comparison with the ones obtained from the new preforms.

“The Coca-Cola Company®” requires specific test procedures to authorize a new package. Among the methods used to test new materials, the gas-barrier properties have a main importance as they are directly linked with the shelf-life of the bottled produces. Shelf-life of carbonated soft drinks is determined by the internal CO_2 level, and limits for this parameter are different for different the types of beverages. Therefore a material with higher CO_2-barrier property would be effective in extending the shelf-life of carbonated drinks. Results evidenced a similar behaviour of the two materials tested for what concerns the characteristics of the bottles (dimensions, appearance, distribution of the material, stress cracking test), showing, in particular, comparable CO_2 internal pressure after 12 weeks of storage.

Key words: carbonated beverages, CO_2 internal pressure, shelf-life, PET

INTRODUCTION

PET (Polyethyleneterephthalate) is one of the most important resins used for the package of carbonated water and soft drinks, thanks to the specific characteristics of clarity, lightness, simple transformability and also the possibility to recycle.The final price of PET preforms is highly influenced by the price of the raw material, the resin, that can reach the 80% on the final total cost. In front to this prices increments, all the factory try to make better the own production, trying all the possible solutions to make lower the incidence of raw material's cost.

Preforms (or parisons) are intermediate products in the production of bottles. They are manufactured in a wide range of designs and colours by injection moulding, and are then blow-molded to obtain the final bottles with volumes ranging from 0.5 to 2 litres.

Sibeg s.r.l., authorized producer of "The Coca-Cola Company" products, located in the province of Catania, follows a specific matrix that provides the guidelines, requirements, tools, specification, and test procedures needed to authorize a new package, recommended by "The Coca-Cola Company" (Packaging Authorization for Non-Refillable Plastic Bottles).

The shelf-life of soft drinks is determined by the CO_2 level, which decreases due to permeation through the packaging material. Therefore, the choice of bottles with higher CO_2 barrier properties could extend the shelf-life of such products.

Standard shelf-life of "The Coca-Cola Company" products is fixed, as a function of the bottle size, to:

6 months: for PET bottles >1 litre

5 months: for PET bottles <1 litre

12 months: for glass bottlesAccording to the numerous standards fixed by "The Coca-Cola Company", Sibeg s.r.l. decided to test a new PET preform for 1.5 litre bottles, a little bit heavier (0,5 g more), but cheaper than the usual preform which was suggested by a possible new supplier.

Shelf-life tests were performed using two different resins type:•Laser+® (ADVANSA)•Alphapet – sd

Fig. 1. Zahm and Nagel piercing device for CO_2 retention test.

MATERIALS METHODS

*Carbonation Retention Test*The test is performed to assess the carbonation loss rate of a plastic beverage bottle and to extrapolate shelf life to a 21.4% loss when bottles are initially carbonated to 4.2 volumes, and to a 17.5% loss for bottle initially carbonated to 4.0 volumes (1 volume= 1.98 g/L)

Sealed not-refillable PET bottles must retain the carbonation in the beverage when initially carbonated to a level of 4.2 ±0.1 volumes. That level must not fall below 3.3

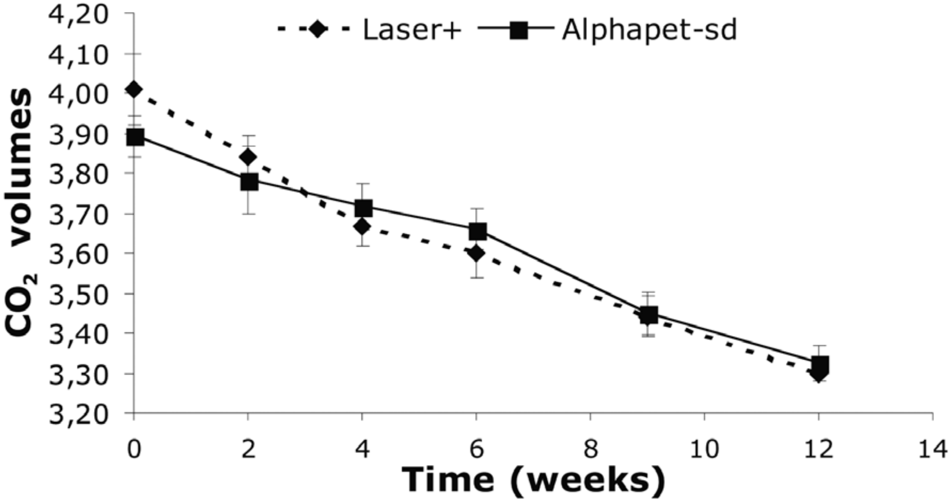

Fig. 2. Variation of CO_2 internal pressure in bottles obtained from different preforms.

volumes within established storage periods under standard laboratory testing conditions of 22° ± 1°C at 50% relative humidity. Storage periods are referred to as the "standard shelf-life" of the bottles and depend on bottle size. For bottles smaller than 1 liter, storage time is 12 weeks, for 1 liter bottles and larger it is set to 14 weeks.

Twelve bottles were randomly chosen 24 hours after the seal, and the CO_2 content was evaluated on each bottle by means of a calibrated Zahm and Nagel piercing device with pressure gauge (Figure 1), collocated on the cap of the bottles. Before the measurement of the CO_2 concentration, the air in the headspace was eliminated, then the cap was pierced by means of the device; the valve was opened in order to discharge the pressure, and the device was calibrated to zero. Bottles were then agitated and let rest until stabilization of the manometer. The test was repeated after 2, 4, 6, 9 and 12 weeks.

RESULTS AND DISCUSSION

The mean CO_2 loss rate (Figure 2) amounted to 0.075 g/L per week for Laser+ and to 0.073 g/L per week for Alphapet-sd, however the loss was much higher in the first 24 hours, and corresponded to 4.5 and 7.4% respectively. The CO_2 overall loss amounted to 21.5 and 21% for Laser+ and Alphapet-sd, respectively, therefore differences were not significative.

In conclusion, results showed not significant differences between the two tested materials. Despite the lower cost of the new suggested preform, Sibeg s.r.l. decided to keep on using the old material, due to comparable CO_2 retention performances, and especially to better mechanical behaviour and constant yield quality.

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OFFICIAL CONTROLS OF THE MIGRATION LIMITS IN PRODUCT AND MATERIALS DESTINED TO ENTER IN CONTACT WITH FOOD AND BEVERAGE PERFORMED IN USMAF OFFICE

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ABSTRACT

In the legislative decree n°123 3.03.1993 (application of European directive 89/397/CEE) is considered “official control of food products activities, such as inspection, sampling, analysis of the samples, etc. performed by the authority on food products, but also on additives, vitamins, and all those materials and objects made to come in contact with the products already described”.

The control activity has to assure the conformity of the different products to guarantee the prevention of any kind of risk for public healthy and to protect consumers interests.

This activity and vigilance are performed by marine, air and custom health offices (USMAF), satellites office of Health Minister. Vigilance of USMAF offices is aimed at vegetarian foods, but also at products destined to enter in contact with food and beverage.

In the last years, an higher number of arrivals of home and kitchen utensils was registered in the Italian harbors, from extra European countries, so that it was useful to intensify controls, and it was decided to organize the Rapid Alert system.

RASFF: Rapid Alert System for Food and Feed is a communitarian rapid alert system to notify in real time direct and indirect risks for public health, the RASFF system consents authorities to know immediately if, for a particular product, there was some alert from other offices; in this case goods are sampled and analyzed.

As described in the Reg. 178/2002 art.11, food imported in the community states to enter the European markets should respect all the communitarian laws, according to this rules, industrial managers should ensure effective procedures to deal with any food safety hazard and to enable the complete, rapid recall of any implicated lot of the finished food from the market.

The way to proceed in the sampling follows specific rules according to the substances that we are looking for, in particular heavy metals, according to ministerial decree 5.3.2003 “methods to sample and to analyze maximal limits of lead, cadmium, mercury, 3-MCPD in food products for official controls”.

As results from the RASF system data, many utensils destined to come in contact with food and beverage coming from extra-communitarian country, especially from People Republic of China, didn’t respect Italian law, according to maximal residual limits of the most common heavy metals.

Key words: heavy metals, Italian law, maximal residual limits, official control

INTRODUCTION

The first kind of sanitary controls, on the products that have to be nationalized in the Italian territory, is on the documents.

Goods travel always with an original certificate and another kind of certificate with analytical results made in the original country. These analytical certificate, when a product is considered in the “alert state” is required obligatory.

Together with the documental control, the RASFF results are required to guarantee that food is safe and suitable for consumption according with the Reg. 178/2002 art.50.

The RASFF system consents to authorities to know immediately if for a particular product there is some alert from other offices; in this case goods are sampled and analyzed.

The following step is the inspective control, carried out on the 100% of the arriving goods, with custom officers providing to the seal’s removal.

At this moment of the procedure USMAF authorities looked at:

- verify the identity of goods with the documents;
- verify the effective hygiene condition of packaging and goods;
- correctly labelling following the L.D. n°181 23.06.2003 (that specifies more details and gives some modification to the old L.D. n°109 27.01.1992).

If the imported products haven’t had recent notification on the RASFF system, and after the preliminary controls, the necessary hygiene conditions have been found satisfactory, the USMAF official can release the “*nulla osta sanitario*”.

The sampling procedure is needed each time in case of:

1. “first arrival”, goods on the custom for the first time from an extra-communitarian country and/or from a new producer;
2. alerted goods or products pushed back in other UE country;
3. goods in awful hygiene conditions;
4. in all the other cases in which “….the sampling is made by sounding by USMA for a 5% of the goods that are expected in the same week” as explained in art.3 comma 6 Republic President Decree (R.P.D.) n°132 14.07.1995.

LEGISLATION

The Italian legislation has always focused its attention on food and products destined to enter in contact with food and beverage.

The first law that speaks about the problem between foods and the materials of the different kind of containers, was the law 30.04.1962, n°283 “Hygienic discipline on the production and sale of foods and beverages”.

The n°283/62 had a lot of integrations and updating with the R.P.D. 23.08.1982, n°777, following the E.D. CE n°76/893, and the L.D. 25.01.1992, n°108 following the 89/109/CEE on object and materials destined to enter in contact with foodstuff.

The M.D. 21.03.1973 “Hygienic discipline of packing, vessels and utensils destined to come in contact with foodstuff” defines, after general dispositions and definitions, the object’s materials destined to enter in contact with foodstuff, and also new indications on migration and cession problems. This M.D. is the most complete among the previous ones, considering some important rules for the company producing these objects and materials, such as the necessity to control the respect on laws and to be able to show in every moment all the controls activity made on goods.

Each lot must be follow by a producer declaration, in which the producer declares that all the objects follow and respect the legislatives rules.

In the same M.D. are specified the dispositions regarding all the materials (plastic, rubbers, paper, cardboard, glass and metal) and the test substances for each category, but also allowed colorants and analytical results to be suitable for use.

This M.D. had numerous changes with the following ministerial decrees 3.08.1974, 19.11.1974, 27.03.1975, 13.09.1975, 18.06.1979, 2.12.1980, 25.06.1981. The last of previous modifies the plastic materials section regarding the permitted additives.

The R.P.D. 26.03.1980, n°327 concerning the principles of food hygiene applicable from the primary production to the food and beverage sale activity, explaining in the article 2 the object of the control activity: kitchen utensils; boxes to keep food safe; external packaging that normally doesn’t come in contact with food, but in relation with the use condition, they can give away some components to the internal food; boxes, utensils and equipments that come in contact with food during the production and trade steps. So there is a big attention to the surfaces, materials and machines used during the food production process.

In the article 68 the attention is focused more specifically on hygienic conditions of the industry that produces object destined to come in contact with foodstuff. The manager must control that their products follow the law rule, but also he must be able to show in every moment the results of his controls and analysis, when they are requested.

The utilization of every kind of equipment in food industry must be controlled and be safe for food products.

The R.P.D. 327/80 advises in *part A* also the condition to proceed for sampling, respecting the sample quantities, divides up into five parts necessary to make chemical analysis, global migration (time, temperature) or specific migration (metals, plastic materials), but also the kind of samples, if they are objects that can be used only one time or longer, and if the samples are film, laminates, plate, and so on.

The articles 2-bis, 3 and 4 of the R.P.D. 777/1982 were modified by the L.D. 108/1992.

2-bis. 1. It is forbidden the production, detained to sale, to put on sale, or using materials and objects that, when finished, are destined to come in contact with food or water for human consumption, with the following characteristics:

a) lead or alloy with more than 10% of lead;
b) internal tin covered with tin having more than 1% of lead;
c) internal covered with glaze layers, varnish or enamel that, in contact for 24 hour in 1% acid acetic solution, give away lead at 25°C;
d) prepared with materials having in their composition arsenic more than 0,03 g/100 g of material.

In the article 3 are indicated for materials and object destined to come in contact with foodstuff, alone or mixed each other, the allowed components in their production, and when necessary also the required purity limits and migrations tests that must be made on materials and object to be considered suitable to use.

In the article 4 materials and objects of new production, but destined to come in contact with food, must be reported when they are selling the following indications:

– the word «for aliment / for food use» or «can come in contact with food» or something that explains the use, for example : “bottle for wine” or the symbols in enclosure 2.

These warnings must be written in Italian language and they can be written also in other languages.

A letter from Health Minister in date 27.03.2001 on the subject of materials and object destined to come in contact with food recapitulates the most important point of the previous indication, in the following way:

° all materials and objects destined to come in contact with foodstuff must respect the R.P.D. 777/82;
° prohibition on the production, detain to sale, sale or put on sale materials and object, that when finished, are destined to come in contact with food or water for human consumption, and, for composition or for the possibility that they give away some components:
  a) make foodstuff harmful or dangerous for public health;
  b) can modify unfavorable the organoleptic property of food.
° it is applied also on materials and objects that can come in contact with food ingredients or water during the process steps of the food production.

In the M.D. 18.02.1984 there is the regulation of tin band container solder with tin-lead alloy and other materials, in which are the rules for the acceptable substances (enclosure I) to prepare objects in tin band destined to come in contact with foodstuff; in enclosure II we found the maximal lead limits (in mg/kg) permitted in food, present in that containers. In enclosure IIIA, IIIB and IIIC there are the procedures for sampling, how to determine global organic migration and heavy metals as lead, iron and tin.

The M.D. 16.10.1988, n°243 is the regulation of varnish chromium-plated band objects destined to come in contact with food, this M.D. suggest the way to prepare, sampling methods and the analysis to do on chromium-plated band, and also the total chrome limits (for the following 5 years, so until 1993).

With the M.D. 13.07.1995, n°405 we had an updating to M.D. 18.02.1984, concerning the tin minimum purity grade fixed on 99,85%, are also given the methods to prove the aptitude of tin band layer to be used for containers destined to come in contact with food, and to verify the presence of rust, tin scratches, part without tin and so on.

In following M.D. 1.12.2000 n°411, 30.05.2001 n°267, 28.03.2003 n°123 are reported the updating about specific migration limits, new kind of copolymers, limits on permitted whitening, new indications for migration tests with different test liquid or substances, the maximum of «residual substance» allowed in the material, the maximum of «residual substance» allowed in the material or in the finished object expressed in mg for 6 dm^2 of the surface in contact with food, specific migration limits in the product or in fictitious foodstuff, if not differently specified.

VIGILANCE

The control activity has to assure the conformity at Italian legislation of the different products to guarantee the prevention of any kind of risk for public health and to protect consumers' interests.

Control activity has to be made in three parts throughout the food chain:

- in the food industry
- in all kind of food establishments along the food chain
- in sale step to the final consumer.

According to materials and object destined to come in contact with foodstuff, made from recycled materials, it doesn't exist generic disposition, but only specific rules for plastic materials, for paper and cartons.

Plastic materials

The article 13 of M.D. 21.3.73 forbids the use of plastic materials obtained from rejected materials or from used plastic materials objects, according to the directive 94/62/EC.

- it can be used if they don't come in direct contact with food, but in the middle there is another material as barrier.
- along the food chain it is important to have all the documentation and the correct information.

Carton materials and objects obtained from recycling

- only for object destined to come in contact with foodstuff without the compulsory migration tests;
- purity grade according M.D. 18.06.1979 for
  - whitening
  - PCB
  - lead.

MATERIALS AND METHODS

All the analyses follow the DM 21.3.1973 method and its numerous updates. According to DM 28.3.2003 n°123, detectable limits must be determined by an affirmed analytical method, or by an analytical method with suitable characteristics.

