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Edited by

Klinkesorn U., Theerakulkait C. and Harnkarnsujarit N.

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This Special Issue of the Italian Journal of Food Science collects the presentations given at the "SLIM 2017, Shelf-Life International Meeting" organized by the Italian Scientific Group of Food Packaging (GSICA), the Department of Food Science & Technology and Department of Packaging & Materials Technology, Faculty of Agro-Industry, Kasetsart University, Thailand, the ISEKI-Food Association (IFA), and Thailand Convention and Exhibition Bureau (TCEB), held in Bangkok, Thailand, on November 1-3, 2017.
These papers were reviewed by the Scientific Committee of SLIM 2017 before their presentation but they did not undergo the conventional reviewing system of the Italian Journal of Food Science.

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The Italian Journal of Food Science is an international journal publishing original, basic and applied papers, reviews, short communications, surveys and opinions on food science and technology with specific reference to the Mediterranean Region. Its expanded scope includes food production, food engineering, food management, food quality, shelf-life, consumer acceptance of foodstuffs, food safety and nutrition, energy and environmental aspects of food processing on the whole life cycle.

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INTRODUCTION

The current volume includes the selected manuscripts with peer-reviewed by the Scientific Committee of the 8th Shelf Life International Meeting (SLIM 2017), which was held in Bangkok, THAILAND on 1-3 November 2017. The conference was hosted by the Department of Food Science & Technology and the Department of Packaging & Materials Technology, Kasetsart University, THAILAND, in collaboration with the Italian Scientific Group of Food Packaging (GSICA) and ISEKI-Food Association (IFA).

The theme of the 8th SLIM was *Emerging Trends in Food Technology and Packaging for Shelf-life Extension and Sustainability Improvement*. The conference purposed to offer a knowledge sharing platform for experts around the world to disseminate the fundamental aspects, research findings as well as industry applications and innovation on food shelf life and sustainability. We need more scientists making connections with, or even taking the jump to industry, social and development work for the benefit of all.

The Conference Proceedings, published once again with the support of Chiriotti Publisher as a special issue of the Italian Journal of Food Science, contain research reports presented as oral and poster papers during SLIM 2017. The conference was organized into three divisions: Division A: Emerging food technology for shelf-life extension, Division B: Shelf-life assessment and modeling, and Division C: Packaging technology and shelf-life. The conference features multidisciplinary lectures from nine distinguished keynote speakers, 20 oral presentations, and 79 poster presentations; and the participants who contribute to the forum are from twelve countries, which are Australia, Brazil, Cambodia, France, India, Italy, Japan, Korea, Malaysia, Spain, USA, and Thailand.

As it was officially announced within the final remarks of the conference, the 9th Shelf Life International Meeting (SLIM 2019) will be held in Naples, Italy. The University of Naples, in collaboration with the National Research Council confirmed their commitment to organize the next conference. The local organizing committee is chaired by Dr. Elena Torrieri and Dr. Giovanna Giuliana Buonocore. Further useful information will be disclosed soon. Hope to see you at our next SLIM 2019 meeting!

Klinkesorn U., Theerakulkait C. and Harnkarnsujarit N.

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SESSION I

Emerging Food Technology for Shelf-life Extension

EFFECTIVENESS OF CELLULOSE NANOCRYSTALS (CNCS) APPLICATION AS BIO-BASED OXYGEN BARRIER FOR SHELLED WALNUTS SHELF-LIFE EXTENSION

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ABSTRACT

The shelf-life of shelled walnuts is strongly affected by the oxidation of polyunsaturated acids content. Therefore, it is hugely important to use high oxygen-barrier packaging materials in order to maintain as long as possible their quality. Cellulose Nanocrystals (CNCs) isolated from cotton linter offer a very low oxygen permeability, thus can assure a significant shelf life extension. To obtain a fact-based confirmation, a comparative 30 days Accelerated Shelf-Life Test (ASLT) was carried out, using CNCs coated and uncoated packs of shelled walnuts. CNCs were obtained through the oxidative hydrolysis of the cotton linters, then a 7% water dispersion (pH=8) of freeze-dried CNCs was coated onto PET/LDPE film obtaining a 700-nm thick layer. Standard (uncoated) and barrier (CNCs coated) pouches were fabricated, packed with 40 g of shelled walnuts under 100% N_2, and stored at 40°C and 30% RH. The ASLT was carried out by assessing the sensorial changes of the shelled walnuts, the Peroxide Value (PV), Dienes content, TBA test and CIELAB color. As a preliminary and supporting information, the walnuts oxygen consumption rates, at different oxygen partial pressures, in the 0 – 0.19 bar range and at 40 °C, were assessed by gas chromatographic analysis (TCD) performed on experimental samples. The oxygen consumption rate resulted linearly related to oxygen concentration in the head-space, with a R^2 = 0.923. The shelf life of shelled walnuts was significantly extended in CNCs coated pouches, as the sensory analysis carried out showed a significant difference between the products.

Keywords: accelerated shelf life test, bio-based oxygen barrier, cellulose nanocrystals, shelf life extension, shelled walnuts

1. INTRODUCTION

Oxidation is generally considered as the main factor in the development of the fats and oils rancidity. These chemical reactions are very fast in the presence of (poly)unsaturated fatty acids (PUFA) and the oxidation rate depends on many factors such as oxygen availability, temperature, metal catalysts and light. For instance, shelled walnuts are food products with a very high content of PUFA which usually undergo the oxidation process producing rancidity in their oil, with accompanying off-flavours and smells causing the consequent shortage of shelf life (TAO, 2015). Therefore, it is incontestable that the role of the package becomes notable in terms of delaying or preventing the foods degradation which is strongly influenced by the external environment (MEXIS *et al.*, 2009). The main goal of this paper is to report that cellulose nanocrystals (CNCs) coated onto a PET plastic film might be used as bio-based oxygen barrier to extend the shelf life of the shelled walnuts. In particular, that such a novel barrier material may be a real alternative to the synthetic barrier like Ethyl Vinyl Alcohol (EVOH), Polyvinylidene chloride (PVDC), or even inorganic barrier such as aluminum, aluminum oxide or silicon oxide. To demonstrate with experimental facts the effectiveness of the CNCs as oxygen barrier, a comparative shelf life test was carried out on shelled walnuts sealed in uncoated and CNCs-coated pouches, stored in the same conditions of temperature and relative humidity.

2. MATERIALS AND METHODS

2.1. Materials

The walnuts, originally from USA, were bought in the local market; their gross composition is reported in Table 1. Cotton linters, used as raw material to produce CNCs were supplied by Innovhub (Milan, Italy). All the chemical reagents used were purchased from Sigma-Aldrich (Milan, Italy). PET/LDPE film, (individual thickness: 12 μm PET, 55 μm LDPE), was provided by Carta Stampa srl (Briosco, MB, Italy).

Table 1. Walnuts gross composition*.

Component	Amount
Total Fats (g)	68.1
Monosaturated Fats (%)	14.0
Polyunsaturated Fats (%)	59.8
Carbohydrate (g)	5.1
Dietary Fibers (g)	6.2
Proteins (g)	14.3
Calories (kcal)	654

*(data provided by the producer).

2.2. CNCs extraction and PET/LDPE coating

CNCs were obtained by the hydrolyzing-oxidative method proposed in 2011 [Leung et al., 2011], the purification steps and the coating process have been already described in our

previous work (RAMPAZZO *et al.*, 2017); the thickness of the CNCs coating onto PET/LDPE film was 756±22 nm.

2.3. Oxygen permeability

The oxygen permeability measurements (PO2) were performed by an isostatic permeabilimeter (mod. Multiperm, PERMTECH S.r.l., Pieve Fosciana, Italy) according to ASTM standard D-3985. The PO2 of both PET/LDPE film and CNCs coated film was measured at 40°C under 30 % RH.

2.4. Walnuts oxygen consumption rate *vs* oxygen partial pressure

As a preliminary and supporting information, the walnuts oxygen consumption rates, at different oxygen partial pressures, in the 0-0.19 bar range were assessed by gas chromatographic analysis (TCD) performed on experimental samples. Those samples were prepared by filling glass vials (150 mL) with 20 g of shelled and ground walnuts and then closing them with a rubber septum under 5-10-15-19% of oxygen in nitrogen. The vials were stored at 40°C and monitored for the oxygen consumption and concentration changes during time, by GC-TCD analysis.

2.5. Accelerated shelf life test

The quality changes of walnuts packed in standard and barrier pouches, during one month storage at 40°C, were evaluated both by sensory analysis and physico-chemical parameters assessed on the oil extracted from the walnuts; i.e. peroxide value (PV), conjugated dienes quantification, color space by CIE L*a*b* System. The oil color was assessed by a Minolta CR-300 chromameter (Konica Minolta Sensing, Inc., Japan), the PV as reported by VANHANEN and SAVAGE (2006), the dienes according to European Official Method of Analysis (EU, 1991). Sensory changes were assessed by more than 30 untrained tasters, to give a score after mouth feeling, tasting the walnuts and observing the oil color. The sensory judges followed the instructions provided by a chart where, for each descriptor, a 4 points rate scale was considered, the minimum value (0) corresponding to the best appreciation and the highest value (4) the worst.

3. RESULTS AND DISCUSSION

3.1. Oxygen permeability

Oxygen permeabilities of standard PET-PE films and CNCs-coated PET-PE films were 8.5 and 1400 $ml*24h^{-1}*bar^{-1}*m^{-2}$ respectively at 40°C and 30% UR.

3.2. Walnuts oxygen consumption rate

The basic phenomenon which governs the quality decay of the shelled walnuts during time and thus affecting their shelf life is the oxygen reactions with the sensitive components of the product. Therefore, it makes sense to investigate the relationship between the oxygen concentration in the head space and the rate of oxygen consumption. Unfortunately, not many paper deals with this fundamental issue. The results obtained in this work, reported in Fig. 1, are quite clear and might be useful in modelling the shelf life in a flexible permeable packaging (LEE *et al.*, 2008). They show that the oxygen

consumption rate of shelled walnuts, at 40°C, is linearly related to the oxygen partial pressure in the head space and that its increase of just 100 mbar, can lead to a rate 4 times faster.

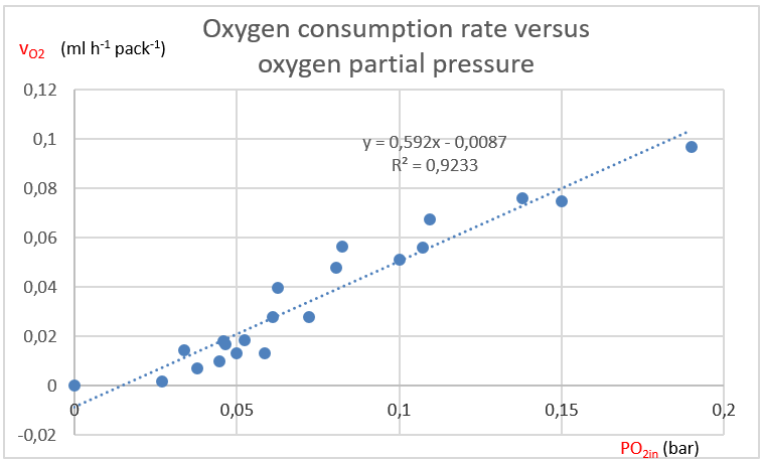

Figure 1. Oxygen consumption rate of shelled walnuts, as a function of oxygen partial pressure, at 40°C.