CONCLUSION

According to RASF system data, many foodstuff utensils from extra-EU countries, especially from the People's Republic of China, don't respect Italian law, according to the maximum residual limits of the most common heavy metals. 88%

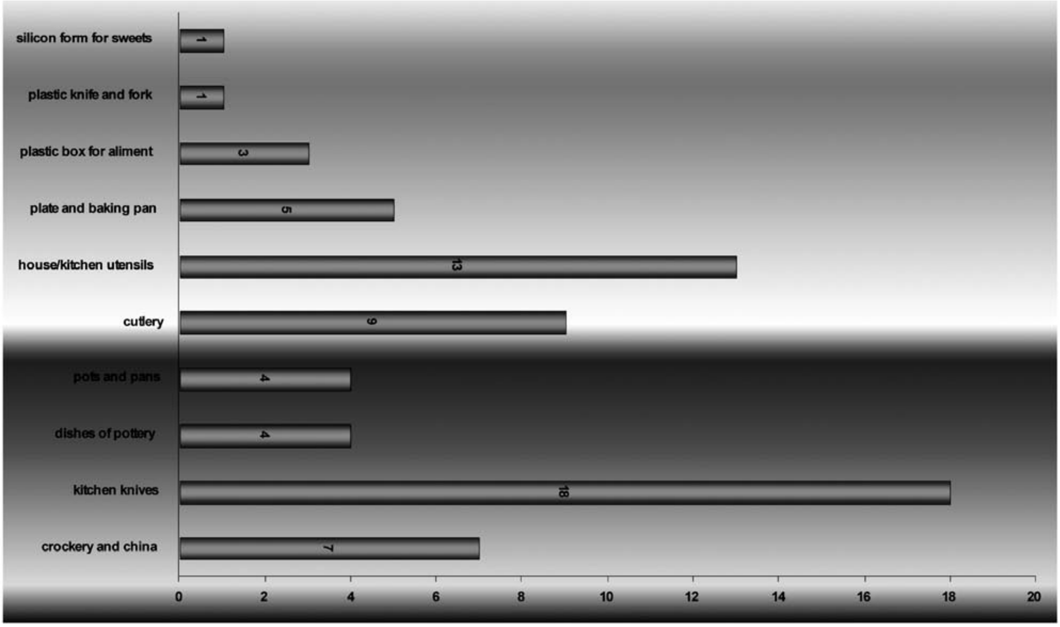

Fig. 1. Principal products blocked for European Trade – Italian RASF System 2004-2005.

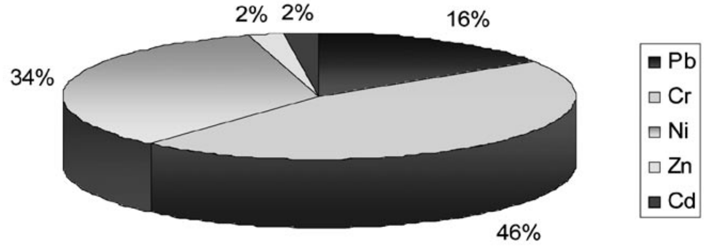

Fig. 2. Rejection for chemical risk by heavy metal according with DM 21/3/1973.

of data shown in figure1 are objects prohibited by China. Pie charts 2 shows the percentage of material and object refusals which fall short of the required purity limits and negative migration tests for every single heavy metal.

89% of cases in pie chart 2 are Chinese products, only 3% of cases come from India, the remainder being from others.

In this period of “globalization”, it is important to reinforce controls on the importation of all food products and all food utensils, to establish an higher product quality, guarantee human consumption safety, and preserve pan-European production quality.

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FLAVOUR AND SHELF-LIFE OF FRESH SPREADABLE CHEESES

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SUMMARY

Aim of the research is to study the volatiles in a fresh spreadable cheese in order to evaluate the shelf-life period in which "freshness" is maintained. The research has been applied to samples of a goat fresh cheese, produced in laboratory using selected cultures; the samples were analysed at the production time and during the refrigeration at + 4°C. The volatile compounds have been extracted using HS-SPME and analysed by GC/MS; the data obtained was correlated with the microbiological and physicochemical characteristics of the product. The used technique allowed the quantification of fifty-seven volatile components, exactly: fatty acids and their ethyl, butyl and hexyl esters, ketones, aldehydes, alcohols, diacetyl, acetoin, hydrocarbons, monoterpenes, aromatic compounds, sulphurated compounds and lactones; spectral data, injection of standards and linear retention indices were used to confirm the identification. ANOVA and PCA concurred to estimate the significant differences of the composition during the storage of the product, to detect the decrease of cheese freshness and to define the critical days during shelf-life.

Key words: fresh spreadable cheeses, flavour, shelf-life, HS-SPME/GC-MS, ANOVA, principal component analysis

INTRODUCTION

Soft cheese varieties which are consumed without a ripening period are commonly defined fresh cheeses. They are slow drainage cheeses which usually are subjected to lactic acid fermentation; they are characterized by a moisture content which usually is > 80%; the three major types of fresh cheeses are cottage, quark and petit suisse; among these, spreadable cheeses are particularly appreciated by consumers for their sensory quality, vs. texture, appearance and flavour (ROBERTSON, 2000). Shelf-life of fresh spreadable cheeses is usually of 1-4 weeks, at refrigeration temperature, depending on the cheese manufacturing and packaging. Deterioration of fresh cheese is primarily caused by microbial growth of psychotropic Gram-negative bacteria of species such as pseudomonas, *Alcaligenes, Proteus, Aerobacter* or *Aeromonads*, which can result in undeliverable off-flavours, odour and slimy curd. Growth of yeasts and moulds (e.g. *Geotrichum, Penicillum, Mucor* and *Alternaria*) may also cause flavour and texture changes, and visual spoilage (GHITTI and OTTOGALLI, 1986).

Flavour is one of the most important quality criteria for cheeses; the typical flavour associated with each type of cheese is due to complex biochemical and chemical processes during processing, ripening and storage; these processes lead to a wide range of volatile compounds which contribute to cheese flavour. Study of flavour is useful to define cheese quality, to characterise the cheese, to detect off-flavour but it could be also important to determine the shelf-life of the product (ASHURST, 1999).

Several studies have been carried out to evaluate fresh cheese shelf-life by using chemical indices and/or innovative techniques (WELLS, 1998; LOBATO-CABALLEROS and AGUIRRE-MANDUJANO, 2000; RIVA and GIANGIACOMO, 2001; TOPPINO *et al.*, 2002; TOPPINO and RIVA, 2003; BENEDETTI *et al.*, 2005); some of these studies regard Crescenza cheese, which covers more than 40% of the Italian fresh market (CATTANEO *et al.*, 2005).

In the current study, volatiles in a fresh spreadable goat cheese were studied by SPME/GC-MS in order to evaluate the shelf-life period in which "freshness" is maintained; goat milk has been used Goat cheeses, since ancient times used for the manufacture of different types of cheeses throughout the world (LOEWENSTEIN *et al.*, 1980; MALLATOU *et al.*, 1994), easily undergo degradation and thus can be used as model of quality modifications. The volatile composition of our fresh cheeses was studied during the refrigeration at 4 °C from the manufacturing to 28 days which would represent the end of shelf-life (FOSCHINO *et al.*, 1999; GALLI *et al.*, 1976); volatile composition was correlated with the microbiological and physicochemical characteristics of the product. ANOVA and Principal component analysis (PCA) were able to detect the decrease of cheese freshness and to define the critical days during shelf-life.

MATERIALS AND METHODS

Cheese Making

Cheese milk was obtained from a flock of "Derivata di Siria" and "Maltese" goat breed. Cheese making trials were carried out on January 2005 at a small dairy of West Sicily; vat pasteurization of milk (72°C, 15-20 s) had the aim to standardize microbial quality. After cooling of milk at 22-25°C, cheese making followed the indications reported by Salvadori del Prato (1998) and referred to fresh "caprino" cheese with slow renneting. The vat was inoculated with a 2% freeze-dried culture of *Lc. lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* strains [R-704 (Chr. Hansen, Corsico, Milan, Italy)] that was suspended in liquid milk; the amount of liquid rennet was 0.5 ml in 10 l of milk. Three different cheese making trials were carried out. Cheese samples, cylindrical shaped, small sized, with a chalk white colour, were individually wrapped in parchment paper and then placed inside some little metal containers. Samples were received in our laboratory under refrigerated conditions (+ 4°C) and analysed immediately upon receipt and after 7, 15, 21 and 28 days of refrigeration at + 4°C. For each cheese making trials, three samples were analysed at each different refrigeration time.

Microbiological Analyses

Representative 10 g cheese samples were homogenized with 90 ml of sterile peptone water in a Laboratory Blender [Stomacher 400 (International pbi, Milan, Italy)] for 2 min. Decimal dilutions were prepared in 9 ml of sterile peptone water and plated in duplicate. Aerobic plate count (APC) and psychotropic bacterial count (PBC) were performed on plate count agar (Oxoid); coliforms and *Escherichia coli* were determined on chromocult coliform agar (Merck). Coagulase positive staphylococci and enterococci were determined on Baird Parker medium, with RPF supplement (Oxoid), and on Slanetz & Bartley agar (Oxoid), respectively. Yeasts and moulds counts were performed on dicholan-rose bengal-chloramphenicol agar (Merck); lactic acid bacteria (LAB) were grown anaerobically (Gas-Pack anaerobic system, Oxoid) on MRS agar and aerobically on M17 agar, respectively.

Physicochemical Analyses

Total nitrogen was determined by the Kjeldahl method and total fat by the Sohxlet extraction, after acid hydrolysis (HCl 4M)(APHA, 1993). The pH of cheese was measured with a Hanna pH meter (Hanna Instr., Padua, Italy); activity water (aw) was determined by an A.O.A.C. approved method (AquaLab, Decagon Devices, Inc., Washington); the moisture and the dry matter was performed according to the Standard Methods for the examination of dairy products (APHA, 1993), as for cottage cheese.

ANALYSIS OF VOLATILE COMPOUNDS

SPME procedures

A 40 ml vial was filled with 6 g of each sample and 12 ml of water. The vial was equipped with a “mininert” valve (Supelco, Bellefonte, PA, USA) that allowed the introduction of the fiber without piercing any septum. The septumless “mininert” vial permitted to avoid extraneous peaks due to possible septum bleeding. Extraction was performed in the headspace vial, keeping it at 40°C, using a commercially available fiber housed in its manual holder (Supelco, Bellefonte, PA, USA). All extractions were carried out using a DVB/CAR/PDMS fiber, 50/30 µm film thickness (Supelco, Bellefonte, PA, USA). The liquid sample was equilibrated for 20 min, and then extracted for 30 min; during the extraction the sample was stirred continuously. After sampling the SPME fiber was introduced onto the splitless injector of the HRGC/MS using the conditions reported below. The fiber was kept in the injector for 3 min for thermal desorption of the analytes onto the capillary GC column. The split-splitless injector port was maintained at 260°C. No artefacts were observed after a SPME analysis of water performed as blank analysis.

HRGC/MS Analysis

A Varian 3800 gas chromatograph, directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy), was used to analyze the volatile cheese components. Injector temperature was 260°C; injection mode, splitless; capillary column, CP-Wax 52 CB, 60 m, 0.25 mm i.d., 0.25 µm film thickness (Chrompack Italy, s.r.l. Milan, Italy); oven temperature, 45 °C held for 5 min, then increased to 80 °C at a rate of 10 °C/min, and to 240 °C at 2 °C/min; carrier gas, helium at a constant pressure of 10 psi; transfer line temperature, 250 °C, acquisition range, 40-200 m/z; scan rate, 1 µ $sec.^{-1}$ Each component was identified using mass spectral data, (NIST library (NIST 92, USA), linear retention indices, literature data and the injection of standard where available. The linear retention indices (LRI) were calculated according to Van den Dool and Kratz. The peak area of each component in total ion current chromatograms was determined during three replicates and the average value was calculated.

The repeatability of the developed method was determined by analysing three different samples of the same cheese under identical experimental conditions; the absolute peak area obtained for each component identified during the three different analyses was tabulated and the coefficient of variation (CV%) was calculated; this was <10% for all the components identified as previously reported (Verzera *et al.*, 2005).

Statistical Analysis

Statgraphic plus software, 5.1 version was used to perform statistical analysis of the data. One way analysis of variance (ANOVA), Duncan's multiple range test and principal components were applied to the data to determine the presence of significant differences between the analyzed samples during the storage; the model was statistically significant with a P-value less than 0.05.

RESULTS AND DISCUSSION

In Table 1 the trend of microbial counts during storage, and in Table 2 the mean values and standard deviation (± s.d) of physicochemical parameters are reported for all the analysed samples. Microbiological characteristics of cheese samples need some remarks. *Escherichia coli* were never detected. Regarding APC and PBC, it would be stressed that a significant fraction of microbial count was constituted by lactic acid bacteria. High counts on MRS agar could have had an environmental source. Coliforms and enterococci counts could be considered post pasteurisation contaminants; salting could be the source of contamination, as indicated for traditional Anevato cheese by pasteurized milk (a spread type traditional Greek cheese made from raw ewe's or goat's milk, or mixture of both) (Xanthopoulos et al., 2000). Low storage temperature (2-3°C), low pH and lactic competitive flora were inhibitory for staphylococci and enterococci, whose number decreased during storage. It wasn't the same for coliforms; this behaviour was considered unusual since coliforms are quite sensitive to acidity conditions (at pH values lower than 4.5). Nevertheless, a false positive result could be stressed, because the acidity of cheese samples would have been sufficient to change the colour of the pH indicator in the agar immediately surrounding the food particles (APHA, 2001).

High moisture percentage and high value of activity water justified the PBC steadiness. Yeasts increase from 15 to 28 days was considered almost obvious for this kind of product. Lowering of pH values from 4.34 to 3.99 was registered up to 10 days; a light increase, from 4.24 to 4.69, until the end of the storage period, was observed. This trend could be explained by fermentation activity from the high

Table 1. Log microbial count ($\log_{10}$ cfu g^{-1}) of the fresh goat cheese samples.

Microbial group	Days of refrigeration at + 4°C				
	0	**7**	**15**	**21**	**28**
APC	12.47	16.79	8.81	13.00	11.84
PBC	11.47	16.90	7.77	11.07	11.84
Coliforms	2.95	3.23	3.20	2.87	3.47
Coag.+ stafilococci	<1	<1	0	0	0
Enterococci	6.47	3.81	4.62	3.97	3.76
Yeasts	0	0	4.44	4.69	4.72
Molds	-	4.11	<1	3.17	<1
LAB (M17 Agar)	>22.47	20.00	12.00	10.47	8.76
LAB (MRS Agar)	>22.47	22.55	15.00	13.64	9.20

levels of lactic acid bacteria, whose counts dropped from 15 to 28 days.

Table 3 reports the average composition by peak area as single components at the production time and during the refrigeration at + 4°C for all the sample analysed; all component identified are listed according to their retention indices calculated on CP-Wax 52 CB column.

Fifty-seven volatile components were identified in each sample analysed; exactly: fatty acids from C_2 to C_{14} and their ethyl, butyl and hexyl esters, ketones from C_2 a C_{15}, diacetyl (2,3-butandione), acetoin and acetophenone; aldehydes from C_5 to C_{10}, alcohols from C_5 to C_8, hydrocarbons, monoterpenes, aromatic compounds, sulphurated compounds and lactones. The compounds identified are well-known in flavour dairy products and the principal pathways for their formation were widely discussed (McSweeney and Sousa, 2000).

The data reported in the Table 3 gave information on the volatile goat cheese composition at the production time and on the behaviour of each component during the refrigeration. From Table 3, main components after manufacturing were free fatty acids, above all hexanoic, octanoic (cheesy, rancid, sweet-like), and decanoic acid (cheesy, rancid), as previously observed in goat cheeses by Woo *et al.* (1984); these components were essential to the intense aroma of goat cheese (Engel *et al.*, 2002). In all the samples analysed, the acids with an even number of carbon atoms were preponderant compared to those with an odd number; in fact, only nonanoic acid, as traces, was detected in the volatile fraction and it is related with a characteristic goat odour (Le Quéré *et al.*, 1996). The other classes of substances were less represented if compared with that of free fatty acids; moreover, diacetyl, acetophenone, limonene, benzoic acid and benzaldehyde were between the main components at the production time. The amount of the single components and, obviously the ratios between the single components seems to be different during the refrigeration period; particularly, evident changes occurred after 15 days. By the Analysis of variance (ANOVA), since the P-value is less than 0,05, there are statistically significant differences between the mean of all the components quantified from 0 to 28 days (95% confidence level); moreover, by the multiple range test were determined for each component the statistically significant differences between the

Table 2. Mean values (±s.d.) of physicochemical characteristics of the fresh goat cheese samples.

Parameters	**Total nitrogen (% w/w)**	**Fat (% w/w)**	**pH**	**Aw**	**Moisture (% w/w)**	**Solids (% w/w)**
Mean values	12.48	18.68	4.36	0.99	66.85	33.15
± s.d.	0.72	0.15	0.22	0.008	5.54	5.48

Table 3. Volatiles[1] in samples of fresh goat cheese during refrigeration at 4°C.

				Days of refrigeration		
Fatty acids	**LRI[2]**	**0**	**7**	**15**	**21**	**28**
acetic acid	1451	141a	161a	154a	189b	195b
butanoic acid	1625	82a	100a	80a	168b	156b
hexanoic acid	1840	441a	451a	449a	585b	588b
octanoic acid	2053	851a	754a	784a	1423b	1556b
nonanoic acid	2162	tr	tr	tr	tr	tr
decanoic acid	2264	609a	619a	645a	1215b	1521b
decenoic acid	2308	40b	48a	38a	61b	58b
dodecanoic acid	2475	52a	67a	45a	179b	243b
tetradecanoic acid	2675	403a	331a	518a	1428b	1309b
benzoic acid	2435	225a	304a	318a	836b	1441c
All		2844	2835	3021	6084	7056
Esters						
ethyl acetate	900	tr	-	-	-	-
ethyl butanoate	1035	39c	41c	29b	-a	-a
butyl acetate	1072	tr	-	-	-	-
butyl butanoate	1218	tr	tr	-	-	-
ethyl hexanoate	1232	98b	tr a	-a	-a	-a
hexyl acetate	1271	tr	tr	tr	-	-
butyl hexanoate	1412	tr	tr	tr	-	-
ethyl decanoate	1635	tr	tr	tr	-	-
All		137	41	29	-	-
Chetones						
acetone	812	tr	tr	tr	tr	tr
5-methyl-2-hexanone	825	tr	tr	tr	-	-
2,3 butanedione	975	189a	221ab	231ab	406c	416c
2-heptanone	1182	25a	66ab	59ab	140c	505d
3-hydroxy-2-butanone	1287	21b	tr a	tr a	-a	-a
6-methyl-5-hepten-2-one	1338	tr	tr	tr	-	-
2-nonanone	1389	47a	64a	44a	105b	456d
2-undecanone	1597	-	-	-	-	tr
acetophenone	1655	126a	119a	170b	183b	194b
δ-decalactone	2196	-	-	-	-	tr
All		408	470	504	834	1571
Aldehydes						
3-methyl-butanal	924	tr	tr	tr	-	-
hexanal	1080	tr	tr	tr	-	-
heptanal	1190	tr	tr	tr	-	-
nonanal	1395	87b	85b	75ab	73ab	69a
2-nonenal	1538	tr	tr	tr	tr	tr
2-decenal	1644	tr	tr	tr	tr	tr
benzaldehyde	1530	135a	137b	168a	303b	1971c
All		222	222	243	376	2040

(continues)

(continues)

		Days of refrigeration				
Alcohols	**LRI[2]**	**0**	**7**	**15**	**21**	**28**
butanol	1152	tr	tr	tr	-	-
hexanol	1344	tr	tr	tr	-	-
2-ethyl-1-hexanol	1483	50b	49b	30a	26a	28a
octanol	1547	24b	20b	21b	tr a	tr a
benzyl-alcohol	1870	tr a	tr a	tr a	150b	2053c
All		74	69	51	176	2081
Hydrocarbons						
methylene chloride	935	49b	tr a	tr a	tr a	tr a
chloroform	1022	115	127	140	135	127
hexadecane	1601	tr	tr	tr	-	-
heptadecane	1701	tr	tr	tr	-	-
octadecane	1801	tr	tr	tr	-	-
toluene	1044	34b	24b	25b	tr a	tr a
p-xylene	1145	46b	48b	38b	tr a	tr a
o-xylene	1147	57b	51b	43b	tr a	tr a
styrene	1261	120	110	149	135	151
All		420	360	395	370	278
Terpenes						
α-pinene	1116	45b	tr a	tr a	- a	- a
β-pinene	1158	75 b	tr a	tr a	- a	- a
limonene	1204	203c	46b	47b	- a	- a
1,8-cineole	1214	tr	tr	-	-	
γ-terpinene	1247	83 b	tr a	tr a	-a	-a
terpinolene	1291	36b	tr a	tr a	-a	-a
All		442	46	47	0	0
Others						
dimethyldisulfide	1077	-	-	tr	tr	tr
methyl-isothiocyanate	1243	185	165	174	158	176

[1] arbitrary units (peak area) were used to evaluate quantities of each compound. Each value is the average of three sample. Each sample in triplicate.

[2] LRI indicates the Linear Retention Indices calculated CP-Wax 52 CB.

[3] a, b, c, multiple range test: different letters in the same row indicate significant between *X* values at $P < 0.05$; Letters are not reported where significant differences did not result ($P > 0.05$).

refrigeration times using homogenous groups. Between 0 and 7, and 0 and 15 days no statistically significant difference resulted for almost all the components identified, except for limonene and other minor components such as ethyl butanoate, ethyl hexanoate, α-pinene, β-pinene, γ-terpinene and terpinolene that dramatically decreased; between 0 and 21 and 0 and 28 days statistically significant differences resulted for most of the components.

The amount of each aliphatic free fatty acid significatively increased after 15 days of refrigeration at + 4°C; this increase was particularly evident for octanoic, decanoic, dodecanoic, tetradecanoic acids and it contributed to intensify the cheesy and rancid flavour. Aliphatic free fatty acids mainly arise from the enzymatic hydrolysis of triglycerides and considering the microbiological data obtained metabolic activity of yeasts and of psychotropic bacteria could have given a certain contribution to the increasing their levels; their increase was not statistically significative between 21 and 28 days. Diacetyl (buttery, pungent, powerful) also showed a similar behaviour to that of free fatty acids; in fact, its increase was statistically significative between 15 and 21 days but not between 21 and 28 days. Diacetyl is one of the principal flavour compounds produced on metabolism of citrate and it is important in a number of cheese varieties especially fresh cheeses such as Cottage and Quark (McSweeney and Sousa, 2000).

Benzaldehyde, benzoic acid and benzyl alcohol (bitter almond, burnt, sugar) significantly increased between 21 and 28 days; these compounds arise from the aromatic aminoacid metabolism. Benzaldehyde may be produced from the α-oxidation of phenyl acetaldehyde or from β-oxidation of cinnamic acid (Casey J. and Dobb R., 1998); it does not accumulate to high concentrations because it is rapidly transformed to alcohol and to the corresponding acid (Dunn H.C. and Lindsay R.C., 1984). The increase of these aromatic substances was related to lactic culture activity; in fact *Lactococcus lactis* subsp. *lactis*, as many other lactococci, has an important peptidase activity: free amino group content increases in cheese cultured with lactococci (Xanthopoulos et al, 2000). When pH is low aminoacids are decarbossilated to amines, when there is an increase in pH, these amines are subsequently oxidized to aldehydes via the Strecker degradation; this behaviour agreed with the light increase in pH observed after 15 days of storage in our samples.

2-Methyl-ketones, showed a significative increase between 15 and 21 and 21 and 28 days of refrigeration; among these, 2-heptanone and 2-nonanone (creamy, fresh), prevailed. 2-methyl ketones with odd carbon-number from C_3 to C_{15} are important compounds in dairy products giving them sweet creamy fermented notes; they occur from free fatty acid oxidation to β-ketoacids and decarboxylation to alkan-2-ones with one less C-atom (McSweeney and Sousa, 2000).

Aliphatic alcohols, aldehydes, ketones and esters, present in the volatile fraction at the production time, together with terpenes and most of hydrocarbons significatively decreased during refrigeration; these compounds were probably due to the goat's feeding. Particularly, monoterpenes such as limonene (citrus, light, weak), 1,8-cineole (fresh, eucalyptus), pinenes (light, pine), etc. are well known components of plant essential oils, especially of citrus species which are widely diffused in Sicily (Dugo *et al.*, 2001). Probably these components, which are less stable in aqueous acid medium, are modified during cheese storage.

Principal component analysis (PCA) was thus performed to calculate the total variance contained in the significant constituents for all the samples analysed. Principal component 1 (PC1) and 2 (PC2) represented 92,5% of the total variance; 81,1% of the total variance for PC1 and 11.3% for PC2. A graphic display of loadings and scores for PC 1 and 2 is shown in Figure 1. Table 4 reports the significant component loadings in decreasing order of importance for PC 1 and 2. The figure shows the fresh cheeses at 0, 7 and 15 days grouped because of limonene, octanol and ethyl butanoate; at 21 because of aliphatic free fatty acids and diacetyl and distinguish 28 days because of 2-heptanone, 2-nonanone, benzaldehyde, benzyl alcohol and benzoic acid.

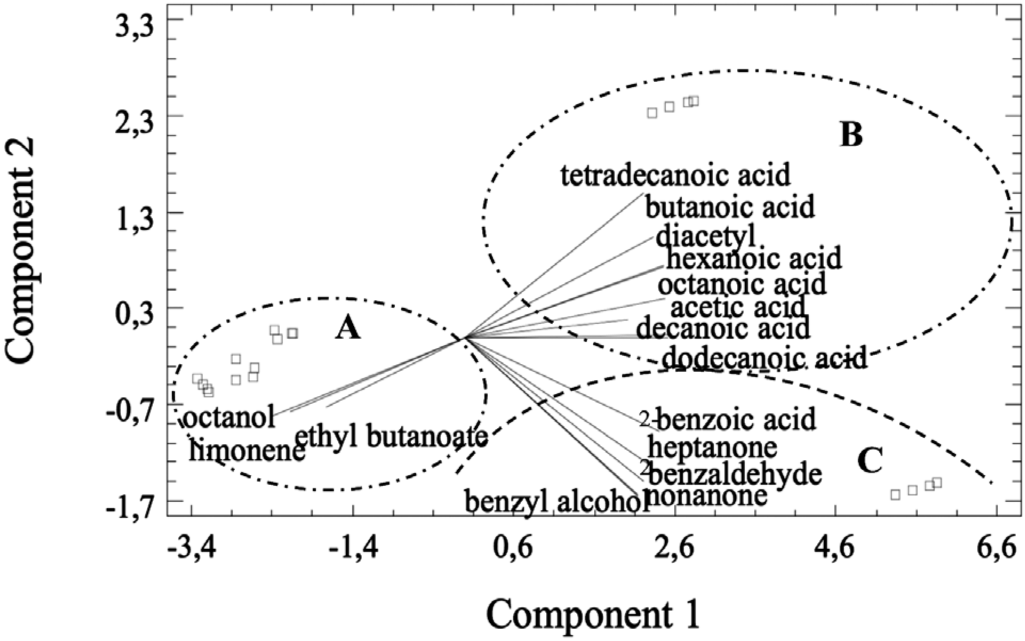

Fig. 1. Principal component analysis (PCA) involving quantities of the significant components of the fresh goat cheeses during the refrigeration. A- 0,7,15 days; B- 21 days; C-28 days.

In conclusion, since freshness of a spreadable cheese is generally associated with low acidity, limited proteolysis and no bitter taste, a shelf life of 15 days could be an acceptable period for this type of cheese. In fact, the quantitative changes that occurred during 15 days of storage proved to be minor for the product's flavour. Anyway the cheese after manufacturing is characterised by a major content of monoterpenes limonene (citrus, light, weak), ethyl hexanoate (apple), octanol (orange, sweet) that gave particular characteristic of fresh, fruity and sweet to the cheese. The amount of these substances in the volatile fraction could give significant information on the freshness of the cheese.

Table 4. Principal components loadings autoscaled data.

Compounds	Loading 1	Loading 2
decanoic acid	0,276866	0,005609
dodecanoic acid	0,275035	-0,001107
octanoic acid	0,270419	0,097553
diacetyl	0,268884	0,177679
hexanoic acid	0,267412	0,182961
benzoic acid	0,262653	-0,237923
ethyl butanoate	-0,262414	-0,200873
butanoic acid	0,255505	0,255538
2-heptanone	0,249626	-0,323135
2-nonanone	0,241334	-0,365686
tetradecanoic acid	0,240336	0,365233
octanol	-0,237877	-0,189683
benzyl alcohol	0,233523	-0,399847
benzaldehyde	0,232907	- 0,402266
acetic acid	0,219485	0,042517
limonene	-0,189162	-0,179243

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INVESTIGATION ON THE *PICHIA ANOMALA* KILLER TOXIN AND ITS EFFECTIVENESS AS AN ANTIMICROBIAL IN PAPER PACKAGING MATERIALS

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ABSTRACT

The use of natural antimicrobial substances as food preservatives has been attracting increasing interest in the last years. We have focused our attention on zymocins produced by *Pichia anomala* that display antimicrobial activity against a wide range of animal and human pathogens (*Candida albicans, Pneumocystis carinii*) and food spoilage yeasts (*Penicillium roqueforti, Penicillium verrucosum, Saccharomycodes ludwigii*) as well.

The toxin was obtained by culturing *P. anomala* DBVPG 3003 since it yields a high specific activity semi-purified extract by means of a simple procedure.

Our aim was to verify whether it was possible to integrate this antimicrobial extract into packaging materials without compromising fungicidal activity. We chose sheets of paper that allowed effective implementation by means of a simple absorption step without requiring additional treatments. We wanted to evaluate toxin effectiveness in association to several different food-packaging papers by testing materials that had undergone several different treatments (calendering, bleaching, smoothing, etc.) and which may be used with different kinds of food matrixes (dairy products, bakery products, etc.).

Key words: *Pichia anomala*, antimicrobial activity, paper packaging materials

INTRODUCTION

Natural antimicrobials meet the producers' needs for preservatives allowing for effective food protection without modifying the sensorial properties of food. Such compounds are also characterized by very high selectivity towards target species and therefore display very low toxicity complying with the consumers' demand for "clean and green" products. Proteinaceous toxins produced by yeasts (and named thereafter "zymocins") were first discovered in the '60s in *Saccharomyces cerevisiae*. Since then, more than twenty zymocins have been identified and characterized in several yeast strains such as *Pichia* spp. and *Kluyveromyces* spp. isolated among others from food related habitats [1]. In addition, there is *Pichia anomala* that display antimicrobial activity against a wide range of animal and human pathogens (*Candida albicans, Pneumocystis carinii*) and food spoilage yeasts (*Penicillium roqueforti, Penicillium verrucosum, Saccharomycodes ludwigii*) [2].

Common-use plastic materials can be implemented with natural antimicrobial compounds [3].

These new generation packaging solutions, known as "Functional Biopackaging", are able to release bioactive substances at different controlled rates depending on the type of materials in which they are incorporated. Therefore, the study of the diffusion kinetic phenomenon of these active substances becomes interesting for suitably enhancing the quality and safety of a wide range of foods during extended storage.

MATERIALS AND METHODS

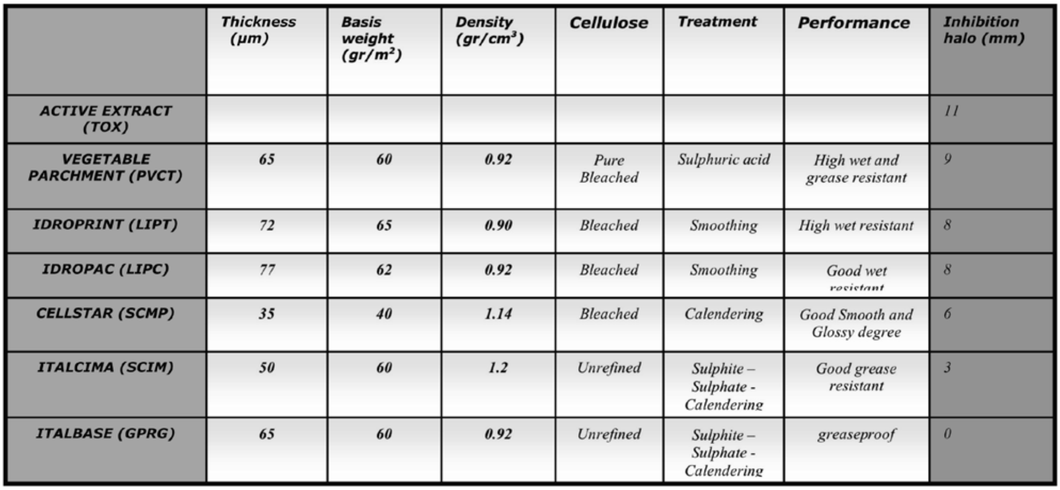
Toxin production

The toxin (PKT) was produced following the method developed by Ciani *et al.* [4]. Toxin production was carried out by culturing *P. anomala* DBVPG 3003 for 48h at 25°C into YPD medium buffered at pH 4.4 with 0.1M citric acid/tri-basic sodium citrate. Yeast cells were then eliminated by centrifugation at 8000 rpm for 30 minutes at 5°C and the supernatant containing the PKT was 25-fold concentrated by using an ultrafiltration cell under N_2 pressure (2 bar) with 10 and 3kDa cut-off membranes. The concentrated supernatants were filter-sterilised through 0.45 μm pore size membrane filter.

Agar well test for antimicrobial activity

Brettanomyces bruxellensis CBS 4481 was cultured into YPD liquid media for

Table 1. Characteristics of commercial paper packaging materials.



	Thickness (μm)	Basis weight (gr/m²)	Density (gr/cm³)	Cellulose	Treatment	Performance	Inhibition halo (mm)
ACTIVE EXTRACT (TOX)							11
VEGETABLE PARCHMENT (PVCT)	65	60	0.92	Pure Bleached	Sulphuric acid	High wet and grease resistant	9
IDROPRINT (LIPT)	72	65	0.90	Bleached	Smoothing	High wet resistant	8
IDROPAC (LIPC)	77	62	0.92	Bleached	Smoothing	Good wet resistant	8
CELLSTAR (SCMP)	35	40	1.14	Bleached	Calendering	Good Smooth and Glossy degree	6
ITALCIMA (SCIM)	50	60	1.2	Unrefined	Sulphite – Sulphate - Calendering	Good grease resistant	3
ITALBASE (GPRG)	65	60	0.92	Unrefined	Sulphite – Sulphate - Calendering	greaseproof	0

24h at 28°C and then inoculated (5%) in agar-malt medium buffered at pH 4.4 at 45°C. Agar-malt was immediately poured into sterile Petri dishes. PKT was then injected into wells cut in the malt agar plates and the killing activity was measured as the diameter of the clear zone of inhibition around the well after incubation for 60h at 25°C with image analysis.