3.3. Oil peroxide value

Standard pouches (W standard) and CNCs pouches (W CNCs) were removed every 3-4 days. Oil was extracted from the walnuts stored in the same temperature (40°C) and the peroxide value (PV) was assessed. The Fig. 2 shows the evolution of the PV of the extracted oil during the storage time.

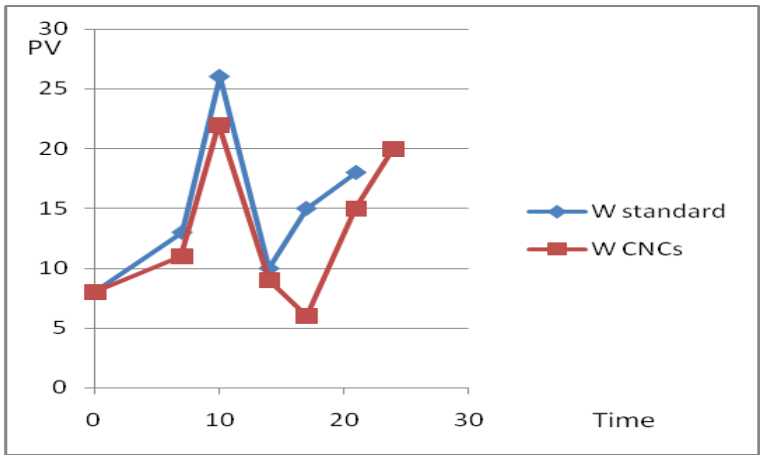

Figure 2. Peroxide Value PV (meq_{ox}/kg_{oil}) *versus* Time (days).

3.4. Conjugated dienes

At 232nm of wavelength, the absorptivity of conjugated dienes of standard pouches (WStandard232) and CNCs pouches (WBarrier232) was evaluated as shown in the Fig. 3.

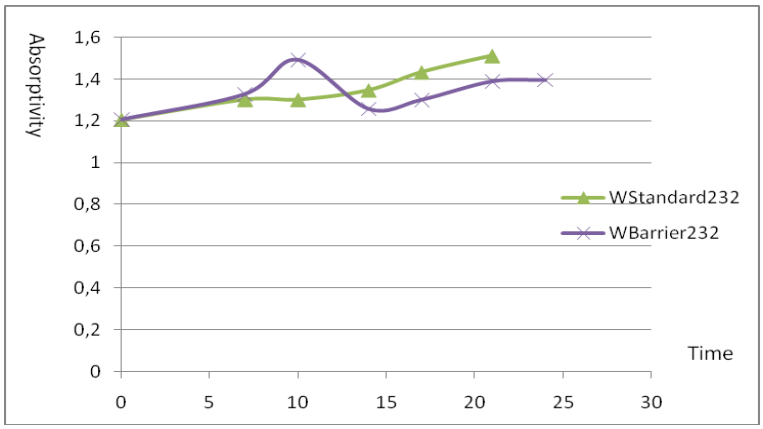

Figure 3. Results of Absorptivity *versus* Time at 232nm.

3.4. Accelerated shelf life test

The storage time considered in this work (one month) at 40°C has been able to demonstrate the advantages of using a high oxygen barrier material in the packaging of walnuts but has been not enough extended to complete the comparison for all the quality parameters considered. In fact, no statistical differences were noted for any physico-chemical attribute investigated except for the color of oil walnuts.
The only certain conclusions arising from the ASLT were those related to the sensory analysis. As well as the color of the extracted oil, all the sensory attributes evaluated clearly demonstrated the best appreciation for the samples stored in the bags, whose oxygen barrier was provided by the CNCs coating. The t-test performed on all the sensory attributes demonstrated high or very high significant differences, as Fig. 4 shows.

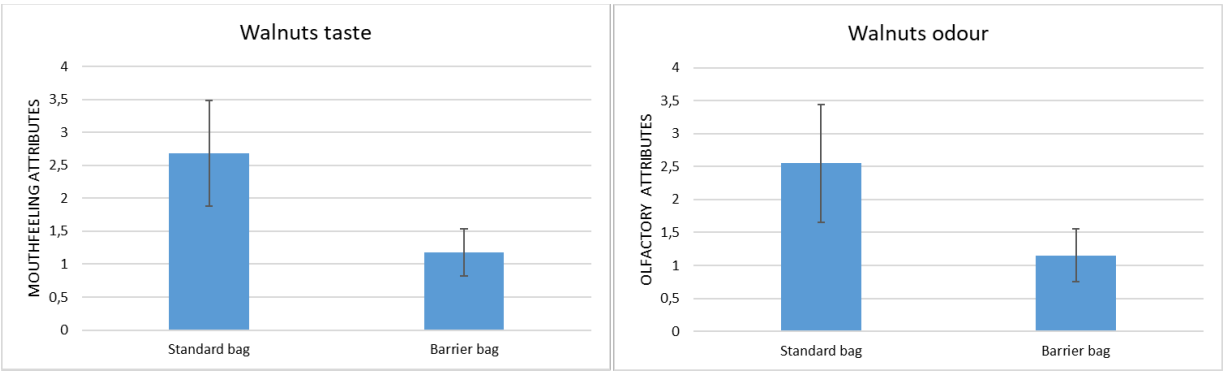

Figure 4. Results of sensory test on walnuts packed in conventional and CNCs coated films.
Mean values ± relative standard deviation.

The color-difference (DE) of oil walnuts that related a measured to a known set of CIELAB coordinates was significant within 20 days of storage. It is worth stressing that the common storage life of walnuts in air is about 6 or more months at room temperature. Moreover, the activation energy of lipid oxidation is not very high, being 15-25 kcal/mole, with acceleration factors (Q10) estimated as 1.7-4 (FRANKEL, 2014). Therefore it is reasonable to admit that the ASLT used, at 40°C and under 100% of nitrogen initially, failed to monitor adequately the quality changes occurring.

4. CONCLUSIONS

Even if the CNCs coating (1μm) provided onto the PET/LDPE film were much lower than the conventional oxygen barrier (EVOH) resulted evident in terms of offering a better conservation of the sensory attributes of shelled walnuts, along 30 days storage at 40°C. The great advantages of using a bio-based, biodegradable and very thin material to provide the required oxygen barrier to sensitive foods, promote a deeper investigation and a further development of such novel packaging material.

REFERENCES

European Union. 1991. Commission Regulation (EEC 2568/91) on the characteristics of olive oil and on the relevant methods of analysis. Official Journal of the European Communities. L 248:1-83.

Frankel E.N. 2014. Shelf life prediction. In "Lipid oxidation" E.N. Frankel (Ed.), p. 179-183 Elsevier Science.

Lee D.S., Yam K.L. and Piergiovanni L. (2008) Shelf Life of Packaged Food Products. In: "Food Packaging Science and Technology". P 479-540. CRC Press, Boca Raton, FL.

Leung A., Hrapovic S., Lam E., Liu Y.L., Male K.B., Mahmoud K.A. and Luong J.H.T. 2011. Characteristics and Properties of Carboxylated Cellulose Nanocrystals Prepared from a Novel One-Step Procedure. Small 7(3): 302-305.

Li F., Biagioni P., Finazzi M., Tavazzi S. and Piergiovanni L. 2012. Tunable green oxygen barrier through layer-by-layer self-assembly of chitosan and cellulose nanocrystals. Carbohydrate Polymers 92(2):2128-2134.

Mexis S.F., Badeka A.V., Riganakos K.A., Karakostas K.X. and Kontominas M.G. 2009 Effect of packaging and storage conditions on quality of shelled walnuts. Food Control 20:743-751.

Rampazzo R., Alkan D., Gazzotti S., Ortenzi M.A., Piva G. and Piergiovanni, L. 2017. Cellulose Nanocrystals from Lignocellulosic Raw Materials, for Oxygen Barrier Coatings on Food Packaging Films. Packaging Technology and Science. Version of Record online: 21 APR 2017 DOI: 10.1002/pts.2308

Tao L. 2015. Oxidation of polyunsaturated fatty acids and its impact on food quality and human health. Advances Food Technology and Nutritional Sciences Open J. 1:135-142.

Vanhanen L.P. and Savage G.P. 2006. The use of peroxide value as a measure of quality for walnut flour stored at five different temperatures using three different types of packaging. Food Chemistry 99: 64-69.

EFFECT OF PASTEURIZATION AND CONCENTRATION ON QUALITY OF MADAN (*GARCINIA SCHOMBURGKIANA* PIERRE) JUICE

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ABSTRACT

Madan (*Garcinia schomburgkiana* Pierre) is a native fruit of Thailand. Generally, it is not consumed as fresh fruit because of its sourness. Due to its high nutritional value and unique sour taste, the processing of the fresh madan to be pasteurized and concentrated juice products was interesting. The objectives of this research were 1) to investigate the effect of pasteurization and concentration processes on the quality of madan juice and 2) to determine the quality of madan juice after 60 days storage at room temperature. There were six treatments in this study comprising the fresh pressed madan juice (MJ), pasteurized madan juice at 95 °C for 30 s (PMJ95), pasteurized madan juice at 85°C and 95°C for 30 s with filtration and adding 0.3% ascorbic acid before pasteurization (FA+PMJ85 and FA+PMJ95), concentrated Madan juice from approximately 6 to 30 °Brix by applying filtration with and without adding 0.3% ascorbic acid before concentration by vacuum evaporation (FA+CMJ and F+CMJ). It appeared that after heat treatments the lightness and redness values of all samples significantly decreased and increased respectively when compared with MJ (p<0.05). Furthermore, the total color difference (ΔE) between MJ and FA+PMJ85 was the lowest. Higher pasteurization temperature and concentration step affected the sample color. The concentration process resulted in the increase of sample viscosity and density. The pasteurization and concentration processes lessened the sensorial scores of all products. After storage for 60 days, ΔE of all samples increased excepting for FA+PMJ95. The viscosities of concentrated samples (FA+CMJ and F+CMJ) were significantly higher while the densities of those of samples and pasteurized samples (PMJ95, FA+PMJ85 and FA+PMJ95) slightly decreased and increased, respectively.

Keywords: concentration, evaporation, filtration, madan, pasteurization

1. INTRODUCTION

Madan (*Garcinia schomburgkiana* Pierre) is sour-tasting fruit that widely found in all parts of Thailand (MEECHAI, 2016). The color of mature fruits is shiny green. Their shapes are ovate to oblong, 5-7 cm long, and 2-3 cm wide (SUBHADRABANDHU, 2001). POOMIPAMORN and KUMKONG (1997) reported that the composition of madan fruits consists of carbohydrates 6.5 g, protein 0.3 g, fat 0.1 g, fiber 0.4 g, calcium 17 mg, phosphorus 7 mg, vitamin A (carotene) 431 IU, vitamin B_2 (riboflavin) 0.04 mg and vitamin C 5.0 mg per 100 g of fresh weight. Madan is natural source of antioxidant compounds and traditionally used for the treatment of coughs and menstrual problems, laxation and diabetes (PHAM, 2006). Madan is commonly processed to be preserved fruit in syrup, pickled fruit and dried fruit. The processing of the fresh madan to be pasteurized and concentrated juice products was interesting in order to attract more consumption and preserve for off-season use. Evaporation is the most widely used method to concentrate liquid foods that offers the advantage of reducing in weight, volume and cost of packaging, storage, transportation and retailing (KAUL and SAINI, 1999). The purposes of this study were to investigate the effect of pasteurization and concentration processes on the quality of madan juice and to determine the quality of madan juice after 60 days storage at room temperature.

2. MATERIALS AND METHODS

2.1. Raw materials

Fresh madan fruit was purchased from Talad Thai Market, Pathum Thani, Thailand. Mature madan fruits with shiny green color and non-skin defects were carefully chosen. Selected fruits were washed in tap water and cut to length into 4 pieces. Pieces of fruit were put into a filter bag before pressing out the juice by using a Hydraulic Juicer (Sakaya Automate Co., Ltd, Thailand).

2.2. Treatment conditions

One portion of fresh pressed madan juice (MJ) (sample no.1) was kept in the sterilized bottle for using as a control sample whereas the rests were exposed to various procedures as illustrated in Table 1.

Table 1. The treatment conditions of madan juice samples.

Sample no.	Treatment	Conditions				
		Filtration	Adding ascorbic acid	Pasteurization		Vacuum evaporation
				85 °C 30 s	95 °C 30 s	
1	MJ	-	-	-	-	-
2	PMJ95	-	-	-	✓	-
3	FA+PMJ85	✓	✓	✓	-	-
4	FA+PMJ95	✓	✓	-	✓	-
5	FA+CMJ	✓	✓	-	-	✓
6	F+CMJ	✓	-	-	-	✓

The pasteurization was carried out using the lab-scale UHT/HTST machine (MicroThermics, Model Lab 25 EHV Hybrid, USA). MJ was pasteurized at 95ºC for 30 s (PMJ95) (sample no. 2). For samples no.3-6, MJ was kept in a cold room at 4ºC for 2 days to form sediment then the sediment was removed by vacuum filtration with Whatman no.4 paper. Filtered MJ was added by ascorbic acid 0.3% w/w prior to pasteurization at 85°C or 95°C for 30 s (FA+PMJ85 and FA+PMJ95) (sample no. 3 and 4). For samples no.5 and 6, filtered MJ with and without adding 0.3% ascorbic acid were concentrated from approximately 6 to 30°Brix by using a small-scale vacuum evaporator (Hisaka model REV-T, Japan) at temperature of 60°C and 71 cmHg of vacuum pressure (FA+CMJ and F+CMJ).

2.3. Quality determinations

All samples were determined their quality after the production was finished (day 0) and after 60 days storage (day 60) at room temperature including color (lightness (L*), redness (a*) and yellowness (b*), pH and viscosity by "HunterLab" colorimeter model Miniscan XE, "ORION" pH meter model 210A and "Brookfield" digital viscometer model RV DV II+, respectively. Total color difference (ΔE) of samples between MJ and other treatments at day 0 and day 60 were calculated. The density of samples was accessed by measuring the volume and mass of samples in a 25 mL graduated cylinder on the lab balance. All quality determinations were conducted in triplicate. The sensorial qualities of all six samples were evaluated in aspects of appearance, color, flavor and overall acceptability using the 7-point hedonic scale test (7 = like very much, 6 = like moderately, 5 = like slightly, 4 = neither like nor dislike, 3 = dislike slightly, 2 = dislike moderately, and 1 = dislike very much) by thirty untrained panelists who were students in the Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand.
All data from quality determinations was analyzed using the analysis of variance (ANOVA) whereas mean comparison was done using Duncan's multiple range test (DMRT).

3. RESULTS AND DISCUSSION

The results of color (L*, a* and b*) and the color difference (ΔE) values of all madan juice samples at day 0 and day 60 were presented in Fig. 1. It was noticed that at day 0; L* and b* values of MJ were high. After heat treatments by pasteurization (PMJ95, FA+PMJ85 and FA+PMJ95) L* and a* values of those samples significantly decreased and increased respectively when compared with MJ (p<0.05). Fruit juices contain colloids that are mainly polysaccharides (pectin, cellulose, hemicellulose, lignin, and starch), protein, tannin, and metals. The filtration process could remove components responsible for the turbidity and cloudiness in fresh juice product (UÇAN *et al*., 2014). In this study, the sediment filtration step prior to pasteurization and the addition of ascorbic acid had little effect to sample color change. However, increasing of pasteurized temperature from 85°C to 95°C (FA+PMJ85 and FA+PMJ95) resulted in significant decrease of L*, a* and b* values. The concentration step affected color values of samples (FA+CMJ and F+CMJ); L* and a* values of those samples significantly decreased and increased correspondingly. Besides, the total color difference (ΔE) between MJ and FA+PMJ85 was the lowest. After storage for 60 days, ΔE of all samples increased excepting for FA+PMJ95 probably due to the browning reaction.

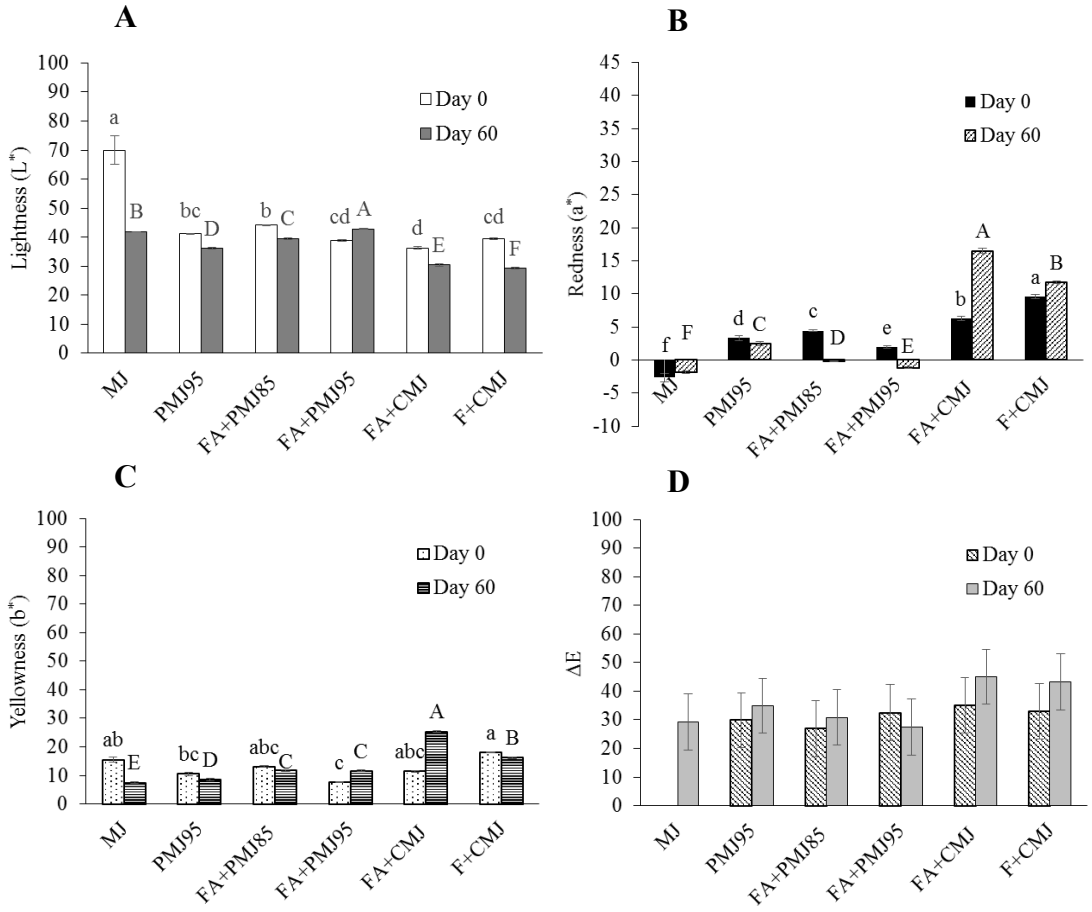

Figure 1. Color and the total color difference of madan juice samples at day 0 and day 60.
[a-f]Different small letters in the day 0 bar graph indicate that values are significantly different ($p < 0.05$).
[A-F]Different capital letters in the day 60 bar graph indicate that values are significantly different ($p < 0.05$).

Table 2 presented pH, viscosity and density values of madan juice samples at day 0 and day 60. It appeared that pH of madan juice products ranged from 1.22 to 1.82 and 1.42 to 2.17 at day 0 and day 60, respectively. An increase in pH was observed after 60 days storage. In accordance with the study of KUMAR *et al*. (2012), a significant increase in pH of guava blended with aloe vera and roselle juice nectars was detected over a period of 120 days of storage at ambient temperature. The concentration process resulted in the increase of sample viscosity and density. After storage for 60 days, the viscosities of concentrated samples (FA+CMJ and F+CMJ) were significantly higher while the densities of fresh juice (MJ) and pasteurized samples (PMJ95, FA+PMJ85 and FA+PMJ95) slightly increased.
The sensorial scores of MJ were significantly higher than other samples (p<0.05) (Table 3). The flavor and overall acceptability scores of MJ were 4.33 indicating that the panelists neither like nor dislike of sample. It was noticed that the pasteurization and concentration processes also lessened the sensorial scores of all products. Madan juice has unique sour taste. The panelists might not be familiar with this product; therefore, product research and development is a necessary issue for further improvement to reach consumer acceptance.

Table 2. pH, viscosity and density values of madan juice samples at day 0 and day 60.