Tests on packaging papers

Tested papers (Cartiera CIMA, Bergamo), with the characteristics shown in Table 1, were cut as 10 mm diameter circles. 100 μl aliquots of PKT were poured onto paper samples and left at room temperature until the solution was completely absorbed into the paper (12 hours at most). Then papers were laid on the surface of Petri dishes and antimicrobial activity was measured as described above. Differences between surface papers were observed using an optical microscope after colouring them with toluidine blue to enhance contrast.

RESULTS AND DISCUSSION

Preliminary trials were performed on the supernatants obtained from *Pichia anomala* cell cultures but no antimicrobial activity was detected; the supernatants were therefore 25-fold concentrated allowing for the production of an active extract that was able to inhibit cell growth both on liquid and solid cultural media inoculated with the test strain (*Brettanomyces bruxellensis* CBS 4481). After testing membranes with 3 and 10 kDa cut-offs to semi-purify the extract, ultrafiltration on a 10kDa pore size membrane allowed for complete retention of the active compounds. The concentrated extract provided repeatable results in the agar-well plate test, giving an inhibition halo diameter of 11±1mm.

Tested paper samples displayed detectable activity against *Brettanomyces bruxellensis* growth as shown (Fig.1).

The pictures (Fig.2) show the inhibition diameter of toxin alone and toxin incorporated in paper sheets on agar plate dishes. Microscope coloured images of

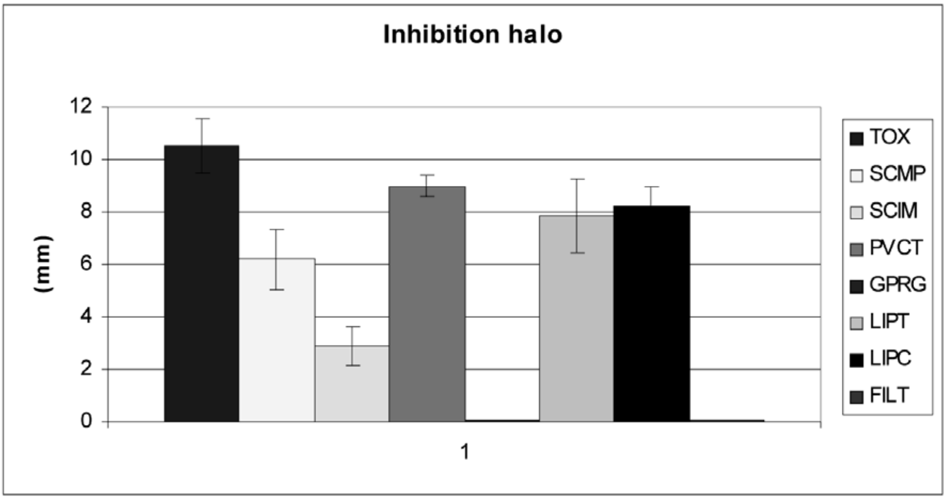


Fig. 1. Diameter of inhibition halo of the toxin against *Brettanomyces bruxellensis*.

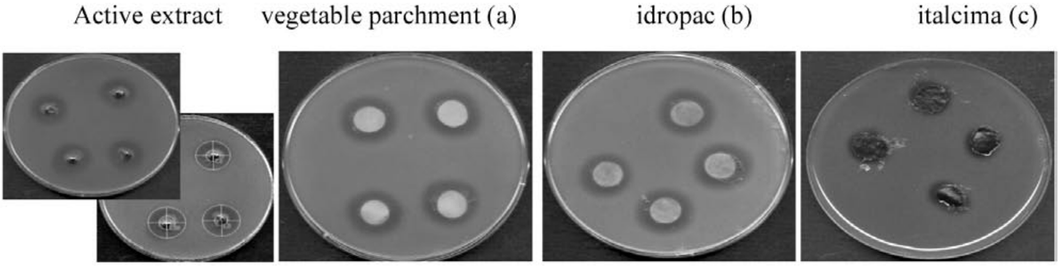
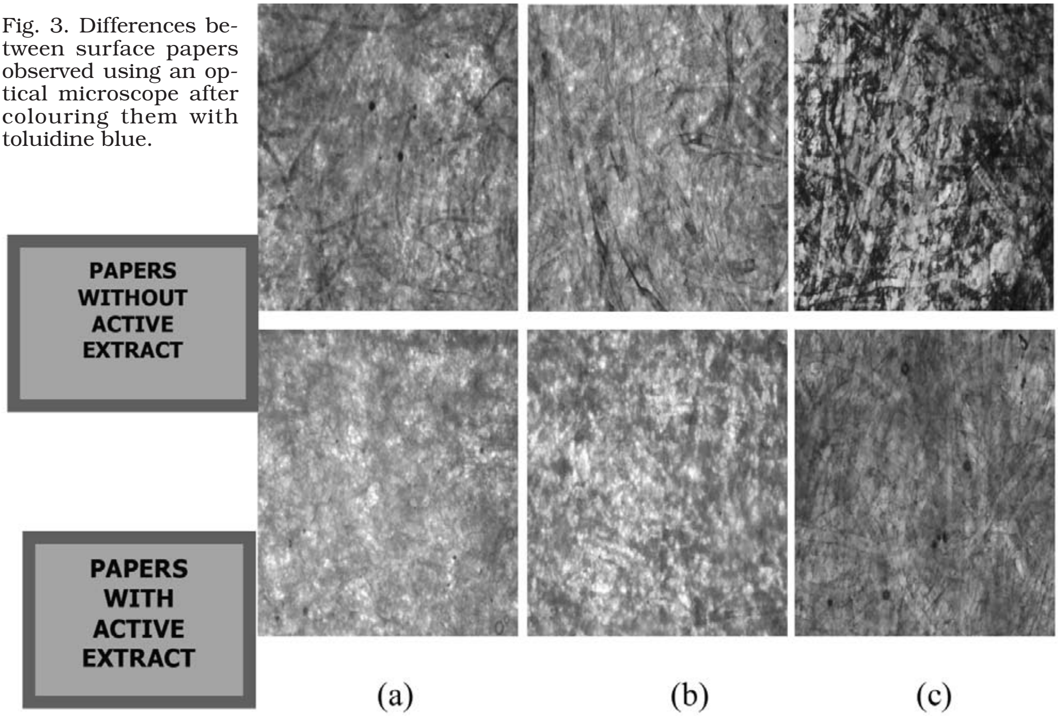


Fig. 2. Inhibition diameter of toxin alone and toxin incorporated in paper sheets.

Fig. 3. Differences between surface papers observed using an optical microscope after colouring them with toluidine blue.



paper samples treated and untreated with killer toxin extract are shown (Fig.3). The treated paper sheets retained less colorant than the untreated ones: this suggests that in the former case the colorant absorption into the cellulose fibres is likely to be hindered by other hydrophilic substances including the antimicrobial principle, that in turn is subsequently released within the hydrophilic medium containing the test microorganism.

The results indicate that the amount of toxin released depends on the type of packaging material employed: the density of the papers and the chemo-physical treatments they underwent seem to play a major role in determining their effectiveness.

Good activity results were obtained with vegetable parchment: this paper is produced by a strong acidic treatment (sulphuric acid bath) that allows for partial dissolution of the fibres. It gives a network consisting of strictly intertwined fibrils that have a very high exchange surface. It is thought that this trimmed structure allows a fast absorption of the semi-purified extract and a controlled release depending on the chemo-physical treatment degree (a).

Much lower antimicrobial activities were provided by calendered papers: these papers are subjected to a mechanical smashing treatment that rearranges and compacts the paper fibres into a material presenting minimal inter-fibre air spaces. Therefore, such a material is likely to retain good amounts of antimicrobial substance even though this is poorly released (c).

Greaseproof paper did not show any antimicrobial activity; it has a very compact structure that results from a complex mechanical treatment and is made up of less refined pulp containing, among others, lignin and additives. The absence of air spaces between fibres accounts for the very limited active principle absorption and release (data not shown). Moreover this paper is made up of less refined pulp containing, among others, titanium oxide and lignin and therefore has different chemical properties.

Idropack and Idroprint are smoothed papers and resulted instead to be very effective: although the smoothing treatment bears some similarities to calendaring, it is milder and therefore allows for larger inter-fibres spaces. Thanks to the presence of air spaces and different dimension fibres, these smoothed have a more effective toxin release than the previous papers (b).

CONCLUSIONS

Pichia anomala toxin shows good antimicrobial activity against the test strain *Brettanomyces bruxellensis* CBS 4481 and can be absorbed and subsequently released by some cellulosic materials. Since toxin release appears to be strictly dependent upon paper properties deriving from the manufacturing process, an effective modulation of antimicrobial activity could be achieved by an appropriate choice of the technological processes the paper is subjected to. For instance, the degree of parchment treatment could be modulated in order to obtain better toxin release within the food matrix and to guarantee the safety of the packaging before and during food contact.

Toxin purification and characterization could finally provide further insight into the absorption and release mechanisms, thus allowing for the development of a carefully targeted implementation protocol.

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THE TRACEABILITY AND LABELLING OF GENETICALLY MODIFIED ORGANISMS (GMOs) IN THE EUROPEAN UNION

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ABSTRACT

Food safety represents a topic of primary importance within the framework of processes of deregulating markets, with effects of important concern, in addition to consumer protection, also with regard to respecting the rules of competition and the safeguarding of local identities.

In the course of the last years, the European Union has defined a specific policy of interventions in this field, having established the principles and general requirements of alimentary legislation through the issuing of numerous provisions. These have led, in 2002, to the institution of the European Authority for alimentary safety.

The present contribution intends examining the measures launched by the EU concerning the traceability and labelling of products obtained by OGM. It intends, in particular, to highlight, with regard to the legislative context of reference, the modalities through which the creation of an "emergency net" is planned to cover all the stages along which the product is developed, from production to marketing, up to the presentation and labelling of the end product, in respect of the consumer's right for information.

Key words: Genetically Modified Organism (GMOs), food security, food labelling, food traceability, precautionary principle

INTRODUCTION

The European Union has legislated on the use, dissemination, marketing and traceability of GMOs both in food intended for both human consumption and in animal feed since 1998. After a five-year moratorium, the Commission once again authorised GMOs in 2004 (Fonte, 2004). So, the question related with the consumer's perception of the GMOs and its impact on food safety is more delicate and present than in the recent past.

EU promote consumers' health, safety, economic and legal interests, and their right to information (Article 153 of the EC Treaty), but it is quite common that the consumer is not able to distinguish all the aspects of the GMOs questions. Actually, in order to be placed on the market, GMOs must first undergo a very strict assessment process. In fact, they must be clearly labelled in line with the latest labelling requirements which include product traceability, a real keyword of the new international food safety policy but also the main action to ensure a high level of protection of the environment and health.

THE FOOD SAFETY POLICY OF THE EUROPEAN UNION

Traceability and food security

Among the fundamental requirements in the management of food safety, currently enforced by Reg. (CE) 178/2002, with which the European Authority for food safety has been instituted and procedures in the field of the food safety have been established, there is *traceability*, by which firms must guarantee to be able to trace every food, feed and alimentary ingredient used, going back along the alimentary chain from the consumer to the producer.

Firms must be able to identify in a clear and unequivocal way their own suppliers and customers, following a well defined path of the one-step-backward, one-step-forward process (European Commission, 2005), that entails the execution of control interventions and observation along the entire alimentary chain "from the farm to the table", with the aim of knowing in detail goods and services supplied in all the exchanges carried out in the sector.

THE TRACEABILITY AND LABELLING OF GMOS

The context

Traces of GMOs in products (their presence is adventitious or technically unavoidable) will continue to be exempt from the labelling obligation if they do not exceed the threshold of 0.9%. So the adventitious presence of GMOs in traditional crops cannot be ruled out. This may presuppose the presence of minute traces of GMOs in traditional foods and feedingstuffs as a result either of an accident or of technically unavoidable contamination during growing, harvesting, transport or processing. The adventitious presence of GMOs is an important point to be noted in this Regulation. The threshold is set at 0.5% for this type of "contamination" for GMOs which have been the subject of a favourable scientific opinion.

Another relevant question is the possibility to identify GMOs easily by a specific code on the product's labelling. It infact could contribute to the traceability of GMOs in general and in particular of those from third countries introduced in the EU market.

GMOs like novel foods and novel food ingredients

The EU regulation are indeed principally oriented to define community procedures for the authorisation and supervision of genetically modified food and feed and carry out provisions for the labelling of genetically modified food and feed according with the precautionary principle (McMahon, 2003). At first the related discipline was principally represented by the Reg. CE n. 258/97 concerning novel foods and novel food ingredients. This Regulation allowed the authorisation of some products derived from GMOs in accordance with a simplified procedure, where the product was substantially equivalent to another which had already been authorised.

The EU approach

After 2003, all foodstuffs that contain genetically modified organisms, including foodstuffs for animals, must be labelled GMO. The compulsory labelling of all GMO products is a fundamental point for the food security. Not only because of the consumer is more informed but also because the product is traceable throughout the food chain. These aspects are regulated with the Reg. CE 1829 and 1830/2003 concerning traceability and labelling, which also establishes a single authorisation procedure. The new regulations cover all food and feed containing GMOs, including products already on the market, but it is also concerned with environmental protection, setting out measures to be taken in the event of environmental problems. These law are strictly related with White Paper on Food Safety and the Directive 2001/18/EC **on the deliberate release into the environment of genetically modified organisms**. The basic concept is that the Commission has reaffirmed its intention to supplement the Community labelling regime in accordance with these rules.

So the simplified procedure for the placing on the market of foods derived from genetically modified products has been definitely abandoned. The regulation provides for a single authorisation procedure for all food and feed containing GMOs and is based on a single scientific evaluation carried out by the European Food Authority's Scientific Committees. The authorisation will be renewable for ten-year periods.

About the labelling the new regulation includes all foods produced from GMOs, without making a distinction between those containing DNA (deoxyribonucleic acid) or protein resulting from genetic modification and those which do not, and also covers all genetically modified feed, giving it the same protection as food intended for human consumption. Consequently, more informations concerning the labelling of genetically modified products, whether for human or for animal consumption are available for the consumer. Traces of GMOs in products, whose presence is adventitious or technically unavoidable, will continue to be exempt from the labelling requirement unless they exceed the 0.9% threshold.

In order to guarantee environmental surveillance and alimentary traceability, the regulation requires operators to transmit an indication that the products consist of or contain GMOs and the unique identifier(s) assigned to the GMOs contained in the products. Operators who place on the market a pre-packaged product consisting of or containing GMOs must, at all stages of the production and distribution chain, ensure that the words "This product contains genetically modified organisms" or "This product is produced from GM (name of organism)" appear on a label affixed to the product. In the case of products, including in large quantities, which are not packaged and if the use of a label is impossible, the operator must ensure that this information is transmitted with the product (i.e. accompanying documents) (http://www.europa.eu.int/scadplus/leg/).

CONCLUDING REMARKS

The EU policy concerning the traceability and labelling of GMOs and products produced from GMOs have two main objectives. To inform consumers about the presence of GMOs through the labelling of food and feed products and, to create a "safety net" based on the traceability at all stages of production and placing on the market. This "safety net" will facilitate the monitoring and checking of the characteristics of food products, but also facilitate the surveillance of the potential effects on human health or the environment and the withdrawal of products if an unforeseen risk to human health or the environment is identified.

consumers will therefore have more information about the labelling of GM products, whether for human or animal consumption.

One of the relevant question related with GMOs is surely the fact that it is not possibile to exclude the adventitious presence of GMOs in traditional crops. Minute traces of GMOs may be present in conventional food and feed, either by accident or as a result of adventitious or technically unavoidable contamination during cultivation, harvest, transport and processing of food products (http://www.europa.eu.int/). This means an high level of efficency in the activities of monitoring and control at different level in all the food chains and in all the member states. It is quite clear, in particular, that are involved technical and economic questions (Loureiro, 2003). It is, in fact, necessary to maintain the effective functioning of the internal market, ensuring a high level of protection of human life and health, animal health and welfare, the environment and consumers' interests in relation to genetically modified food and feed.

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INFLUENCE OF THE PUFFING DEGREE ON THE SHELF LIFE OF HOT-AIR-PUFFED AMARANTH SEEDS

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ABSTRACT

Puffed amaranth seed is commonly used in South American Countries as a breakfast cereal, as an ingredient in nutritional beverages and in a traditional snack called *alegria.* The aim of the present study was to evaluate the effect of the expansion degree of the seeds on the enzymatic and non-enzymatic oxidation of the amaranth oil, after two months storage at 25°C. Amaranth seeds were puffed (9 different experiments) with hot air at a temperature ranging between 270°C and 312°C. After puffing, the products of each trial were classified in three groups for dimensions (class A: mean diameter of 1.95 mm; class B: 1.56 mm; class C: 1.17 mm.) and evaluated for moisture, water activity, bulk density, starch damage, peroxide number and lipase activity. The puffing process, as evidenced by the Scanning Electron Microscope (SEM) analysis, radically changed the original grain organization leading to a very porous and highly disorganized structure. Seeds of class A presented a lower moisture content, a higher degree of starch damage, as evidenced by the viscoamylographic and by the enzymatic analyses, and a high peroxides content. On the contrary, the level of lipase inactivation was similar in the different samples. When observed by SEM, the surface of the less expanded products (class C) appeared partially protected against oxygen uptake by the presence of teguments still stuck to the endosperm. Statistical evaluation of the data evidenced that lipid oxidation was solely related to the degree of expansion independently of the puffing temperature. Therefore the possibility of increasing the shelf-life of puffed amaranth should mainly depend on oxygen removal from the package atmosphere.