Treatment	pH		Viscosity (cP)		Density (g/mL)	
	Day 0	Day 60	Day 0	Day 60	Day 0	Day 60
MJ	1.82^{a}±0.02	2.17^{a}±0.01	1.36^{c}±0.01	1.43^{c}±0.01	0.99^{b}±0.00	1.03^{a}±0.01
PMJ95	1.82^{a}±0.00	1.93$^{b±}$0.01	1.38^{c}±0.01	1.39^{cd}±0.02	0.97^{b}±0.01	1.02^{a}±0.03
FA+PMJ85	1.79^{a}±0.00	1.94^{b}±0.01	1.38^{c}±0.02	1.31^{e}±0.01	0.99^{b}±0.00	1.02^{a}±0.03
FA+PMJ95	1.80^{a}±0.00	1.89^{c}±0.01	1.39^{c}±0.01	1.34^{de}±0.01	0.99^{b}±0.00	1.02^{a}±0.02
FA+CMJ	1.22^{c}±0.01	1.63^{d}±0.01	3.62^{b}±0.07	7.77^{a}±0.10	1.07^{a}±0.01	1.03^{a}±0.01
F+CMJ	1.52^{b}±0.00	1.42^{e}±0.02	5.74^{a}±0.11	6.76^{b}±0.03	1.10^{a}±0.00	1.03^{a}±0.01

$^{a-e}$Different letters in the same column indicate that values are significantly different ($p < 0.05$).

Table 3. Mean sensory scores of madan juice samples at day 0.

Treatment	Appearance	Color	Flavor	Overall acceptability
MJ	5.50^{a}±1.25	5.63^{a}±1.16	4.33^{a}±1.81	4.33^{a}±1.86
PMJ95	3.77^{b}±1.67	3.63^{b}±1.61	2.50^{b}±1.55	3.07^{b}±1.66
FA+PMJ85	3.33^{b}±1.60	3.73$^{b±}$1.66	2.70^{ab}±1.72	3.03^{b}±1.61
FA+PMJ95	3.73^{b}±1.63	3.40^{b}±1.65	2.67^{ab}±1.77	2.83^{b}±1.55
FA+CMJ	3.60^{b}±1.49	3.53^{b}±1.41	3.00^{ab}±1.90	3.30^{b}±1.62
F+CMJ	2.93^{b}±1.41	3.20^{b}±1.58	2.67^{ab}±1.62	3.00^{b}±1.62

$^{a-b}$Different letters in the same column indicate that values are significantly different ($p < 0.05$).

4. CONCLUSIONS

It was possible to process the fresh madan to be pasteurized and concentrated juice products. The pasteurization and concentration processes affected the quality of madan juice. Those processing steps provided darker color and more viscous samples. The panelists liked fresh madan juice more than processed juice. After 60 days of storage at room temperature, the quality of madan juice significantly changed in some extent. The level of quality change depended on the processing methods applied to the samples. The pasteurization and concentration by vacuum evaporation were deemed feasible for preservation of madan juice in commercial.

REFERENCES

Kaul R.K. and Saini S.P.S. 1999. Compositional changes during storage and concentration of Kagzi lime juice. J. Sci. Ind. Res. 59: 395-399.

Kumar S.N.S., Sreenivas K.N., Shankarappa T.H. and Ravindra V. 2012. Standardization of recipe for value added nutraceutical beverage of guava blended with Aloe vera and roselle. Environ. Ecol. 30:995-1001.

Meechai I., Phupong W., Chunglok W. and Meepowpan P. 2016. Antioxidant Properties and Phytochemical Contents of *Garcinia schomburgkiana* Pierre. J. App. Pharm. Sci. 6:102-107.

Pham H.H. 2006. Vietnamese Medicinal Plants. p. 92. Youth Publishing House: Ho Chi Minh City, Vietnam.

Poomopamorn S. and Kumkong, A. 1997. Edible multipurpose tree species. 486 pp. Faung Fa Printing. Bangkok. (in Thai).

Subhadrabandhu S. 2001. Under-utilized tropical fruits of Thailand. 70 pp. FAO Regional Office for Asia and the Pacific. Bangkok.

Uçan F., Akyildiz A. and E. Ağçam E. 2014. Effects of Different Enzymes and Concentrations in the Production of Clarified Lemon Juice. J. Food Process. 2014, (2014), Article ID 215854, 14 pages.

EFFICACY OF GOAT LIVER TRYPTIC HYDROLYSATES AS PRESERVATIVE IN MEAT EMULSION

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ABSTRACT

Goat liver tryptic hydrolysate was ultrafiltered to recover <1 kDa peptides and evaluated for antioxidant & antimicrobial potential to utilise as an alternate of chemical preservatives in meat products. Peptide fraction was vacuum dried at 50°C & 600 mm Hg and incorporated in goat meat emulsion at three levels viz. T1 (0.03%), T2 (0.06%) and T3 (0.09%) and compared with C1 (0.02% butylated hydroxy toluene; BHT) and C2 (without any additive). All the samples were packaged aerobically in LDPE bags and stored under refrigeration (4±1°C). The samples were drawn on 0th, 2nd, 4th and 6th day for evaluation of physico-chemical (pH, a_w, emulsion stability, Extract release volume; ERV), instrumental colour profile, antioxidant properties (1, 1 Diphenyl-2picrylhydrazyl radical scavenging activity; DPPH, 2-2-Azinobis-3ethylbenthiazoline-6-sulphonic acid; ABTS^{+}, Ferric reducing antioxidant power; FRAP, Peroxide value; PV, Thiobarbituric acid reactive substances; TBARS, Free Fatty acids; FFA), and microbial quality parameters (SPC, Coliforms, Psychrophiles). The pH followed an increasing trend whereas a_w, emulsion stability and ERV decreased ($P<0.05$) with storage period. Though, there was a significant ($P<0.05$) decline in the values of radical scavenging activity (DPPH, ABTS^{+}, FRAP) and increase in PV, TBARS and FFA values during storage in all the samples. However, all these parameters varied in dose dependent manner and C2 exhibited poorest and T3 highest antioxidant quality attributes. The values of T3 were significantly ($P<0.05$) higher than C1. Instrumental colour profile characteristics; Lightness (L^*), redness (a^*), yellowness (b^*) and Hue decreased throughout storage. SPC and coliform count though increased during storage in all the samples; however, the values of T3 were 2.8 log cycles lower than C1 and C2 on 6th day. Psychrophiles of T2 and T3 were significantly ($P<0.05$) lower than C1, C2 as well as T1 on 6th day. Results concluded that 0.09% bioactive peptide fraction of <1 kDa generated from trypsin hydrolysed goat liver significantly improves the oxidative stability and microbial quality of goat meat emulsion during refrigeration storage. Therefore, it can be used as a natural preservative in a meat system.

Keywords: bioactive peptide, natural meat preservative, ultrafiltration, vacuum drying

1. INTRODUCTION

Meat products are mostly marketed under refrigerated temperatures (2-5°C) and they spoil at refrigeration due to microbial growth and oxidative rancidity (SEBRANEK *et al.,* 2005). Oxidation of lipid is initiated by the abstraction of hydrogen atom in unsaturated fatty acids and it gets propagated as a radical mediated chain reaction (VAYALIL, 2002). During processing the grinding of meat disrupts the integrity of muscle membranes and exposes lipid membranes to metal ions, facilitating interactions of pro-oxidants with unsaturated fatty acids. Meat can be contaminated by micro-organisms during slaughter and processing, though the meat from healthy animals is sterile at the time of slaughter (GILL, 1979). The micro-organisms bring undesirable changes in the quality of meats, especially with regard to lactic acid bacteria, a major spoilage factor in meat (EGAN, 1983). Lipid oxidation and microbial growth during storage can be controlled by application of antioxidant and antimicrobial agents, leading retardation of spoilage and maintenance of safety and quality in meat products. Many synthetic preservatives, such as BHT, butylated hydroxyanisole (BHA) and propyl gallate (PG), are used to protect foods, although their use is restricted due to possible carcinogenic effects. Therefore, the interest in alternative additives from natural sources has been increasing, provided impetus to eliminate the synthetic preservatives (SHAN *et al.,* 2009).

Bioactive peptides are specific protein fragments that have a positive impact on the health living beings (KORHONEN and PIHLANTO, 2006). These beneficial effects are attributed to various properties of peptides such as antioxidant (SARMADI and ISMAIL, 2010), antimicrobial (RAJANBABU and CHEN, 2011), immunomodulatory activities (GAUTHIER *et al.,* 2006) and antihypertensive (ERDMANN *et al.,* 2008) among others.

Liver is the largest edible nutritive gland in animals and weighs nearly 0.5 kg in goat. Its nutrient density exceeds that of muscle meats and 100 grams of liver provides 25 grams of high-quality protein, while contributing only 160 calories to the diet (ROMANS *et al.,* 2001).

Taking into consideration of the above facts, an experiment was conducted to extend the shelf life of aerobically packed goat meat emulsion under refrigeration by incorporating the trypsin hydrolysates of goat liver.

2. MATERIALS AND METHODS

Beetal Goat liver was obtained from University Experimental Abattoir. Deboned meat chunks of about 1 $inch^3$ were minced through meat mincer (Mado Eskimo Mew-714, Mado, Germany) and emulsion was prepared by prestandardized method in bowl chopper (Model: TC11, Scharfen, Germany). The tryptic goat liver hydrolysates were subjected to ultrafiltration (Millipore 8400 UF unit; cellulose membranes, Amicon, Millipore, USA) to obtain peptide fractions of <1 kDa, 1-3 kDa, 3-5 kDa, 5-10 kDa and >10 kDa, thereafter compared for their antioxidant and antimicrobial efficacy to select best (<1 kDa) liver peptide fraction (LPF) (results not included). Groups T1 (0.03% <1 kDa LPF), T2 (0.06% LPF) and T3 (0.09% LPF) were compared with control (C1: emulsion with 0.02% BHT), and (C2: emulsion without LPF or BHT). All the samples were stored in aerobically packaged Low density polyethylene (LDPE) bags under refrigerated conditions (4±1°C) and evaluated for physico-chemical (pH, water activity, emulsion stability, ERV), instrumental colour profile (Lovibond Tintometer; Model: RT-300, The Tintometer Limited, Amesbury, UK), antioxidant properties (DPPH, ABTS, FRAP and TBARS), and microbial quality parameters (SPC, Coliform count and Psychrophilic counts) on 0^{th}, 2^{nd}, 4^{th} and 6^{th} day.