Key words: amaranth, lipid oxidation, puffing, SEM, storage

INTRODUCTION

Amaranth was a major grain crop in the pre-Columbian Aztec empire. The grain was then forbidden by the Spanish as it was associated to pagan rituals and human sacrifice during religious ceremonies. It consequently fell into obscurity for hundreds of years until the mid-1970s when the scientific community re-discovered this grain whose cultivation nevertheless continued over the centuries in a few remote areas of the Andes and Mexico. The word *Amaranthus* contains the Greek root “αμαρᾶντινος" that means everlasting, "that it doesn’t wilt"; the plant is actually quite drought-resistant and it grows both on rich and on rather poor soil, from sea level to about 3500 m (Berghofer and Schoenlechner, 2002). The genus *Amaranthus* consists of approximately 60 species, but the three principal species considered for grain production are: *A. hypochondriacus*, *A. cruentus*, and *A. caudatus*. Amaranth seeds are small and lenticular in shape, with each seed averaging 1.0-1.5 mm in diameter and 1000 seeds weighing 0.6-1.2 g (Catenacci, 2004). Their colours range from off-white to brown and a large embryo surrounds the starchy endosperm in a form of a ring. The seeds of *Amaranth hypochondriacus* L., the species most widely cultivated in Mexico, contain 13-18% proteins rich in lysine (3.2-6.4 g/100g protein), 57-63% starch, 2-6% crude fibre and 3-11% lipids with a high degree of unsaturation (Berghofer and Schoenlechner, 2002). It has also a high level of calcium (250mg/100g – ten times more than wheat) and iron (15mg/100g – four times more than wheat) and the unsaponifiable fraction of amaranth oil is comprised primarily of squalene, a highly unsatured triterpene. Amaranth is used in various cultures in different ways: it can be cooked as a cereal, ground into flour, popped like popcorn, sprouted, or toasted. In Mexico it is puffed and mixed with honey or molasses to make a traditional confection called "*alegria*" (happiness). Whereas, on the one hand puffing treatment makes this pseudo-cereal crunchy and thus suitable to be used without further cooking, on the other hand it promotes the emergence of defects due to lipid oxidation.

The aim of the present study was to evaluate the effect of the degree of expansion of seeds on the enzymatic and non-enzymatic oxidation of puffed amaranth. This work is a part of a research project performed by DiSTAM of the University of Milan in collaboration with two Mexican organization l’Organización Civil de Desarollo *Alternativas* and the Cooperative *Quali*. This project named "The rediscovery of Amaranth” is partially financed by the Fondazione Cariplo, Italy.

MATERIALS AND METHODS

Amaranth seeds were puffed (9 different experiments) with hot air at a temperature ranging between 270°C and 312°C by the Cooperative *Quali* (Tehuacán - Mexico). Other thermal treatments were also applied before puffing in order to reduce the native enzymatic activity (Table 1). After puffing, the products of each trial were classified in three groups by size (class A: mean diameter of 1.95 mm; class B: 1.56 mm; class C: 1.17 mm). The analyses were performed after 2 months storage at 25°C.

Bulk density. Raw and puffed grain bulk density (g/L) was evaluated (n=3) by measuring the volume reached by 15g of the sample poured into a graduated cylinder and gently tapped ten times.

Moisture content. Moisture content (%) was determined (n=2) immediately after grinding, according to the AACC Official Standard Method 44-15A (1983).

SEM. Raw and puffed grains were observed as whole kernels or fragments; samples were mounted on aluminium stubs and sputter-coated with gold; their ultrastructure was imaged in the SEM LEO438 VP (Leo Electron Microscopy Ltd. Cambridge, UK), under high vacuum conditions (10^{-4} Pa) at an accelerating voltage of 20 kV.

Peroxide value. The oxidation of the lipids in the early stage was measured through the evaluation of peroxide value according to the NGD Db 6 procedure (1989) modified as reported by Gerletti (2002).

Lipase activity. The lipase activity was determined by measuring with an automatic titrator (Mettler DL 25 titrator) the μmoles of NaOH necessary to maintain, for 10 minutes, the pH of a dispersion containing amaranth flour (100-200mg in 20mL water) and glycerol tributyrate (0.2mL) to a constant value of 7. NaOH μmoles correspond to the μmoles of H^+ released by the lipolytic enzymes. Results were expressed as μmol H+/g min.

Visco-amylograph test. The analysis was carried out using a Micro Visco-Amilograph (Brabender OHG, Duisburg, Germany) under the conditions reported by Mariotti et al. (2006). For the characterization of the pasting properties of the samples, initial viscosity and peak viscosity were taken from the curves.

Statistical analyses. Data were subjected to Principal Components Analysis (PCA) using The Unscrambler® software (Camo A/S, Trondheim, Norway) in order to describe the samples and to find similarities between them.

RESULTS AND CONCLUSIONS

The puffing treatment caused the grain moisture content to decrease from its original value, ranging from 11.5% for untreated seed (experiment 1) and 6.5% for seeds treated for 10 minutes at 100°C (experiment 4) or 122°C (experiment 8), to values that are strictly related to the expansion degree (mean value: 4.13±0.07% for samples A, 4.68±0.06% for samples B and 7.02±0.04% for samples C) (Fig. 1). Water activity was related to the final moisture of puffed seed and ranged from 0.155±0.003 to 0.397±0.002, thus assuring a good microbial stability.

In all treatments, puffing promoted a significant increase in the size of the grains for samples of classes A and B, while only a limited expansion was proved for samples of the class C. These last samples showed only a limited decrease of bulk density from 93.7±0.01 g/100mL of the raw grain to an interval ranging between 75.00±0.01 and 57.04±2.57 g/100mL as reported in Fig. 2.

The higher size increase was obtained in experiment 1: in fact bulk density values of 16.93±0.48 g/100mL and 28.66±0.31 g/100mL were obtained for samples A and B, respectively. Among the products belonging to category A, samples 4A and 9A, grains that before puffing had been subjected to more drastic treatments, showed the highest bulk density (lower expansion degree) due to the lower water content of the seeds before puffing, as reported in Table 1.

The SEM images of amaranth before puffing show an internal structure for the raw grain that was compact and homogeneous together with a well defined germ surrounding the endosperm (Fig. 3). As expected, the puffing treatment radically changed the ultrastructure of the grain creating a highly porous matrix made up of numerous cavities of different sizes separated by a very thin wall. More specifi-

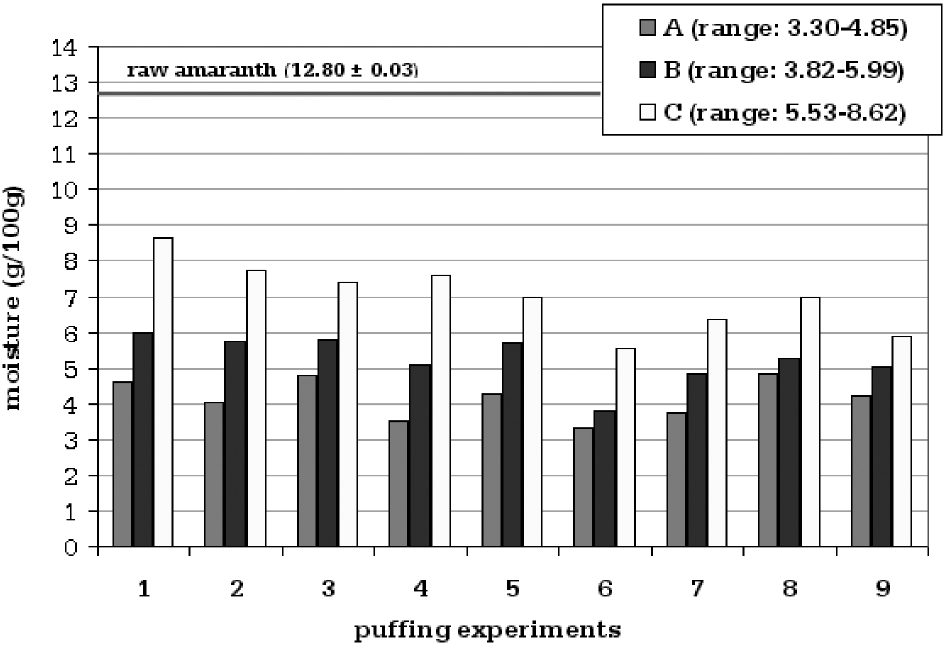

Fig. 1. Moisture content of the different amaranth samples.

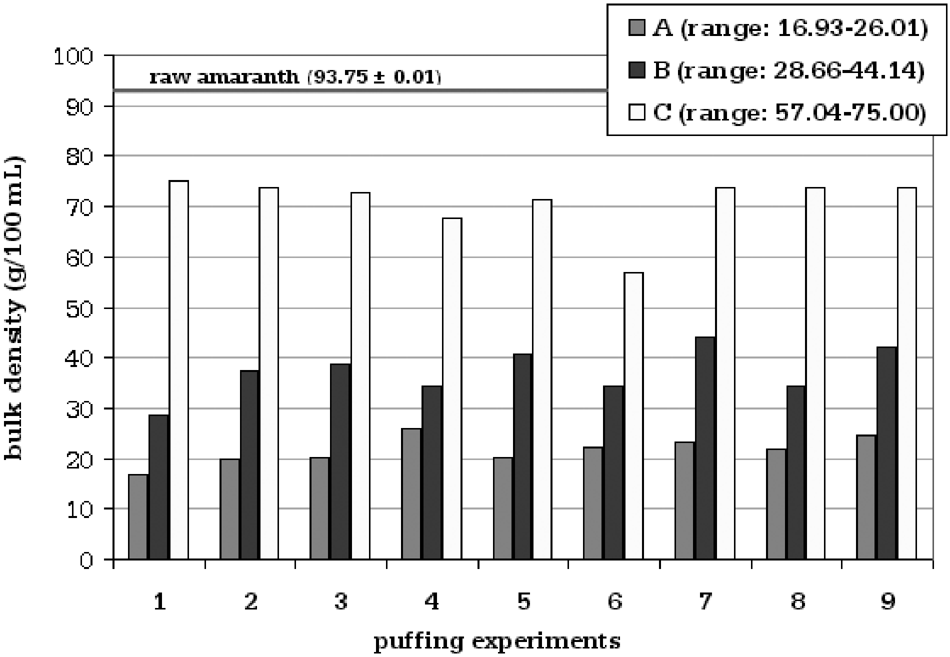

Fig. 2. Bulk density of the different amaranth samples.

Table 1. Processing conditions of puffing treatments (dh=direct heat).

	1	2	3	4	5	6	7	8	9
Pre-treatment									
- method	none	oven	oven	oven	oven	dh	dh	dh+steam	dh+steam
- time (min)	-	5	10	15	5	5	7	5+5	7+3
- temperature (°C)	-	100	100	100	150	106	120	122	122
Moisture before puffing (%)	11.5	9.0	8.0	6.5	7.5	-	7.0	8.0	6.5
Puffing temperature (°C)	279	279	279	311	279	312	279	279	270

cally, for the samples of class A, puffing caused a complete disorganization of the tegumental portion of the seed and the formation of the cavities distributed all along the grain, that reached a maximum size of 100-130 μm. In samples C many grains presented a partially disorganized structure, almost completely wrapped in the teguments. The class B samples showed an intermediate situation. It is worth noting that in samples A, which were characterized by the higher expansion levels, the germ is completely disorganized making the oil more susceptible to lipid oxidation.

The considerable porosity of the puffed seeds provides a large contact area between oxygen and the highly unsatured amaranth oil that contains 45% linoleic acid. This condition has determined an increase of the peroxide value from 5.88±0.92 meq O_2/kg oil of the untreated seed to much higher values for the products after 2 months of storage (Fig. 4). Results show an important fat oxidation, and in particular in samples A and B. Lower values obtained for products 4A, 4B and 6A may be connected to the higher temperature used in the puffing process (Table 1). Any positive result is, on the contrary, associated to the thermal treatments applied to the seeds before puffing, in particular for trials 2, 3 and 5. As regards the lipolytic enzyme inactivation, both thermal treatments applied to the seeds before

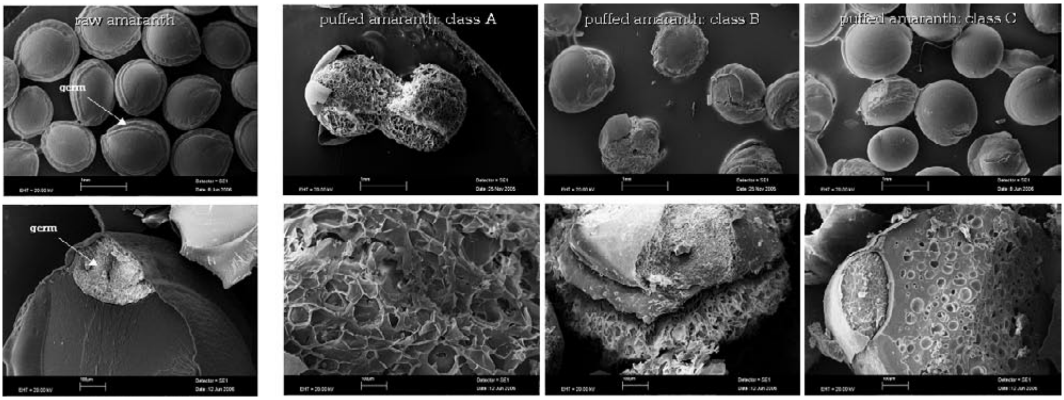

Fig. 3. SEM images of the different classes of puffed amaranth: at the top, the whole grain appearance: 18x; at the bottom, the corresponding internal structure of the grains: 100x.

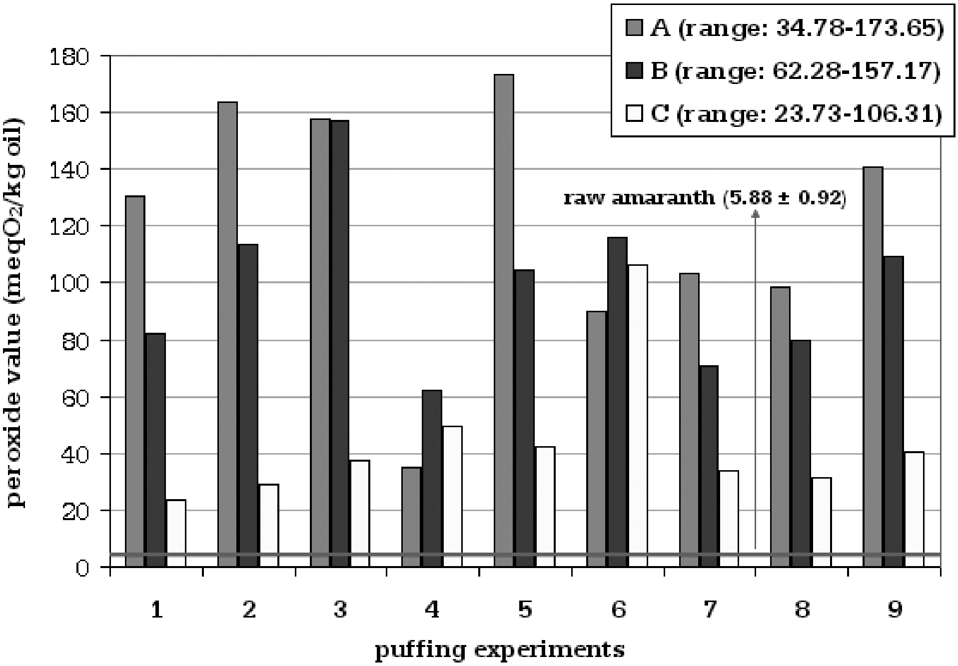

Fig. 4. Peroxide value of the different amaranth samples.

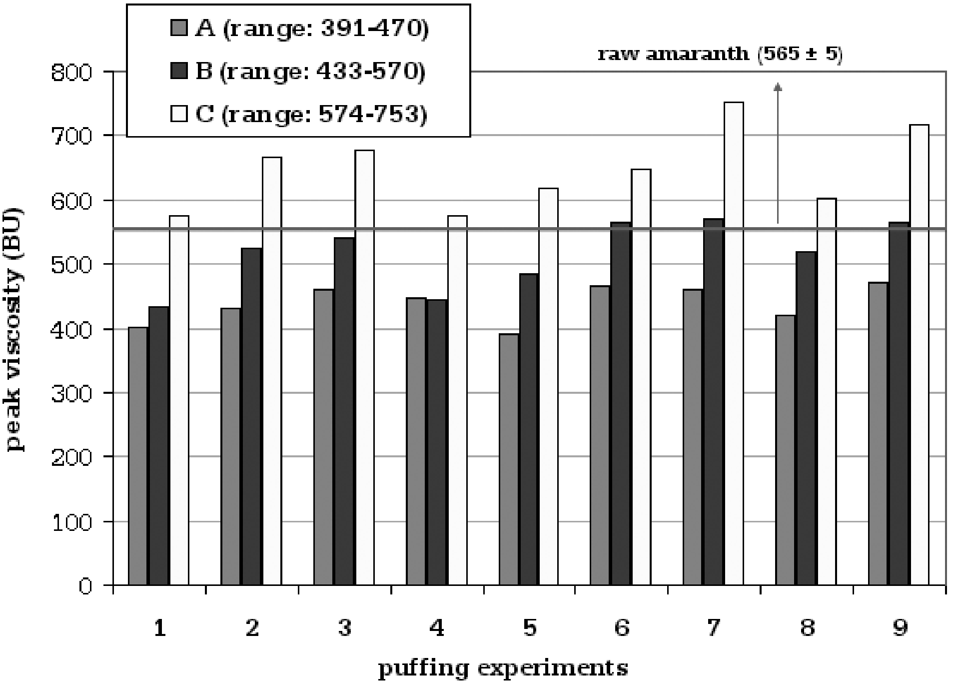

Fig. 5. Viscoamilographic peak viscosity of the different amaranth samples.

puffing and puffing itself appeared to be less effective in samples 8 and 9 as the lipase activity remained very close to the value of 0.314±0.076 μmol H^+/g min of the raw seed. A higher inactivation was obtained for the other experiments and in particular in trial 2.

Puffing induced important changes to the starch characteristics that can be evidenced by the viscoamylographic analyses: raw seeds presented low initial viscosity and high peak viscosity; on the contrary, in the thermally-treated products, peak viscosity decreased as the degree of expansion increased to indicate a higher level of starch damage (r=0.898) (Fig. 5).

A PCA model was made including all puffed samples (27 samples) and all the variables (Fig. 6). The explained variances on PC1 and PC2 account for 82% of variance. As regards the PC1, the variability is related to parameters both associated to the expansion of the products such as bulk density and moisture and to heat damage (viscoamylographic initial and peak viscosity) and negatively associated to lipid oxidation (peroxide value). As regards the PC2, only the lipase activity appears to be relevant. The score plot shows that puffed seeds with a different expansion degree (class A, B and C) are placed in different positions on the PCs plane. Samples A are positioned on the left in a plane area characterized by high values of both viscoamylographic initial viscosity and peroxide indices, while samples C are in an area identified by high values of bulk density, moisture and peak viscosity. An

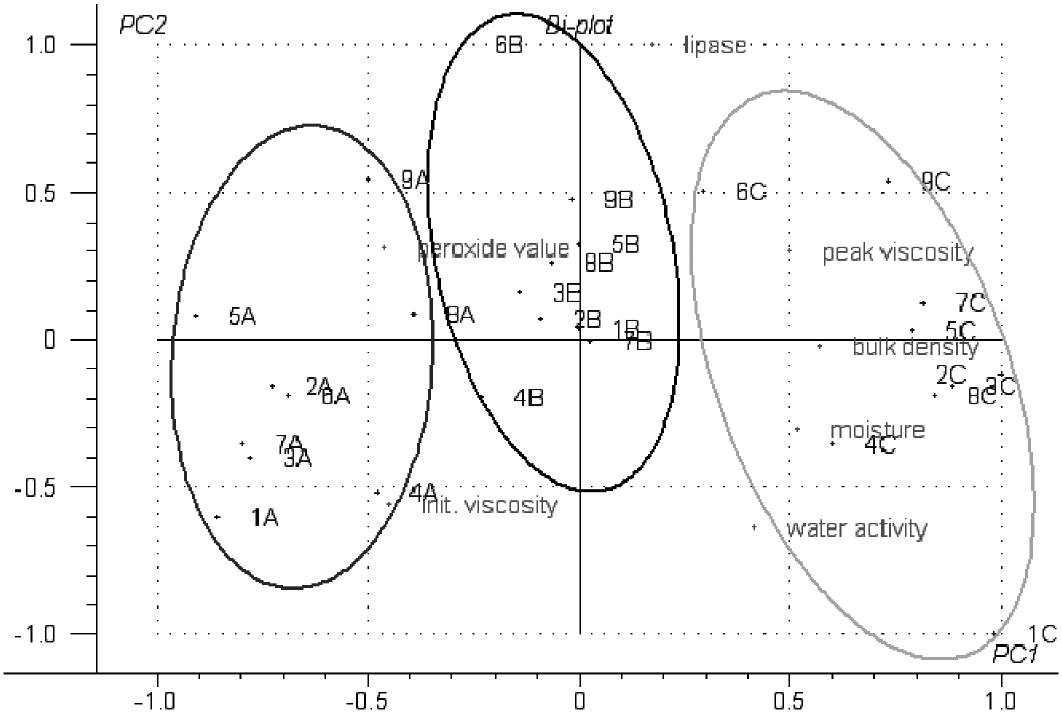

Fig. 6. Principal Component Analysis (PCA) (PC1: 62%; PC2: 20%) (init. viscosity: viscoamylographic initial viscosity; peak viscosity: viscoamylographic peak viscosity).

intermediate position is occupied by samples B. Lipolytic activity, on the contrary, does not have any bearing on samples discrimination.

The results obtained in this study demonstrate that the early stage of lipid oxidation of puffed amaranth was solely related to the degree of expansion, apart from of both the treatments applied to the seeds before and during puffing. Therefore the possibility of increasing the shelf-life of puffed amaranth should mainly depend on the removal of oxygen from the package atmosphere.

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SHELF LIFE DETERMINATION OF THREE KOREAN SAVOURY SAUCES

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ABSTRACTS

A variety of savoury sauces are used in Korean homes and food service to enhance palatability and satisfaction of Korean foods. Various combinations of preservation hurdles are being used to keep the products stable for extended period of time. The product characteristics affect the storage stability and shelf life. Primary quality attribute limiting the shelf life depends on the product composition and property. Three typical savoury sauces used with Korean foods were submitted to storage test to provide general picture of their shelf life behaviour. The tested products include a liquid sauce of meat extract for cold-immersing noodle, soybean paste seasoning for cooked rice and semi-liquid sauce for sandwich bread. The liquid meat extract sauce for noodle processed and packaged with hot fill shows absence of aerobic bacteria and has a little low pH of 4.3 and high water activity of 0.98. Soybean paste seasoning for cooked rice having low pH of 4.0 and low water activity of 0.78 has initially some microbial load of 2.8 in log CFU/g coming from fermented soybean paste ingredient. The semi-liquid sauce for bread has low initial pH of 4.0 and intermediate water activity of 0.88. All the product packages stored at 5, 15, 25 and 35°C were taken out periodically to measure the quality attributes including pH, water activity, microbial growth and lipid oxidation degree.

For liquid meat extract sauce the primary quality change determining shelf life was found to be lipid oxidation measured in thiobarbituric acid value, while those for soybean paste seasoning and semi-liquid sauce for bread were pH decrease and colour change, respectively. Water loss was significant for flexible pouch package of liquid meat extract sauce, and there was some noticeable increase in microbial count of soybean paste seasoning for rice. Some pH decreases were also observed for liquid meat extract sauce for noodle and semi-liquid sauce for bread. The temperature dependence of the primary quality attributes could be described by Arrhenius equation to give the shelf life estimation as function of temperature. The activation energies for liquid meat extract sauce, soybean paste seasoning and semi-liquid bread sauce were 20.3, 28.0 and 53.7 kJ/mol.

Key words: savoury sauce, storage stability, shelf-life, primary quality index, temperature effect

SHELF-LIFE OF SLICED BLOOD ORANGES: MULTIVARIATE ANALYSIS OF MICROBIOLOGICAL AND SENSORY DATA

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ABSTRACT

The study considered fruits packaged with a low permeability film under three different modified atmospheres.

The aim of this work was to extract by statistical analysis more information from microbiological and sensory data obtained by profile.

ANOVA with factor cultivar showed significant differences for all microbiological parameters and for sensory attributes except off-flavour. PCA showed no correlation between microbiological and sensory data.

Key words: microbiological data, multivariate analysis, sensory data, shelf-life, sliced blood oranges

INTRODUCTION

Marketplace in part because of the value added to the product through its preparation and delivery in a ready-to-eat condition. The primary warranties of this added value are low temperature and Modified Atmosphere Packaging (MAP). These two factors work in concert to maintain freshness, extend shelf life (10 days), ensure safety, and promote sale. Oranges on the basis of their morphological and physiological characteristics, show good potentiality to be minimally transformed (Pretel *et al.*, 1998). In a preliminary study (Caggia *et al.*, 2003) was individuate two most suitable Blood clones ('Tarocco Gallo' and 'Tarocco Scirè'). Further studies investigated packaging influence on orange slices film permeability (Caggia *et al.*, 2004) and modified atmosphere (Rapisarda *et al.*, 2005). No correlation was found between microbial growth and changes in sensory characteristics with respect to the permeability of the films tested. The aim of this work was to extract by statistical analysis more information from microbiological (mesophilic and psychrotrophic viable counts, yeasts and moulds, acetic acid bacteria, acidophilic bacteria counts) (Davenport,1996; Maimer and Busse, 1992) and sensory data obtained by profile.

MATERIALS AND METHODS

The study considered fruits packaged with a low permeability film under three different modified atmospheres α, β and γ as shown in Table 1, and evaluated at five different times (0, 3, 5, 7, 10 days).

For the sensory evaluation of the slices during storage, the profile method was used (ISO 13299, 2003) by a trained panel in a laboratory (UNI ISO 8589, 1990). The judges were required to estimate the selected attributes (freshness, aroma, firmness, juiciness, acidity sweetness, off-flavour) using a numerical unipolar scale from 1 (absence) to 9 (maximum intensity).

Table 1. Modified atmosphere compositions (%).

Atmosphere	O_2	CO_2	N_2
α	5	15	80
β	2.5	8	89.5
γ	10	20	70

The microbiological analyses of each trial were performed in triplicate. Mesophilic viable counts (MVC) were performed on Plate Count Agar (Oxoid, CM325), after 48 h incubation at 32 °C (PCA32); psychrotrophic viable counts (PVC) were done with the same medium but incubated at 15 °C for a week (PCA15). Yeasts and moulds counts (SAB) were determined with Sabouraud Dextrose Agar (Oxoid, CM41) supplemented with chloramphenicol (Oxoid, SR0078), after incubation at 25 °C for 4 days; acetic acid bacteria (GYCA) were determined using medium (50 g/L glucose, 10 g/L yeast extract, 30 g/L calcium carbonate, 25 g/L agar) after incubation at 32°C for 48 h; acidophilic bacteria counts (OSA) were performed using Orange Serum Agar (Oxoid, CM657) after incubation at 32°C for 48 h. Colony forming units were manually counted.

Table 2. F values of all variables and for three factors.

	F values		
Variables	Cv	MAP	DAYS
Freshness	16.93***	2.36 n.s.	34.37***
Aroma	64.83***	3.87*	57.92***
Acidity	18.10***	3.19*	2.09 n.s.
Juiciness	9.72**	0.89 n.s.	20.88***
Sweetness	10.12**	3.57*	20.40***
Firmness	29.57***	2.04 n.s.	28.14***
Off-flavour	0.01 n.s.	1.19 n.s.	6.62***
OSA	11.69***	2.28 n.s.	14.99***
PCA15	9.64**	0.22 n.s.	17.79***
PCA32	5.42*	0.56 n.s.	18.69***
GYCA	12.16***	2.19 n.s.	24.77***
SAB	10.45**	0.63 n.s.	15.15***

*** significant difference for $p < 0.001$
** significant difference for $p < 0.01$
* significant difference for $p < 0.05$
n.s. no significant difference

Microbiological and sensory data was submitted to one way analysis of variance (ANOVA). In order to correlate better these parameters Principal Component Analysis (PCA) was carried out on all data set.

The statistical package STATGRAPHICS Plus version 4.0 was used.

RESULTS AND CONCLUSIONS

ANOVA with factor cultivar showed significant differences for all microbiological parameters and for sensory attributes except off-flavour. Also considering time effect all microbiological variables and sensory attributes, except acidity, were found

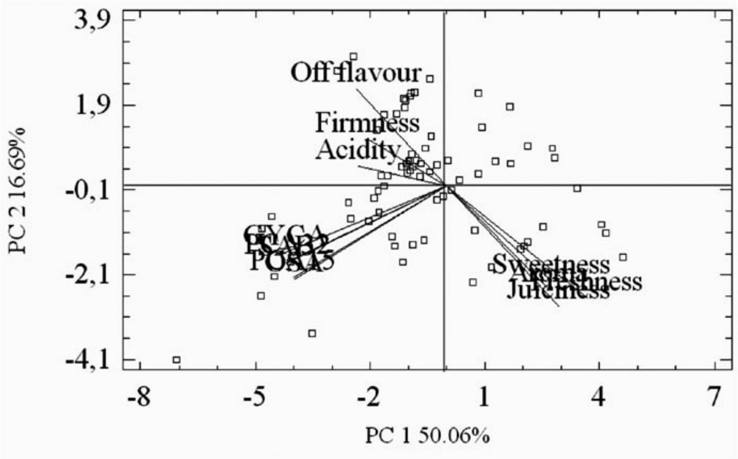

Fig. 1. Biplot of microbiological and sensory variables.

significantly different. On the contrary, modified atmosphere factor influence only the sensory attributes aroma, acidity and sweetness, while maintain microbiological variables unchanged (Table 2). PCA showed no correlation between microbiological and sensory data (Figure 1).

The variables are in different position defining three groups, two with sensory attributes (off-flavour, firmness, acidity, sweetness, freshness, aroma and juiciness) and another with microbial parameters. These are influenced by characteristic and cultivars of fresh fruits, by time of storage, but they are not influenced by characteristics of MAP. So the choice of Cv, MAPs only depend by sensory evaluation regarding that the MAP does not influence the safety of the product.

ACKNOWLEDGEMENTS

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PRELIMINARY STUDY OF ETNA PACKAGED PRICKLY PEARS BY SENSORY EVALUATION

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ABSTRACT

The fruit of opuntia is edible, although it has to be peeled carefully to remove the small spines on the outer skin before consumption, in fact the scarce diffusion of its market is principally own to the difficult of peeling. So, the possibility of finding a new market segment for the Sicilian prickly pear, has suggested to study the shelf-life of Etna prickly pears by evaluation of sensory profile varying films, conditions of package and technological treatment.

On the basis of microbiological analyses, sensory evaluation was not possible for all the samples beside the 5th day. In fact, up to 8th day the samples A, B, C, D, G, while up to 10th day only A, B, C, G have been evaluated. From the gotten results, for the storage of prickly pear like fresh-to-cut fruits it is advisable to use film at low permeability and a packaging to contained lower part of O_2.

Key words: packaged, preliminary study, prickly pears, sensory evaluation

INTRODUCTION

The prickly pear cactus (*Opuntia ficus Indica*) is a plant highly distributed in the Mediterranean area, Central and South America. In fact, owing to its crassulacean acid metabolism, this plant is characterized by a high potential of biomass production with very low water consumption. It is, therefore, extremely drought tolerant and grows abundantly under semiarid conditions. The content of proteins, carbohydrates, minerals and vitamins (mostly vitamins A and C) in the fleshy stems (cladodes) is nutritionally significant (Gurrieri *et al.*, 2000) and the fruits have good sensory characteristics. Sicilian cultivars of prickly pear produce yellow, red and white fruits. The fruit of opuntia is edible, although it has to be peeled carefully to remove the small spines on the outer skin before consumption, in fact the scarce diffusion of its market is principally own to the difficult of peeling. It is often used to make candies and jelly.

The prickly pear fruit normally ripens and is ready for harvest during the late summer and early fall months.

The growing commercial success of fresh-cut fruits and vegetables, linked to consumer demand for convenient food products which give a perception of freshness and natural taste, and the possibility of finding a new market segment for the Sicilian prickly pear, have prompted research into the production of ready-to-eat prickly pear.

So, the aim of this research is to study the shelf-life of Etna prickly pears yellow variety by evaluation of sensory profile varying films (low and high permeability), conditions of package (modified atmosphere) and technological treatment. The sessions have been carried out after 1, 3, 5, 8 and 10 days of storage at 4°C.

MATERIALS AND METHODS

Seven samples have been evaluated:

A: 2.2 O_2/2.6 CO_2/film at low permeability;
B: 5.0 O_2/2.0 CO_2/film at low permeability;
C: 2.0 O_2/5.0 CO_2/film at low permeability;
D: blanching of fruit in water at 80°C x 10s/film at low permeability;
E: blanching of fruit in water + citric acid 2% at 80°C x 10s/film at low permeability;
F: film at high permeability;
G: film at low permeability.