Enzyme, standards, media and chemicals were procured from standard firms like SRL, Fisher Scientific, Loba Chemicals, MP Bio-Medicals, Hi-Media and Sigma-Aldrich etc. LDPE bags of 200 gauge was exposed to UV light in a laminar flow for 30 minutes for sterilization before use. The Elico pH meter (Model LI 127) and water activity meter (Rotonix HYGRO Palm AW1 Set) was used. The spectrophotometric analysis of ABTS radical scavenging activity was performed as per Salami *et al.*, (2009), FRAP (Benzie & Strain, 1999) 2, 2 diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams *et al.*, 1995). TBARS (Witte *et al.*, 1970), peroxide value and free fatty acids (Koniecko, 1979), titratable acidity (Shelef & Jay, 1970), extract release volume (ERV) (Jay, 1964) and microbiological quality (APHA, 1984). The data were statistically analysed by 2-way analysis of variance (ANOVA) using SPSS version 22 and significance was tested by Duncan's multiple range tests with $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The pH increased with storage period in both the treated and control groups. The pH was lowest for T3 and highest in C2 on 6th day. This might be due to the growth of spoilage micro-organisms and accumulation of bacterial metabolites (JAY *et al.*, 1962). a_w was significantly ($P<0.05$) higher in T2 and T3 than T1 and controls (C1; C2) however, a_w decreased during storage. ERV decreased with storage time in all the groups, and this might be due to increasing rate of spoilage during storage (JAY *et al.*, 2005). However, with increased levels of peptide fraction ERV decreased at a lower rate. This might be due to the antimicrobial effect of LFP, slowing down the rate of spoilage of meat emulsion. Emulsion stability (ES) increased significantly ($P<0.05$) in T2 and T3 compared to C2 and T1 attributed to better protein and fat binding in the emulsion with LFP. VIOQUE *et al.*, (2000) also reported emulsifying and stabilizing effect of rapeseed protein hydrolysates. ES decreased with storage time; however, the treated samples recorded higher values than C2 on 6th day. The oxidative deterioration and microbial spoilage might have caused this decrease in values.

T3 continued to show better ABTS+ % inhibition throughout the storage period but ABTS decreased during storage. The quenching affinity of peptide fraction towards the ABTS radical was concentration dependent. T3 and C1 showed the highest DPPH scavenging activities, irrespective of days; while C2 exhibited the lowest activity. FRAP increased with increasing levels of LFP. T3 was significantly ($P<0.05$) higher than C1 throughout the study. FRAP decreased with storage in all the groups. However, the rate of drop in the values of treated was slower than C2 and C1. TBARS increased progressively with storage period and decreased with increasing level of incorporation of LFP (Fig. 1).

TBARS was highest for C2 and lowest ($P<0.05$) for T3 on all the storage days. TBARS value varied inversely with the level of LFP in the formulation attributed to antioxidant properties (SUETSUNA, 2000).

Redness (a^*) increased significantly ($P<0.05$) in treated samples than control attributed to the innate reddish brown colour of LFP. The a^* values increased with increasing level of LFP, however the values decreased during storage. This might be due to oxidation of emulsion. It might be due to antioxidant effect of bioactive peptides in the fraction. During storage yellowness (b^*) decreased at a higher rate in C1 and C2 compared to treated groups. On 6th day the values of b^* were significantly ($P<0.05$) higher for the treated groups than C2. Similar results were reported by DEKKERS *et al.* (2011) in mahimahi red muscle treated with tilapia protein hydrolysates and by JIN *et al.* (2015) in chicken meat hydrolysate treated sausages.

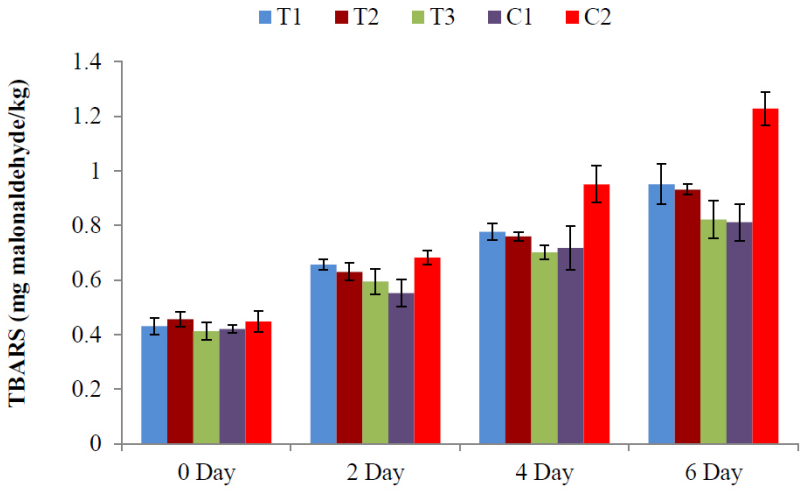

Figure 1. Effect of incorporation of different levels of trypsin generated goat liver peptide fraction (<1 kDa) on the TBARS (mg malonaldehyde/kg) of goat meat emulsion, on different days, during aerobic storage under refrigerated conditions.

SPC were lowest for T3 and highest for C2 on all the days (Fig. 2). SPC was more than 2.5 log cycle higher in control than T3. SPC decreased with the increase in level of LFP in the emulsion. ADJE *et al.* (2011) also reported dose dependent antimicrobial effect of peptides. There was decrease in the values of coliforms count upto 4th day in the treated groups and increased thereafter, whereas the count kept on increasing for C2 and C1 throughout storage. The antimicrobial peptides in the fraction might have caused this decrease in the counts. SUBBALAKSHMI and SITARAM (1998) also reported the antimicrobial efficacy in bioactive peptides. Psychrophills were detectable on day 2nd in C2, C1 and T1 groups while it continued to be undetected for T2 and T3 upto 4th day. However, the values were lower in the treated groups compared to the C2 and C1 attributed to antimicrobial activity of LFP.

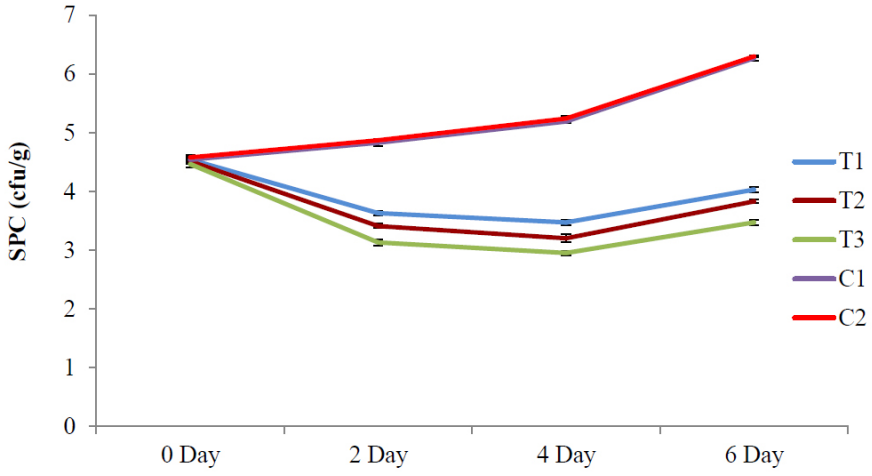

Figure 2. Effect of incorporation of different levels of trypsin generated goat liver peptide fraction (<1 kDa) on the SPC (cfu/g) of goat meat emulsion, on different days, during aerobic storage under refrigerated conditions.

4. CONCLUSIONS

From the results it is evident that goat liver tryptic hydrolysates can be utilized to generate bioactive peptides that have antioxidant as well as antimicrobial effects, and could effectively replace the chemical preservatives such as BHT, in the meat system.

REFERENCES

Adje E.Y., Balti R., Kouach M., Dhulster P., Guillochon D. and Nedjar-Arroume N. 2011. Obtaining antimicrobial peptides by controlled peptic hydrolysis of bovine hemoglobin. Int. J. Biol. Macromol. 49:143-53.

APHA.1984. "Compendium of Methods for Microbiological Examination of Foods" 2nd ed. American Public Health Association, Washington, DC.

Benzie I.F.F. and Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Annals Biochem. 239:70-77.

Brand-Williams W., Cuvelier M.E. and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28:25-30.

Dekkers E., Raghavan S.K., Hordur G., Kristinsson, Maurice R. and Marshall. 2011. Oxidative stability of mahimahi red muscle dipped in tilapia protein hydrolysates. Food Chem. 124:640-45.

Egan A.F. 1983. Lactic acid bacteria of meat and meat products. Antonie van Leeuwenhoek. 49(3):327-36.

Erdmann K., Cheung B.W.Y. and Schroder H. 2008. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. J. Nutr. Biochem. 19(10):643-54.

Gauthier S.F., Pouliot Y. and Saint-Sauveur D. 2006. Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. Int. Dairy J. 16(11):1315-23.

Gill C.O. 1979. Intrinsic bacteria in meat. J. Appl. Bacteriol. 47(3):367-78.

Jay J. M., Kittaka R. S. and Ordal Z. J. 1962. The effect of temperature and packaging material on the storage life and bacterial flora of ground beef. Food Tech. 16:95.

Jay J.M. 1964. Beef microbial quality determined by extract release volume (ERV). Food Technol. 18:1637-41.

Jay J.M., Loessner J.M. and Golden D.A. 2005. "Modern Food Microbiology" 7th ed. pp. 101-18, Springer publications, New York.

Jin S.K., Choi J.S., Choi Y.J., Lee S.J., Lee S.Y., Hur S.J.. 2015. Development of sausages containing mechanically deboned chicken meat hydrolysates. J. Food Sci. 80: S1563-S67.

Koniecko R. 1979. "Hand Book for Meat Chemists" Avery Publishing Group, Inc. Wayne, New Jersy, pp. 53-55.

Korhonen H., Pihlanto A. 2006. Bioactive peptides: production and functionality. Int. Dairy J. 16(9):945-60.

Rajanbabu V. and Chen J.Y. 2011. Applications of antimicrobial peptides from fish and perspectives for the future. Peptides. 32(2):415-20.

Romans J.R., Costello W.J., Carlson C.W., Greaser M.L. and Jones K.W. 2001. "Meat we Eat; Packing house by-products", Interstate Publishers, Inc./Danville, Illinois, USA, pp. 284.

Salami M., Yousefi R., Ehsani M.R., Razavi S.H., Chobert J.M. and Haertle T. 2009. Enzymatic digestion and antioxidant activity of the native and molten globule states of camel α-lactalbumin: possible significance for use in infant formula. Int. Dairy J. 19:518-23.

Sarmadi B.H. and Ismail A. 2010. Antioxidative peptides from food proteins: a review. Peptides. 31(10):1949-56.

Sebranek J.G., Sewalt V.J.H., Robbins K.L. and Houser T.A. 2005. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Sci. 69(2):289-96.

Shan B., Cai Y.Z., Brooks J.D. and Corke H. 2009. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. J. Sci. Food Agric. 89(11):1879-85.

Shelef L.A. and Jay J.M. 1970. Use of a titrimetric method to assess the bacterial spoilage of fresh beef. Appl. Microbiol. 19:902-05.

Subbalakshmi C. and Sitaram N. 1998. Mechanism of antimicrobial action of indolicidin. FEMS Microbiol. Lett. 160:901-10.

Suetsuna K. 2000. Antioxidant peptides from the protease digest of prawn (*Penaeus japonicus*) muscle. Marine Biotechnol. 2:5-10.