In order to define the attributes characterizing the product and to investigate the sensory changes of fruits during the storage period, the sensory profile method (ISO 13299, 2003) was applied. The trained panel (5 females and 5 males) was selected among students of Foods Science and Technology Department at the University of Catania. Some preliminary sessions were performed to chose the descriptors (at least 70% of frequency of citations), to validate the assessors, to familiarize them with scales and procedures and to develop a common vocabulary, which allowed assessors to use the same terms to describe their perceptions. Each term was fully described and explained to remove any doubt about its meaning.

The fourteen descriptors used included: Freshness and Turgidity (appearance), Typical aroma and flavour, Grassy aroma and flavour, Off-odor, Off-flavour (olfactive and flavour), Resistance to cut, Crispness, Juiciness (reological properties), Sweetness and Acidity (taste) and Overall. All sensory evaluations were performed in a laboratory according to ISO 8589, asking to judges to quantify the intensity of each attributes by assigning a score between 1 (absence of the sensation) and 7 (extremely intense). Within each session the design was balanced for carry over effects (MacFie *et al.*, 1989).

On the basis of microbiological analyses, in contemporary effectuated to guarantee the safety of products, sensory evaluation was not possible for all the samples beside the 5th day. In fact, up to 8th day the samples A, B, C, D, G, while up to 10th day only A, B, C, G have been evaluated.

The sensory data for each attribute was submitted to one way Analysis of Variance (ANOVA). The significance was tested with F test. The mean values were submitted to the multiple comparison test using the procedure LSD (Least

Significant Difference) that allows to determine what attributes differentiate the samples.

The statistical package STATGRAPHICS Plus version 4.0 was used.

Table 1. F values of sample and means of the score among the 14 sensory attributes for the 5 samples at t8. The values marked with different letters in the same line are significantly different ($p<0.05$).

		LSD				
		Samples				
Attributes	**F values**	**A8**	**B8**	**C8**	**D8**	**G8**
Freshness	0.35 n.s.	3.7	3.4	4.3	3.8	3.9
Turgidity	0.90 n.s.	3.9	3.4	3.9	3.4	4.5
Typical aroma	0.54 n.s.	4.1	3.2	3.9	4.0	3.9
Grassy aroma	0.85 n.s.	3.2	2.8	2.5	3.2	3.4
Off-odor	1.20 n.s.	1.6	2.6	2.2	2.4	2.5
Resistance to cut	2.24 n.s.	4.1	3.2	3.4	3.4	4.5
Crispness	5.83***	3.4^{bc}	2.3^{a}	2.7^{ab}	2.3^{a}	4.0^{c}
Juiciness	0.55 n.s.	3.8	3.3	3.6	3.3	4.1
Sweetness	0.45 n.s.	4.2	3.7	4.1	3.4	4.2
Acid	0.33 n.s.	2.1	2.2	1.9	2.4	2.1
Typical flavour	0.76 n.s.	4.0	3.6	3.3	4.1	4.2
Grassy flavour	0.38 n.s.	2.6	2.9	2.9	2.6	3.1
Off-flavour	3.33*	1.8^{a}	3.3^{b}	3.3^{b}	3.0^{b}	2.3^{ab}
Overall	2.32 n.s.	4.2	3.2	3.4	3.3	4.3

Table 2. F values of sample and means of the score among the 14 sensory attributes for the 4 samples at t10. The values marked with different letters in the same line are significantly different ($p<0.05$).

		LSD			
		Samples			
Attributes	**F values**	**A10**	**B10**	**C10**	**G10**
Freshness	0.74 n.s.	4.5	3.8	4.2	4.8
Turgidity	2.03 n.s.	4.3	3.2	3.8	5.0
Typical aroma	1.56 n.s.	4.2	3.0	3.5	4.7
Grassy aroma	1.03 n.s.	3.6	2.7	3.2	3.7
Off-odor	1.45 n.s.	2.7	3.1	2.9	1.9
Resistance to cut	3.99*	4.3^{b}	3.1^{a}	3.0^{a}	4.5^{b}
Crispness	5.37**	4.2^{c}	2.0^{a}	2.5^{ab}	3.7^{bc}
Juiciness	0.85 n.s.	4.3	3.7	3.7	4.4
Sweetness	0.98 n.s.	3.7	3.8	4.0	4.8
Acid	0.23 n.s.	2.2	2.3	2.2	2.0
Typical flavour	2.69 n.s.	3.8	2.9	2.6	4.3
Grassy flavour	0.09 n.s.	2.9	2.7	2.6	2.7
Off-flavour	0.78 n.s.	3.2	3.5	3.1	2.5
Overall	2.34 n.s.	3.7	2.8	3.1	4.4

*** significant difference for $p<0.001$
** significant difference for $p<0.01$
* significant difference for $p<0.05$
n.s no significant difference

RESULTS AND CONCLUSIONS

Results show, at 1st, 3rd and 5th day of sensory evaluation, no significant differences among samples for all attributes.

At 8th day only samples A, B, C, D and G were submitted to ANOVA, and the results (Table 1) show no significant differences among the samples except Crispness ($p<0.001$) and Off-flavour ($p<0.05$). The sample A shows the less value for the attributes Off-flavour, Off-odor and the sample G shows the greater value for Crispness, Turgidity, Resistance to cut, attributes correlated to reological properties.

At 10th day only sample A, B, C and G were submitted to ANOVA, and the results (Table 2) show no significant differences between the samples for all attributes except Resistance to cut ($p<0.05$) and Crispness ($p<0.01$). At t10 like t8 the sample G shows the greater value for Freshness, Turgidity, Resistance to cut and besides for Typical aroma and flavour, Sweetness and Overall and the less for Off-odor and Off-flavour.

From the gotten results

(some data aren't reported), it is possible to conclude that sample G maintains unchanged its sensory attributes in particulare its reological attributes during the storage followed from samples A and C.

For the storage of prickly pear like fresh-to-cut fruits it is advisable to use film at low permeability and a packaging to contained lower part of O_2.

This research is a preliminary study about the alternative storage of prickly pear that it will start again at the end of the next summer.

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EFFECT OF STORAGE METHOD ON POSTHARVEST QUALITY OF HIGHBUSH BLUEBERRY

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ABSTRACT

Highbush blueberry fruits (*Vaccinium corymbosum* L.) are highly perishable and must be marketed soon after picking. Applying modern techniques of storage, fruit harvested in a particular season, may be consumed months later, preserving decay and quality. Therefore, in order to guarantee a top quality product and to lengthen its shelf-life, it is of primary importance to develop suitable storage techniques. The purpose of this work was to investigate the influence of postharvest method to maintain berry quality. Blueberry samples were stored for 6 weeks in Normal Atmosphere (3°C, 90-95% R.H.) and in Modified Atmosphere (3°C, 90-95% R.H.+0.3 ppm of O_3). During postharvest period firmness was measured with Durofel® instrument and with a Texture Analyzer TAXT2i®. To evaluate berry quality, total soluble solids content, titratable acidity and pH were measured. Little or no quality advantage was evident with the use of ozone on fruit firmness, soluble solid content and acidity. During postharvest storage Normal Atmosphere treatment was more effective to lengthen blueberry shelf-life compared to Modified Atmosphere. These preliminary results showed that ozone treatments seems to be no effective to extend postharvest life of blueberry fruit.

Key words: titratable acidity, bluecrop, Durofel®, R.S.R., Texture Analyzer TaxT2i®

INTRODUCTION

Highbush blueberry culture is increasing in Piedmont Region due to its high market profitability and nutritional value (Bounous *et al.*, 2005). But blueberry is a non-climateric fruit and it must be harvest at full maturity to achieve the maximum quality in relation to flavour and colour. As a consequence, blueberries should be harvested ready to consumption. For this reason cold storage and innovative atmospheres have been used to extend marketing season, reduce losses and increase profitability (Machado *et al.*, 2004, NeSmith *et al.,* 2005).

Ozonation is a new technology that can be used to reduce the decay of fresh fruits and sanitize the produce, but results have been variable (Forney *et al.*, 2001, Palou *et al.,* 2001).

The main characteristics related to the quality of ripe blueberry fruit are texture, flavour and colour. Change in texture is a consequence of the natural process of senescence and also of the atmosphere in which the fruit is stored (Sanford *et al.,* 1991).

The aim of this work was to study how texture and quality attributes of Blueberry fruits changes during 6 weeks of cool storage and to investigate the influence of ozone to maintain berry quality.

MATERIALS AND METHODS

The trial was carried out during the summer of 2004, using the highbush blueberry cultivar Bluecrop. Berries were harvested by hand from commercial plantings situated in Cuneo Province (Piedmont region, north-west of Italy), placed directly into plastic clamshell containers and cold stored under two different storage conditions:

✓ Traditional storage (N.A.): normal atmosphere (3°C, 90-95% of R.H.);

✓ Innovative storage (M.A.): modified atmosphere (3°C, 90-95% of R.H. + 0,30 ± 0,05 ppm O_3). Ozonation was created by AgroCare™ (Grupo Interozone, Cile).

Firmness was evaluated at each picking date and once a week during storage by:

✓ a penetrometer test by Durofel® (Copa Technologie), a dynamometer with a bolt of 3 mm ∅ (0.10 cm^2), on a scale of 1 (soft) to 60 (firm), a rapid nondestructive instrument;

✓ a penetrometer test by TA-XT2i Texture Analyzer® (Stable Micro System) equipped with a 5 Kg loadcell. The probe penetrated 3 mm into the berry at a crosshead speed of 3 mm/s, with P-3mm Dia Cylinder Stainless probe. Force max (N) of penetration was calculated.

For chemical measurements of soluble solid content (°Brix), titratable acidity (meq/l) and pH, 100g samples were weighted and centrifuged (Rotofix 32) at 3000 rpm for 10 min at 20°C. Soluble solid content was measured by placing a few drops of the filtered juice on an digital refractometer PR-32 α (Atago). Titratable acidity and pH were measured by titrating 1:10 diluted juice using 0.1 N NaOH by an automatic titrator (Compact 44-00, Crison).

Data were analyzed using the analysis of variance (ANOVA), Tukey's test HSP was used to determine significant differences among treatment means. Means values were considered significantly different at $P \leq 0.05$. Data was analysed using the program package STATISTICA ver. 7.1 (Statsoft Inc., Tulsa, OK, USA).

RESULTS AND CONCLUSION

As expected, decrease in firmness occurred throughout the storage period regardless of the treatment due to the normal softening of the tissues associated with senescence of the fruit. In particular, M.A. treatment showed significant reduction in the firmness of the berries during postharvest storage period. Data in figure 1 report the results of measurements of force max of penetration (N) by TA-XT2i associated with storage conditions and postharvest weeks. Both sets of data (N.A. and M.A.)

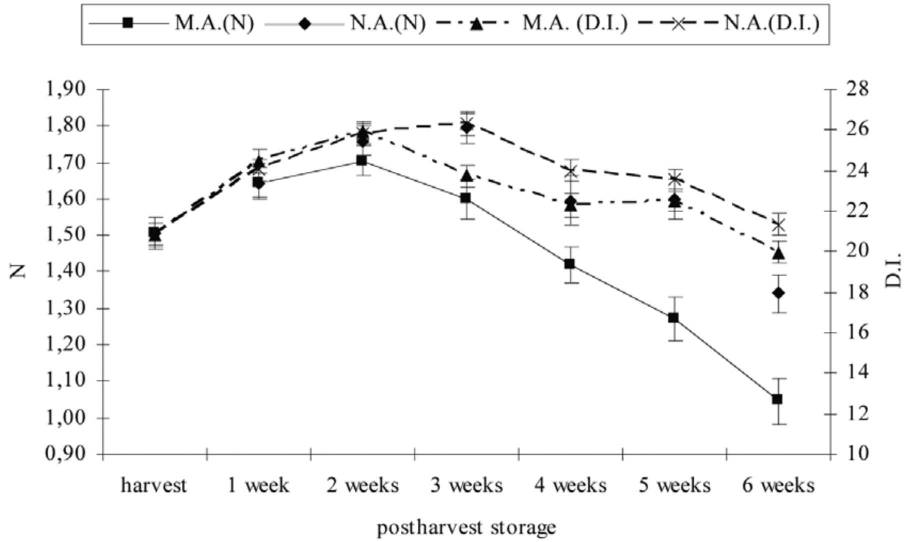

Fig. 1. Fruit firmness as a function of postharvest storage. The figure shows the mean values and the standard error of force max (N) by TA-XT2i and Durofel Index (D.I.) by Durofel instrument. Samples stored in Normal Atmosphere (N.A.) and Modified Atmosphere (M.A.).

shows an apparent rise in force during the first two weeks of storage, but began to fall significantly later in the postharvest weeks. The rate of fall decreased and almost levelled out in the last weeks of storage in particular in the samples stored in N.A.. This trend is probably due to the lost of moisture during the first weeks of postharvest storage due to transpiration and respiration. Similar results are obtained by Durofel Index (fig.1). Wide variability of a same fruit sample, in particular for the firmness measured by TA-XT2i® (fig. 1) is present and it is caused by the higher sensibility of this instrument and the high fruit variability. Probably due to the high fruit variability there are not significant difference ($p<0.05$) for firmness of fruits between the two atmosphere/storage methods. Firmness values covered the range of 0.75 to 2 N for samples stored in M.A., and 0.85 to 2.1 N for N.A.. These results are in disagreement with other experiments (Guzel-Seydim *et al.*, 2004; Machado *et al.*, 2004; Palou *et al.*, 2001) but the effectiveness of ozone may vary significantly depend on treatment concentration, treatment length and produce physiology (Song *et al.*, 2003).

Storage methods evaluated had no detectable effect on chemical parameters. According with Ristow *et al.*, (2004), in general, solid soluble content and titratable acidity were not affected by storage treatment with ozone.

Total soluble solid content increase during postharvest storage, and is higher in the samples stored in M.A. (tab. 1). Statistical testing show no significant differences between the two type of storage methods; only berries stored for five weeks in M.A. were more sweet as compared with other samples (tab.1). Titratable acidity of the blueberries is an indicator of potential storage quality, and decline gradually over the storage period (Spayd *et al.*, 1990).

In this work, only the first week of storage showed significant differences among treatments; in the other weeks of storage, titratable acidity values recorded were decreased similarly in the two storage conditions evaluated. The pH values showed the same tendency, increased corresponding with the slight decline in T.A. during storage in either type of storage method.

Table 1. Effect of storage time and conditions on quality parameters. R.S.R. (°Brix), T.A. (meq/l).

	harvest	*1 week*	*2 weeks*	*3 weeks*	*4 weeks*	*5 weeks*	*6 weeks*
R.S.R.							
N.A.	10.19	9.62n.s.	10.54n.s.	10.17n.s.	10.68n.s.	10.2b	10.69n.s.
M.A.	10.19	9.57n.s.	10.18n.s.	9.88n.s.	11.08n.s.	11.67a	10.97n.s.
T.A.							
N.A.	189.74	163.7a	154.49n.s.	147.46n.s.	119.38n.s.	124.64n.s.	125.75n.s.
M.A.	189.74	141.52b	151.11n.s.	150.45n.s.	124.65n.s.	131.36n.s.	139.29n.s.
pH							
N.A.	2.51	2.57n.s.	2.67a	2.68n.s.	2.76n.s.	2.76n.s.	2.75n.s.
M.A.	2.51	2.58n.s	2.59b	2.69n.s.	2.73n.s.	2.74n.s.	2.74n.s.

Means separated by Tukey's test. Means in columns with different letters are significantly different at $p \leq 0.05$.

Ozone treatment seem to be not effective in preserve blueberry quality and extend postharvest life. There was no detectable effect of ozone on fruit firmness, soluble solid content and acidity. Further investigation of ozone storage may be useful to clarify the actual response on berry quality and also to evaluate the effect of ozone on the development of the most important postharvest pathogens of blueberry.

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EVALUATION OF SHELF-LIFE AND ORGANOLEPTIC CHARACTERISTICS OF EXTRA VIRGIN OLIVE OILS FLAVORED WITH CHILLY PEPPER AND GARLIC

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ABSTRACT

The shelf-life and organoleptic characteristics of "Peranzana" extra virgin olive oils flavoured with chilly pepper and garlic during 7 months of storage were investigated. Gourmet olive oils were prepared by separately adding oily extracts of chilly pepper and garlic at three different levels corresponding to 10-20-40 for chilly pepper and 20-30-40 g/L for garlic. The following quality indices were monitored during the storage: acidity, peroxide value (PV), K_{232}, $K_{270,}$ (E)-2-hexenal/hexanal ratio and sensorial analysis. At the end of the storage all gourmet oil samples showed PV and K_{232} values lower than those of the control sample, whereas similar values of acidity, K_{270} and (E)-2-hexenal/hexanal ratio were observed. The addition of spices extracts gave more stability to the extra virgin olive oil. The tasters were able to differentiate among the addition levels and, at the end of the storage, they preferred the oils flavoured at 40 and 30 g/L of chilly pepper and garlic, respectively.