Vayalil P. K. 2002. Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaceae). J. Agric. Food Chem. 50(3):610-17.

Vioque J., Sanchez-Vioque R., Clemente A., Pedroche J. and Millan F. 2000. Partially hydrolyzed rapeseed protein isolates with improved functional properties. J. Am. Oil. Chem. Soc. 77:447-50.

Witte V.C., Krause G.F. and Bailey M.E. 1970. A new extraction method for determining 2-Thiobarbituric acid values of pork beef during storage. J. Food Sci. 35:582-85.

EFFECT OF *CYNARA CARDUNCULUS* EXTRACT ON THE SHELF LIFE OF AUBERGINE BURGERS

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ABSTRACT

Vegetable burger is a convenient processed food product exclusively prepared from non-meat ingredients. This research was intended to improve the shelf life of aubergine burgers with the addition of *Cynara cardunculus* extract characterized by a considerable presence of bioactive compounds. Two concentrations of extract were used for the preparation of aubergine burgers. Burger samples without *Cynara cardunculus* extract were prepared as control. Microbial load and sensory changes of vacuum-packed aubergine burgers were analyzed after 1 day, 30 days and 105 days of cold storage. The microbiological analyses were carried out during the shelf life test. The sensory profile method was used to measure any change in the sensory characters of samples, as a result of *Cynara cardunculus* extract treatment. Microbial growth observed values did not exceed the recommended limit, in particular yeasts were absent. The best sensory results were for samples prepared with 3% of *Cynara cardunculus* extract, especially on the attributes off-odour, off-flavour and overall. The control sample and the sample prepared with 1% of extract presented a low intensity of colour and firmness.

Keywords: aubergine, *Cynara cardunculus*, microbial load, sensory profile, vegetable burger

1. INTRODUCTION

Red meats are a rich source of animal fats that contain high amount of triglycerides, of saturated fatty acids as well as cholesterol. Recently, vegan substitutes for animal-based foods have become available; in particular, alternatives to cow's milk, including soy, almond and rice drinks. There are also yogurt, cheese, chicken and red meat vegan substitutes made of soy and/or other vegetable-based ingredients. Many of these products are made to resemble specific animal-based foods (MCILVEEN *et al.*, 1999). In the last years, the demand of consumers for veggie burgers has increased rapidly: in this context, vegetable burgers are convenient processed food products exclusively prepared from non-meat ingredients (ADISE *et al.*, 2015). Also, the demand for non-synthetic preservatives is increasing worldwide, such as antimicrobial compounds of natural origin, which should be not toxic for humans, environmentally safe, inexpensive and available in the market (MOHANKA and PRIYANKA, 2014). There is growing interest in using natural antimicrobial compounds, especially extract from plants, for the preservation of foods. Among these, *Cynara cardunculus* L. leaves, characterized by a considerable presence of bioactive compounds, is widely recognized for medical purpose, and the potential use of their extracts to control the growth of food pathogenic and/or spoilage microorganisms is at the beginning of investigation (ZHU *et al.*, 2004).
For vegetable burgers, the cooking process should significantly reduce the number of vegetative microbial cells and inactivate degradative enzymes. Consequently, spoilage of these products is primarily due to post-cooking contamination by microorganisms, which can be minimized by good hygiene and handling. The use of vacuum and long-term storage at refrigerated temperatures may promote the growth of psychrotrophic anaerobic/facultative anaerobic bacteria and yeasts allowing them to become dominant and deteriorate the product.
This research was intended to improve the sensory and microbiological shelf life of aubergine-based burgers with the addition of *Cynara cardunculus* extract characterized by a considerable presence of bioactive compounds.

2. MATERIALS AND METHODS

Two concentrations of extract were used (1% and 3%) for the preparation of aubergine-based burgers, indicated as Burger 1 and Burger 3, respectively. Burger samples prepared without *Cynara cardunculus* extract were taken as control (Burger C).
Microbial load and sensory changes of vacuum-packed aubergine-based burgers were analyzed at the processing day (t0), after 30 (t1) and 105 days (t2) of cold storage (4±1°C).

2.1. Preparation of extract

The *Cynara cardunculus* extract was prepared according to PANDINO *et al*. (2013).

2.2. Preparation of aubergine-based burgers

The following ingredients were added to prepare burgers for boiled and shredded eggplants: potatoes, onions, parsley, garlic, black pepper, nutmeg, dried tomato, thyme, salt, sugar, flour and maize starch, according to a consolidated industrial receipt (Terranèo Emozioni Siciliane srl, Vittoria, RG). The mixture obtained was given the form of 100g burgers, which were subsequently fried in sunflower oil for 40 seconds, cooled, vacuum-packed and refrigerated at 4°C.

2.3. Sensory analysis

The sensory profile method UNI 10957 (2003) was used to measure any change in the sensory characters of samples, as a result of *Cynara cardunculus* extract treatment. Twelve judges were trained (ISO 8586, 2012) in 4 sessions to familiarize with scales and procedures. The evaluation sessions were conducted in a sensory laboratory (UNI EN ISO 8589, 2010) from 11:00 a.m. to 12:00 a.m. in individual booths illuminated with white light. Randomized samples were evaluated by assigning a score between 1 (absence of sensation) and 9 (extremely intense), using five attributes (colour, firmness, off-odour, off-flavour and an overall assessment, expressed by considering all of the attributes). All data were acquired by a direct computerized registration system (FIZZ Byosistemes. ver. 2.00 M, Couternon, France).
The sensory data for each attribute were submitted to one-way ANOVA by the software package Statgraphics® Centurion XVI (Statpoint Technologies, INC.) using samples as factors. The significance was tested by means of the F-test. To differentiate the samples, the mean values were submitted to the multiple comparison test using the least significant difference (LSD) procedure.

2.4. Microbiological analysis

Total mesophilic and psychrotrophic bacterial counts, yeast count were determined on the samples. An aliquot (10 g) of burger was sterilely sampled from each package and homogenized with 90 mL of sterile physiologic solution in a Stomacher (Lab-Blender 400, Brinkmann, Westbury, NY, USA) for 30 s. The same diluent was used for subsequent decimal dilutions. The total mesophilic and psychrotrophic bacteria counts were performed on Plate Count Agar (PCA, Oxoid Ltd., Basingstoke, UK) with cycloheximide 0.1% solution (Oxoid), incubated, respectively, at 32°C for 24-48 h and at 4°C for 10 d; yeast count was carried out on Sabouraud Dextrose Agar (SDA, Oxoid) supplemented with chloramphenicol (0.1 g/L) incubated at 25°C for 48-72 h.
The microbiological counts, performed in triplicate, were expressed as log10 CFU/g of burger.

3. RESULTS AND DISCUSSION

3.1. Sensory analysis

Table 1 reports sensory attributes that significantly differentiated the burger samples during storage. The intensity (mean score) was reported only for the significantly different attributes.
All the attributes except for off-odour significantly differentiated the control burgers. At 105 days of storage there was a decrease of significative attributes. The attributes colour, firmness and overall significantly differentiated the Burger 1, while only the attribute colour and overall were significantly different for the Burger 3. Samples with 3% of extract had the highest intensity of overall score compared to the other samples at t1 and t2.

3.2. Microbiological analysis

Mesophilic bacterial counts of control burger (Burger C), burger supplemented with 1% extract (Burger 1) and with 3% extract (Burger 3) are shown in Table 2. Psychrotrophic

bacteria and yeasts were not detectable in any of the analyzed samples over the considered shelf life period.
The addition of *C. cardunculus* extract at 3% (v/w) completely inhibited the growth of mesophilic bacteria up to 30 d of refrigerated shelf life; in addition, the antibacterial effect of the extract persisted up to 105 d, since it significantly reduced mesophilic count by more than 1 log cfu/g, with respect to the unsupplemented burger sample.

Table 1. Mean scores of the significant sensory attributes.

Sample	Attribute	t0	t1	t2
Burger C	Colour	5.50±1.73^{b*}	4.87±1.17^{b}	3.75±0.48^{a}
	Firmness	5.87±1.69^{b}	5.25±0.83^{b}	3.50±1.50^{a}
	Off-flavour	5.12±2.03^{b}	4.87±1.90^{b}	2.87±0.78^{a}
	Overall	6.12±1.05^{b}	5.75±1.30^{b}	3.50±1.00^{a}
Burger 1	Colour	6.50±1.41^{b}	4.87±1.05^{a}	3.62±1.41^{a}
	Firmness	6.50±1.58^{b}	5.12±0.78^{a}	3.87±1.05^{a}
	Overall	6.50±1.00^{c}	5.12±0.93^{b}	3.87±1.05^{a}
Burger 3	Colour	6.00±1.32^{b}	5.25±0.97^{ab}	4.12±1.27^{a}
	Overall	8.00±1.39^{b}	6.00±1.00^{a}	6.00±0.99^{a}

*Values marked with different letters in the same row are significantly different ($p \leq 0.05$) according to the LSD multiple comparison test.

Table 2. Microbial counts of different burger samples throughout the refrigerated storage.

	t0	t1	t2
		log CFU/g	
Mesophilic bacteria			
Burger C	2.53	3.36	2.91
Burger 1	1.95	2.59	2.66
Burger 3	nd	nd	1.85

nd: not detectable (below the detection limit of plate count technique)

4. CONCLUSIONS

The present study showed the possibility of preparing a vegetable product without chemical preservatives. The addition of *Cynara cardunculus* extract has improved the sensory characteristics ensuring at the same time the microbiological stability of the product. As an additional advantage, the use of *C. cardunculus* extract allows to improve the nutritional quality of the product, with special regards for the antioxidant potential.

REFERENCES

Adise S., Gavdanovich I. and Zellner D.A. 2015. Looks like chicken: exploring the law of similarity in evaluation of foods of animal origin and their vegan substitutes. Food Qual. Prefer. 41:52-59.

ISO 8586 2012. Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors.

McIlveen H., Abraham C. and Armstrong G. 1999. Meat avoidance and the role of replacers. Nutr. Food Sci. 99:29-36.

Mohanka R. and Priyanka 2014. Plant extract as natural food preservative against spoilage fungi from processed food. Int. J. Curr. Microbiol. App. Sci. 3(8):91-98.

Pandino G., Lombardo S. and Mauromicale G., 2013. Globe artichoke leaves and floral stems as a source of bioactive compounds. Ind. Crops Prod. 44:44-49.

UNI 10957 2003. Analisi sensoriale – Metodo per la definizione del profilo sensoriale degli alimenti e bevande, Ente Nazionale Italiano di Unificazione, Milano, Italia.

UNI EN ISO 8589 2014. Analisi sensoriale – Guida generale per la progettazione di locali di prova, Ente Nazionale Italiano di Unificazione, Milano, Italia.

Zhu X., Zhang H. and Lo. R. 2004. Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. J. Agric. Food Chem. 52:7272-7278.