Key words: shelf-life, extra virgin olive oil, chilly pepper, garlic

INTRODUCTION

The addition of some spices such as garlic and chilli pepper, containing molecules with antimicrobial, antioxidant and antiinflammatory activities (Chipault *et al.,* 1956; Farag *et al.,* 1989; Economu *et al.,* 1991), is an ancient tradition and leads to particular products used to dress fresh dishes or in traditional preserved ("olio santo", gourmet oil, etc.). The presence of spices besides affecting the organoleptic characteristics, has an impact on the oil shelf-life and nutritional value, as reported in some studies carried out on oils from different origins. De Felice *et al.* (1993) evaluated the antioxidant activity of some herbs freshly added to rectified sunflower oil, during 4 months of storage at room temperature. The authors observed a slower increase of the oxidation, by means of Peroxide Value (PV) and K_{232} analyses. In particular, basil showed the highest antioxidant activity among the added spices, followed by parsley, mint, sage, rosemary, garlic, bay, onion, oregano and chilli pepper. Antoun and Tsimidou (1997) evaluated the stability of olive oils (mixture of virgin and refined olive oil) flavoured with 2% w/w oregano or rosemary and with a garlic infusion, during storage at 37°C in glass bottles for about 6 months. They found that rosemary and oregano flavoured oils were more stable than the same olive oil, whereas the garlic addition did not affect the oil stability; as regards the consumer acceptability and preference, they found that all flavoured oils were preferred by the consumers rather than olive oil.

The aims of this work were: a) to evaluate the shelf-life of extra virgin olive oils flavoured with chilly pepper or garlic; b) to determine their sensorial characteristics in order to identify the spice type and concentration more appreciated by the consumers.

MATERIALS AND METHODS

Extra virgin olive oil

An extra virgin olive oil ("Peranzana" cv., used for the production of PDO "Dauno" oil) produced in the 2004/2005 year by means of a three phases continuous extraction system was used for the flavoured oils preparation.

Preparation of the Extracts

Dried and chopped chilly pepper and garlic were purchased from local market. One kg of each spice was separately added to 5 L extra virgin olive oil that was then packed in sealed conical flasks and stored at room temperature in the dark, with daily shaking. Every 15 days the extracts were subjected to sensorial analysis by a panel made up of 3 experts to verify the flavour intensity. After 2 months, the extracts were ready for the preparation of the flavoured oils.

Preparation of flavoured oils

The flavoured oils were prepared by adding different aliquots of the filtered extracts to the extra virgin olive oil. Products at low, medium and high spice concentration (10, 20 and 40 g/L for chilly pepper, and 20, 30 and 40 g/L for garlic) were obtained. The samples were then transferred into 1 L dark glass bottles (10 bottles for each type of spice and for each concentration), leaving an head-space of about 2 %. Bottles were then sealed and stored at room temperature in the dark. Ten dark glass bottles containing the extra virgin olive oil were stored at room temperature in the dark and used as controls. The storage period was 7 months.

Sampling was carried out monthly by preparing sub-samples, as follows:

- One 500 ml bottle to be used for the sensorial analysis;
- One 250 ml bottle for volatile compounds analysis (ratio E-2-hexenal/hexanal);
- Two 125 ml bottles, one for the physical and chemical analyses and another one, stored at –20 °C, to be used for any repetition.

Quality characteristics of the oils

Acidity, peroxide value (PV) and specific extinction coefficients K_{232} and K_{270} were determined according to the European Official Method of Analysis (EC 1991), whereas the volatile compounds were determined according to the method reported by Angerosa et al. (1997).

Sensorial analysis

The sensorial analysis was carried out by a panel made up of 30 tasters, previously trained and coordinated by a panel leader. A specific profile sheet for the flavoured oils was set up. Tasters assigned to each sample a score from 0 to 5 coming out from the mean value.

Statistical analyses

The chemical analyses were carried out in triplicate and the results expressed as mean value ± standard deviation. As regards sensorial analysis, the one-way variance test was carried out to verify the significance of the differences.

RESULTS AND DISCUSSION

Quality characteristics of the extra virgin olive oil

The initial quality characteristics of the extra virgin olive oil used for the preparation of the flavoured oils were the following:

Acidity (% oleic acid): 0.34 ± 0.01;
Peroxide Value (meq O_2/kg of oil): 7.6 ± 0.1;
K_{232}: 1.75 ± 0.01;
K_{270}: 0.110 ± 0.000;
Organoleptic score: 8.5 ± 0.1;
(E)-2-hexenal/hexanal: 29.7 ± 2.1.

Evolution of acidity

Fig. 1A and 1B show the % increase in acidity of chilly pepper and garlic flavoured samples, respectively. According to this index, all the samples preserved their extra virgin quality (acidity less than 0.8%) after 7 months of storage.

No differences were found between the flavoured oils and the control; however, all samples showed a slight increase of this parameter (from about 0.35 to 0.50 g/100 g for control and chilly pepper, and from about 0.40 to about 0.50 g/100 g for garlic).

Evolution of peroxide value (PV)

The % increases of this parameter in the oils flavoured with chilly pepper and garlic, are reported in Fig 2A and 2B, respectively. Concerning the control oil, the value increased of about 230 %, whereas the absolute values ranged from 7.6 to 25.23 meq O_2/kg. The chilly pepper-flavoured oils showed an increase of about 50 % for 20 g/L and 40 g/L flavoured samples (5 times lower than control) and of 90 % for sample having the lowest spice concentration (10 g/L), whereas the absolute values were higher at the beginning and lower at the end of storage. The same considerations can be made for garlic-flavoured oils, which showed an increase of about 80 %. The addition of spice extracts determined an initial increase of the oil PV, but the enrichment in antioxidant compounds limited the hydroperoxydes formation in the successive storage.

Evolution of specific extinction coefficients K_{232} and K_{270}

Fig. 3A and 3B show the K_{232} % increases in chilly pepper and garlic flavoured samples, respectively. At the end of the storage, all samples had lower K_{232} values than those established by the UE regulation as the highest limit for the extra virgin olive oil (≤ 2.50). In particular, the control showed an increase from 1.75 to 2.43 (about 40 %), whereas all the flavoured oils did not show any significant increase of this parameter (max value reached was 2.20) even if starting from K_{232} values higher than the control, so indicating a greater stability during storage. This behaviour could be explained by the fact that extracts contained a greater amount of primary oxidation compounds than the control as a consequence of the prolonged maceration time (2 months). Therefore, their addition to the extra virgin olive oil determined an initial increase in the PV and K_{232} but also brought antioxidant compounds that, together with those coming from the oil, limited the hydroperoxydes production during the storage.

Concerning the K_{270} values (Fig. 4A and 4B), all samples showed values lower than those established by the UE regulation for the extra virgin olive oil (≤ 0.22). As expected, an increase in this parameter was detected for all samples but the

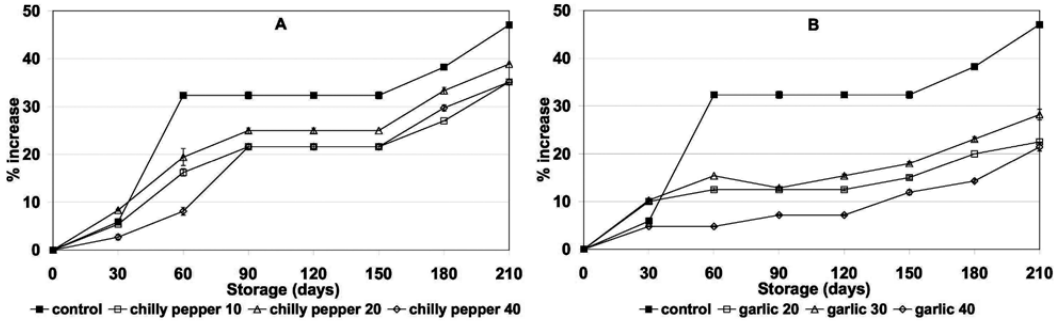

Fig. 1. Acidity of the oils flavoured with chilly pepper (A) and garlic (B) plotted as a function of the storage time.

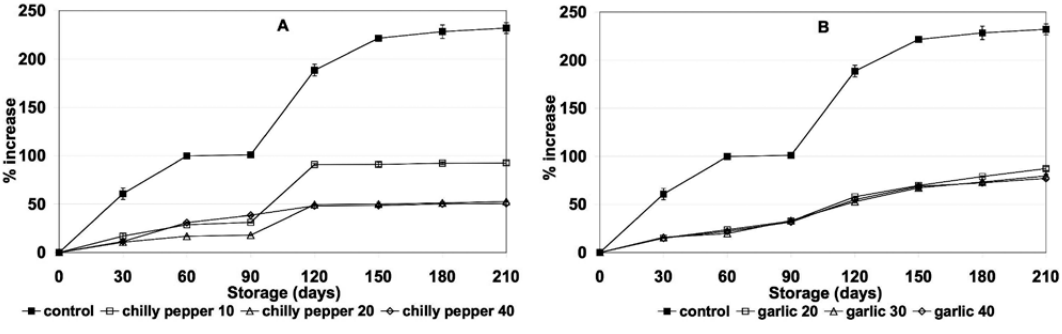

Fig. 2. PV of the oils flavoured with chilly pepper (A) and garlic (B) plotted as a function of the storage time.

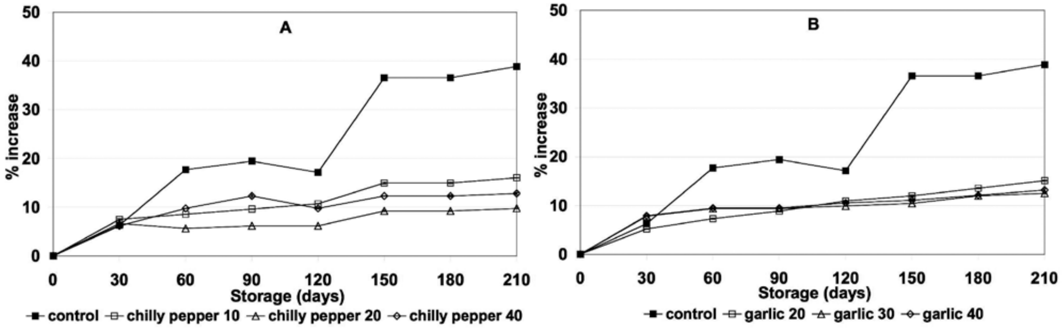

Fig. 3. K_{232} of the oils flavoured with chilly pepper (A) and garlic (B) plotted as a function of the storage time.

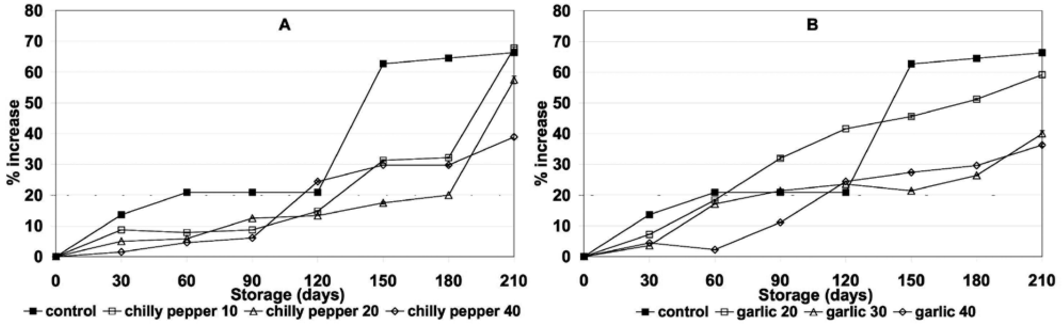

Fig. 4. Time course of K_{270} of the oils flavoured with chilly pepper (A) and garlic (B).

control sample showed the highest value. Chilly pepper-flavoured oils showed similar trends with an increase of 40, about 60 and about 70 % in 40 g/L, 20 g/L and 10 g/L flavoured oils, respectively. Concerning the garlic-flavoured oils, the 30 and 40 g/L flavoured samples showed a similar increase, which was lower (about 40 %) than that of 20 g/L flavoured sample (60 %).

Evolution of (E)-2-hexenal/hexanal

The (E)-2-hexenal/hexanal ratio decreased from little less than 30 to little more than 10 (Fig. 5A and 5B) in all samples. It is well known that values of this ratio lower than 1 indicate loss of volatile compounds with herbaceous odorous impact and formation of compounds coming from hydroperoxydes degradation causing the rancid defect. Therefore, the values found in both the flavoured oils and the control demonstrate the absence of organoleptic defects.

Sensorial analysis

Figure 6 and 7 show the sensorial profiles at 30, 120 and 210 days of chilly pepper and garlic flavoured oils, respectively. All samples resulted free from organoleptic defects. At 30 days of storage, the control showed a very intense olive fruity (>3), a strong olive taste (>3) and a good balance of the bitter, sweet and spicy characteristics, for a good total score of 3.17. During the storage, the control sample kept the fruity, developed the sweet, but lost the olive taste and bitter and

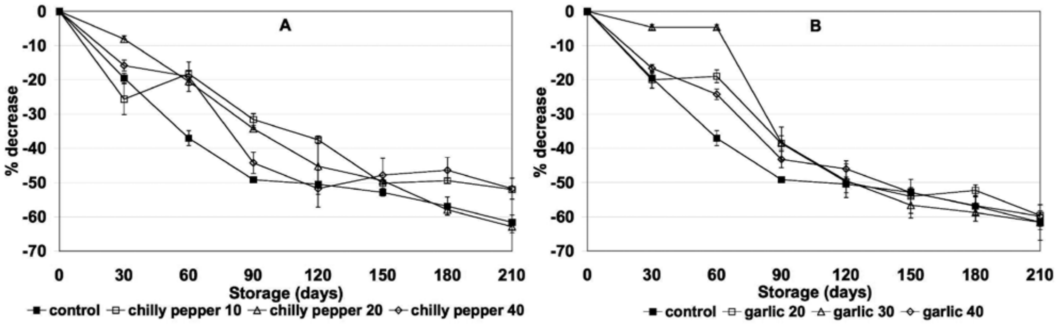

Fig. 5. Trend in (E)-2-hexenal/hexanal of the oils flavoured with chilly pepper (A) and garlic (B) during the storage time.

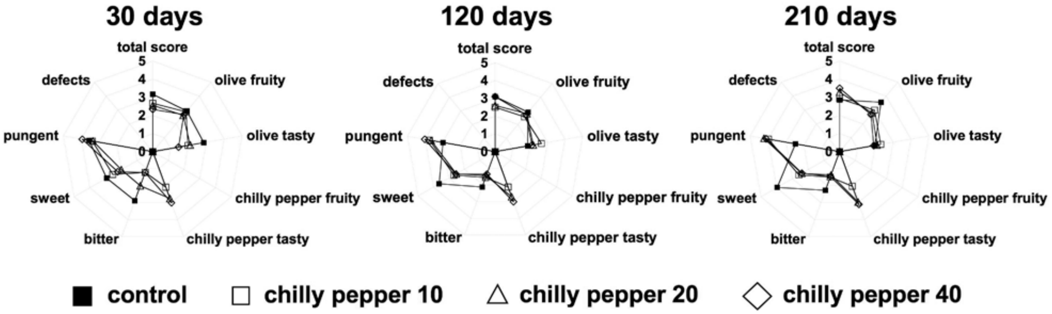

Fig. 6. Sensorial profiles of the oils flavoured with chilly pepper.

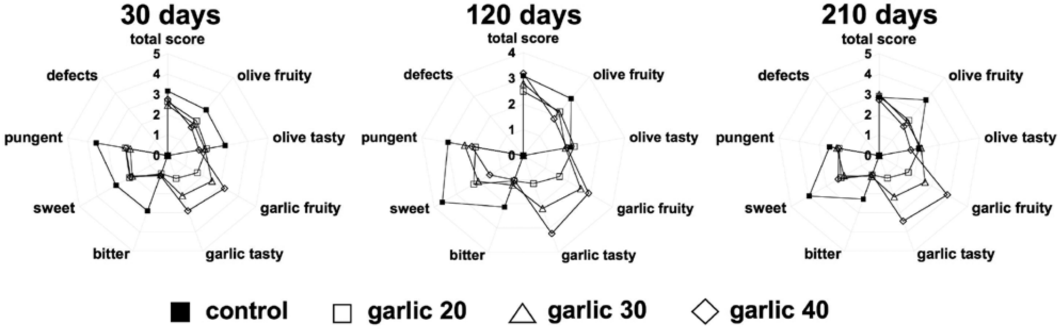

Fig. 7. Sensorial profiles of the oils flavoured with garlic.

spicy characteristics for a total score slightly lower respect to the initial value. In the chilly pepper-flavoured oils the tasters identified the spice added for the "chilly pepper tasty" attribute (intensity of about 2 for 10 g/L and about 3 for 20 and 40 g/L concentrations), whereas any perception was detected for "chilly pepper fruity". Concerning the garlic-flavoured oils, the tasters identified the spice added for both tasty and fruity of garlic at the three different concentration levels. As concerns the sample acceptability, at the beginning the control was more appreciated than chilly pepper and garlic flavoured oils, whereas these latter ones were more appreciated after 210 days of storage except for garlic at the highest concentration.

CONCLUSIONS

The present work shows that the added spices, at all the considered concentrations, had an antioxidant activity that held back the formation of primary oxidation products. Furthermore, these spices did not affect acidity and secondary oxidation products evolution (K_{270} and (E)-2-hexenal/hexanal). Tasters were able to identify the added spice at the tested concentration levels. The chilly pepper-flavoured oils obtained the highest acceptance at the end of the storage.

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