INFLUENCE OF STERILIZE CONDITION AND STORAGE TIME OF CANNED BAMBOO SHOOT IN YANANG JUICE

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ABSTRACT

The objective of this work was to study the sterilization condition for producing canned bamboo shoot in Yanang juice by retort sterilization at 110, 120 and 130°C, F_0 5 minutes. All samples were stored at room temperature with no light exposure for 6 months for shelf-life study. The effect of storage time on the quality of Yanang juice was determined regarding its viscosity, stability, color and microbiological content. The results showed that the stability of product decreases with increasing storage time for all samples. The higher sterilization temperature exhibited high stability that was taken shorter time to reach F_0 5 minutes leading to higher stability when comparing with lower sterilization temperature. On the other hand, viscosity, $\triangle E^*$ value decreased when high sterilization temperature was applied. During storage time for 6 months, there was no yeast and molds. The results showed that all microbiology tested are less than standard and no indicated flat sour bacteria could be detected after sterilization. By comparing with fresh Yanang juice, the sterilization at 120°C, F_0 5 minutes was recommended condition for canned bamboo shoot in Yanang juice. The results could be used as guidance for producing sterilized bamboo shoot in Yanang juice with a good quality.

Keywords: aroma, cabbage, carrot, minimally processed, modified atmosphere

1. INTRODUCTION

Thai people are very proud of their traditional and unique cuisine. The popular dishes are made of local vegetables, fruits and other ingredients. For example, bamboo shoot and Yanang juice are basic content to achieve the typical Thai flavor. In the Isan culture of northeast Thailand, Yanang juice is used in the preparation of "Kaeng no mai" that includes bamboo shoots, Yanang juice, chilis, salt and sometimes oyster mushrooms, cha-om. Yanag juice is used to make the broth, primarily as a thickening agent rather than for its flavor (PHUNGAMNGOEN *et al.*, 2016).

It is possible to storage bamboo shoot and Yanang juice up to one week without losing its quality. An improvement in shelf life would be a benefit for the local chefs as well as a chance for exporting the product to other countries. For extension of the self-life and preserve the quality of low acid canned food, "Thermal processing" is the most widely used for these objectives (CHAROEN and PHUNGAMNGOEN, 2016). The recommended conditions for effective sterilization of low acid food (pH > 4.6) in retort were 10 psi above atmospheric pressure for sterilization at 116°C, 15 psi at 121°C and 20 psi at 127°C (POTTER and HOTCHKISS, 1998). SEOW and GWEE (1997) recommends a minimum F_0 value is 5 minutes for low acid canned food. During storage, quality changes may occur causing from the non-enzymatic browning reaction and nutritional degradation including low stability of Yanang juice. Separation of Yanang juice into an aqueous phase and pigment phase is commonly occurred and this leads an unacceptably physical defect (PHUNGAMNGOEN *et al.*, 2016).

As mentioned above, there are many work studies on quality change during thermal process of canned bamboo shoot in Yanag juice. However, no information is so far available on the effect storage time on stability of sterilized Yanang juice. Therefore the objective of this work was to study the effect of storage on the quality of Yanang juice.

2. MATERIALS AND METHODS

2.1. Sample preparation

Fresh bamboo shoots were purchased from a local market. The bamboo shoot was washed, peeled, and sliced with dimensions of 3×10 ×0.3 cm. A sliced bamboo shoot was blanched in boiling water for 15 min.

Yanang leaves were purchased from a local market. The ratio Yanang leaves to water was 1:6 and then sample were blending at 7000 rpm for 1 min using blender (HR 2115 600W 2L, Philips, Indonesia). Xanthan gum 0.06 %w/v was added in Yanang juice while the samples were heated and stirred continuously until sample temperature reached 80°C for 2 min.

2.2. Retort processing

Approximately 500 g of sample (bamboo shoot and Yanang juice at the ratio 1:1.5 was filled in a can of size 307×409. The samples were drained and filled with Yanang juice maintaining a headspace of 1 cm. The cans were fixed with thermocouple glands (Ellab, Denmark), and the thermocouple probe (Ellab, Denmark) was inserted through it. The can was fitted with a thermocouple at cold point of the can. The cans were exhausted in steam for 10 minutes to remove the residual air, and immediately double seamed. The sealed cans were loaded inside the horizontal still retort (Patkol, Thailand) and processed at temperatures of 110, 120 and 130°C under pressure to the standardized F_0 value of 5

minutes. Time-temperature data were collected during heat processing using an Ellab data recorder (CTF 9008, Ellab, Denmark).

2.3. Percentage of separation

To evaluate the percentage of separation, the sterilized samples were kept at room temperature (~ 30°C) for 3 days and determined as follow;

$$\%PS = \frac{\text{Height of water}}{\text{Height of whole Yanang juice}} \times 100 \quad (1)$$

2.4. Determination of physical properties

Color of samples was measured in a Hunter colorimeter (Hunter Lab, Model Colorflex 45/0, Virginia). Reflection spectra were registered and Hunter Lab color parameters for 10° vision angle and D65 illuminant were calculated. The total color change (ΔE^*) was calculated as:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

The apparent viscosity of the sample was determined at 25°C using Brookfield viscometer, Model LVDV-II+ Pro (Brookfield laboratories, Massachusetts) at 100 rpm. All determinations were made in triplicate and the results were expressed as the mean values.

2.5. Microbiological analysis

Microbiological analysis conducted on these containers showed their safety for thermal processing. The viable microbial numbers were enumerated by pour plating onto Plate Count Agar (PCA, Difco, USA) incubated at 37 °C for 48 h for total bacteria and onto Potato Dextrose Agar (PDA, Difco, USA) incubated at 30 °C for 72 h for yeasts and molds. The finial products were sent to The Institute of Food Research and Product Development of Kasetsart University to investigate flat sour bacteria (mesophile and thermophile).

2.6. Statistical analysis

All the experiments were conducted in three replications. Significant differences between means were determined using statistical package for social science (SPSS).

3. RESULTS AND DISCUSSION

3.1. Retort processing of product

Figure 1 shows the temperature profiles of sample during sterilization process. Sterilization process with different temperature showed the same behavior (data not shown). The initial temperature of the samples prior to heating was approximately 60°C. Temperature of sample increased continuously as the heating time increased and approached the set target value (F_0 = 5 min). During heating process, the heat transfer mechanisms in products were conduction and convection (POTTER and HOTCHKISS,

1998). Yanang juice is heated more quickly than the pieces of bamboo shoots (AWUAH *et al.,* 2007). Processing time of canned bamboo shoot including come up time, heating time and cooling time was 205, 105, 80 min for 110, 120 and 130°C, respectively.

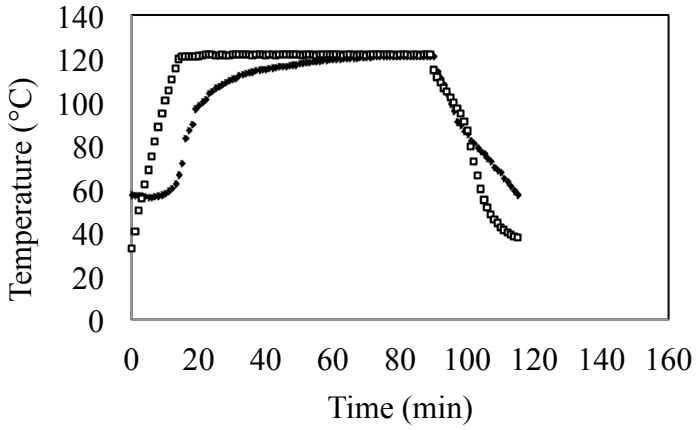

Figure 1. Temperature profile at cold point of product during sterilization process in retort.

3.2. Quality of Yanang juice

Table 1 shows stability of Yanang juice with different sterilized temperature and after storage for 6 months. During storage, %PS increased with increasing time. Heating at 110°C showed the highest %PS but at 120 and 130 °C, %PS were not observed for stability of sample. After 3 months %PS exhibited stable because Xanthan gum (stabilizing agent) can caused an increase in viscosity of water phase therefore it could protect the gravitational separation of the pigment phase (AUTAPAB and CHAROENKUL, 2012).

Table 1. Percentage of separation of Yanang juice.

Storage time (month)	Percentage of separation (%)		
	110°C	120°C	130°C
0	18.86±3.11^{cd}	15.32±2.04^{bc}	14.02±2.44^{cd}
1	19.63±3.65^{bc}	17.33±4.17^{abc}	15.15±3.01^{bcd}
2	20.88±1.09^{bc}	19.89±2.34^{ab}	17.77±3.33^{ab}
3	24.31±1.88^{ab}	21.22±1.67^{a}	19.63±3.23^{ab}
4	24.50±3.54^{abc}	22.24±3.06^{ab}	21.65±2.11^{a}
5	25.21±1.77^{ab}	22.52±2.33^{a}	21.33±2.07^{a}
6	26.23±2.67^{a}	22.63±2.04^{a}	21.76±1.97^{a}

Values in the same column with different superscripts mean that the values are significantly different (p<0.05).

Table 2 shows ΔE^* of sample that was calculated by comparing with the color of fresh Yanang juice. ΔE^* decreases linearly with increased storage time. This was due to the heat stability of chlorophyll in Yanang juice (SUWANKANID *et al.,* 2006; PHUNGAMNGOEN *et al.,* 2016). ΔE^* value at 110 °C was higher than 120 and 130°C, respectively. Because

process at 110°C takes a lot more time than the process at 120 and 130°C. Low temperature and long time of sterilization process result in high dark green color of Yanang juice.

Table 2. Total color change of Yanang juice.

Storage time (month)	Total color change (△E^*)		
	110°C	120°C	130°C
0	20.07±0.45^{b}	14.33±0.98^{b}	13.01±2.03^{bc}
1	19.66±0.88^{b}	14.69±0.77^{b}	12.65±1.98^{c}
2	20.53±1.23^{b}	15.21±1.02^{b}	12.98±1.02^{c}
3	20.26±1.17^{b}	16.22±1.05^{b}	13.29±0.96^{c}
4	20.88±1.97^{b}	19.47±1.31^{a}	15.63±0.59^{b}
5	27.96±1.78^{a}	19.42±1.52^{a}	17.43±1.43^{a}
6	29.87±2.01^{a}	18.78±1.67^{ab}	18.27±1.08^{a}

Values in the same column with different superscripts mean that the values are significantly different (p<0.05).

Table 3 shows viscosity of Yanang juice. The product was retorted at 130°C showed almost the same range with the product from 120 and 110°C. There was no recognizable distinction between different temperatures or in progress of storage time.
Table 4 shows total bacteria in product after canning process. There was no yeast and molds detected. According to the THAILAND NATIONAL STANDARD (2014) the canned ready to eat food should contain the numbers of total bacteria, yeast and molds less than 100 CFU/g. The results showed that all microbiology tested are less than standard and no indicated flat sour bacteria could be detected after sterilization.
Canning of bamboo shoot in Yanang juice was compare with fresh Yanang juice. Heating at 120 and 130 °C exhibited the same value in %PS, △E^*, viscosity and total bacteria. Therefore, sterilizing temperature at 120 °C under pressure to obtain F_0 value of 5 min was the recommended condition for producing canned of bamboo shoot in Yanang juice.

Table 3. Viscosity of Yanang Juice.

Storage time (month)	Viscosity (cP)		
	110°C	120°C	130°C
0	45.02±2.77^{a}	43.86±3.01^{a}	42.97±2.37^{a}
1	45.03±1.97^{a}	43.11±2.98^{a}	42.53±2.76^{a}
2	44.50±1.65^{b}	42.40±3.29^{a}	40.79±2.53^{abc}
3	43.33±2.03^{ab}	42.54±3.22^{ab}	41.35±1.89^{ab}
4	42.79±2.55^{abc}	38.97±3.18^{b}	37.02±2.07^{c}
5	42.53±2.87^{abc}	38.54±2.09^{b}	37.77±2.09^{bc}
6	42.89±2.67^{abc}	38.93±2.78^{b}	37.12±2.33^{c}

Values in the same column with different superscripts mean that the values are significantly different (p<0.05).

Table 4. Total bacteria in product after canning process.

Storage time (month)	Total bacteria (CFU/g)		
	110°C	120°C	130°C
0	ND	ND	ND
1	ND	ND	ND
2	ND	ND	ND
3	17±2.67	ND	ND
4	53±1.88	28±3.17	22±2.45
5	57±401	13±2.12	18±2.97
6	62±5.63	27±2.18	23±2.67

ND = Not detected.

4. CONCLUSIONS

Quality changes of Yanang juice during storage canned bamboo shoots in Yanang juice were investigated. The results showed that high-temperature sterilizing caused quality degradation less than low-temperature; longer sterilizing time to obtain F_0 = 5 min. All microbiology tested was less than standard. After kept the product for 3 months, all qualities showed the same value during storage. Optimum set of conditions were obtained to achieve required goals at sterilizing temperature 120°C by comparing with fresh Yanang juice. Next target would be to retain maximum amount of antioxidants using different sterilizing conditions.

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REFERENCES

Autapab D. and Charoenkul N. 2012. Chemical properties of carbohydrate-hydro coriol and industrial application. Carb Tech. KMUTT. Thailand. Available at: SLIM2017_PHUNGAMNGOEN.docxhttp://eu.lib.kmutt.ac.th/elearning/Courseware/BCT611/chapter4.html.

Awuah G. Ramaswamy H. and Economides A. 2007. Thermal processing and quality: Principles and overview. Chem. Eng. Process 46:584-602.

Charoen R. and Phungamngoen C. 2016. Product development of canned Thai food by thermal processing. Int. Food Res. J. 23(3):320-1326.

Phungamngoen C., Eadmusik S., Chenin S. and Aichayawanich, S. 2016. Study of factors affecting stability of Yanang juice in canned bamboo shoot in Yanang juice process. The 9th TSAE internation conference, Bangkok, Thailand 8-10 September.

Potter N. and Hotchkiss J.H. 1998. Food Science. 5th Ed. Philadelphia: Aspen Publisher Inc.

Seow C.C. and Gwee C.N. 1997. Review, coconut milk: chemistry and technology. Int. J. Sci. Tech. 32: 189-201.

Suwankanid C., Chaungchaichana V. and Srisupho S. 2006. Production of bamboo shoot and Yanang Juice in suitable packaging. Suan-Dusit University. 72 pages.

Thailand National Standard 2014. "Hermectically sealed container" Notification of the Ministry of Public Health (No. 355), Bangkok, Thailand.

USE OF CHITOSAN AND CHITOSAN-CASEINATE COATING TO PROLONG SHELF LIFE OF MINIMALLY PROCESSED APPLES

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ABSTRACT

The effect of biopolymer coatings (1% chitosan, 2% chitosan, caseinate/chitosan blend at ratio 2/1) in combination with anti-browning agent (1% citric acid, 1% l-ascorbic acid agents, 1% $CaCl_2$) on the shelf life of minimally processed apples was studied. The experimental works were divided in two phases: in the first phase, the coating composition has been selected on the bases of microstructural analysis of the coatings on the fruit surface (SEM), respiration rate, water resistance, and color. In the second phase of the study, the shelf life of packed minimally processed apples coated with the selected coating was studied during storage at 5°C. The uncoated sample dipped in anti-browning solution was used as control. The following quality attributes were monitored after 1, 3, 7, 11 and 14 days: pH, hardness, relative humidity (RH %) and color. All coatings effectively reduced respiration rate of minimally processed apples but only caseinate/chitosan one was not significantly different from the control sample in terms of color attributes ($p<0.05$). Shelf life study showed that the chitosan-caseinate coating was able to extend the product shelf life from 7 days to 11 days at 5°C.

Keywords: edible coating, minimally processed fruits, quality indices, respiration rate, shelf life

1. INTRODUCTION

The growing potential of minimally processed fruits market is still very low due to the limiting shelf life of the product (3-5 days at 5°C). The shelf life of minimally processed fruits depends on respiration rate, transpiration rate and enzymatic browning. A way to prolong product shelf life is to use edible coating. Coating based on carboxymethyl cellulose, tapioca starch, chitosan, alginate in combination with antioxidant additive has been used to improve the shelf life of minimally processed apples (QI *et al.*, 2011; PAN *et al.*, 2013; ROJAS-GRAU *et al.*, 2008; SABA *et al.*, 2016). Milk proteins, such as caseinates, have special properties that make them highly suitable for biopolymer films. Films made of sodium caseinate are colorless, tasteless, odorless, transparent, flexible, highly impermeable to oil and oxygen and resistant to thermal denaturation (SCHOU *et al.*, 2005). To our knowledge, no publication has been reported on caseinate-chitosan blend films applied on fresh-cut apples. In this work chitosan film and blend of chitosan and caseinate has been studied. The objective of the work was to evaluate the effect of biopolymer coatings of different composition on product shelf life. To this aim, the structure of the coatings on apples was studied by electron microscopy (SEM); the quality of the product was evaluated by studying the respiration rate, the water loss, the color and the mechanical properties of the product.

2. MATERIALS AND METHODS

2.1. Preparation of film forming solutions

The chitosan (CH) solution (1% and 2%) was prepared by weighing 1 g or 2 g of chitosan in 100 mL of 1% acetic acid solution (v / v) and stirred for 16 h. The sodium caseinate solution (SC) (4%) was prepared by weighing 4g of SC in 100 mL of PBS buffer and meanwhile agitated with a magnetic stirrer for 4 h. Blend solution (SC/CH=2), was prepared by adding drop wise the solution of SC to CH with 1: 1 ratio. To each solution it was added an aliquot of glycerol, such as to obtain a glycerol / total solids ratio equal to 0.1.

2.2. Coating application

Apples were selected for uniform size and appearance, without damages and gently washed with tap water for 3 minutes. Then, apples were peeled and cut in 16 slices and dipped in the following solutions for two minutes : (1) Anti-browning solution of 1% citric acid, 1% ascorbic acid, 1% calcium chloride (A), (2) solution A + 1% of chitosan (CH1%), (3) solution A + 2% chitosan (CH2%), (4) solution A + SC/CH blend (SC/CH). Coated and uncoated apple slices were put on a grid and allowed to dry into an air tunnel (Armfield tray drier) at 30°C for 40 minutes.

2.3. Characterization of fresh product

After processing, the minimally processed apples were characterized in terms of microstructure, respiration rate, water resistance and color surface.

2.3.1. Microstructure analysis

Microstructure analysis of the surface and of the section of the apples slide was examined using an LEO EVO 40 scanning electron microscope (Zeiss, Oberkochen, Germany). Lyophilized samples of apples slide were metallized with a metallizer (AGAR B7340, Agar Scientific Ltd, Stansted, UK) and examined with a 20kV accelerating voltage to a variable magnification from 200 to 1500 X.

2.3.2. Respiration rate measurement

Apple slices (500 g), coated or uncoated, were stored in a steel container (0.004 m^3) at 5°C, 10°C and 20°C in air for a variable time. Respiration rate has been measured as reported in TORRIERI *et al.* (2010).

2.3.3. Water resistance

Apple cylinder samples (surface area: 12.6 cm^2) were equilibrated for 24 h at 20±1°C and 97% of relative humidity. After 24 h, the samples were placed in two different conditioned systems: (1) at 20°C and 60% of RH; (2) at 5°C, 10°C and 20°C into desiccators containing silica gel. Weight was taken periodically during a 24 h period and the water vapor flux per unit of surface area (g s^{-1} cm^{-2}) was determined as follow:

$$WF = \left(\frac{dP}{dt}\right) \times \left(\frac{1}{A}\right) \quad (1)$$

Where dP/dt is the water vapor flux (gs^{-1}); A is the area exposed. The water vapor resistance (WVR) which is the resistance of the coating to water diffusion (s cm^{-1}) was calculated as:

$$WVR = \left[\left(\frac{a_w - \frac{RH}{100}}{RT}\right) \times P_{WV}\right] \times \left(\frac{1}{WF}\right) \quad (2)$$

Where: a_w is the water activity of the sample; RH is the relative humidity of the environment; P_{WV} is the saturated water vapor pressure (mmHg); R is the gas constant (mmHg cm^3 K^{-1} g^{-1}); T is the temperature (K). Each measurement was conducted in triplicate. Water activity of the apples was measured with an Aqua lab (Water activity meter 4TE, USA) at 20°C.

2.3.4. Color measurement

The color of apple slices was measured with a colorimeter (Minolta Chroma MeterMod. CR 300, Osaka, Japan). The degree of browning was expressed as the change in the L* value, a*, b*, ΔE*.

2.4. Packaging and storage condition

Apples samples (88g) were packed in air by using polypropylene pouch and stored at 4°C for 14 days. After 1, 4, 7, 11, and 14 days the color, the pH, the mechanical properties were studied as reported in the following sections.

2.4.1. Color, pH, mechanical analysis

The color has been measured as reported in section 2.3.4. PH has been measured by using a pH meter (pH 8 series, Eutech Instruments Pte Ltd., Singapore). The mechanical properties have been measured by using a texture analyzer (TMS-Pro Texture Analyzer Food Tech Corporation) and by performing a penetration test (probe with round head; load cell: 50 N; penetration rate: 30 mm min^{-1}).

3. RESULTS AND DISCUSSION

3.1. Microstructure analysis, respiration rate and water resistance analysis

Samples CH2% and SC/CH blend covered the apple surface in a homogenous way with a thickness of approximately of 10mm (Fig. 1).

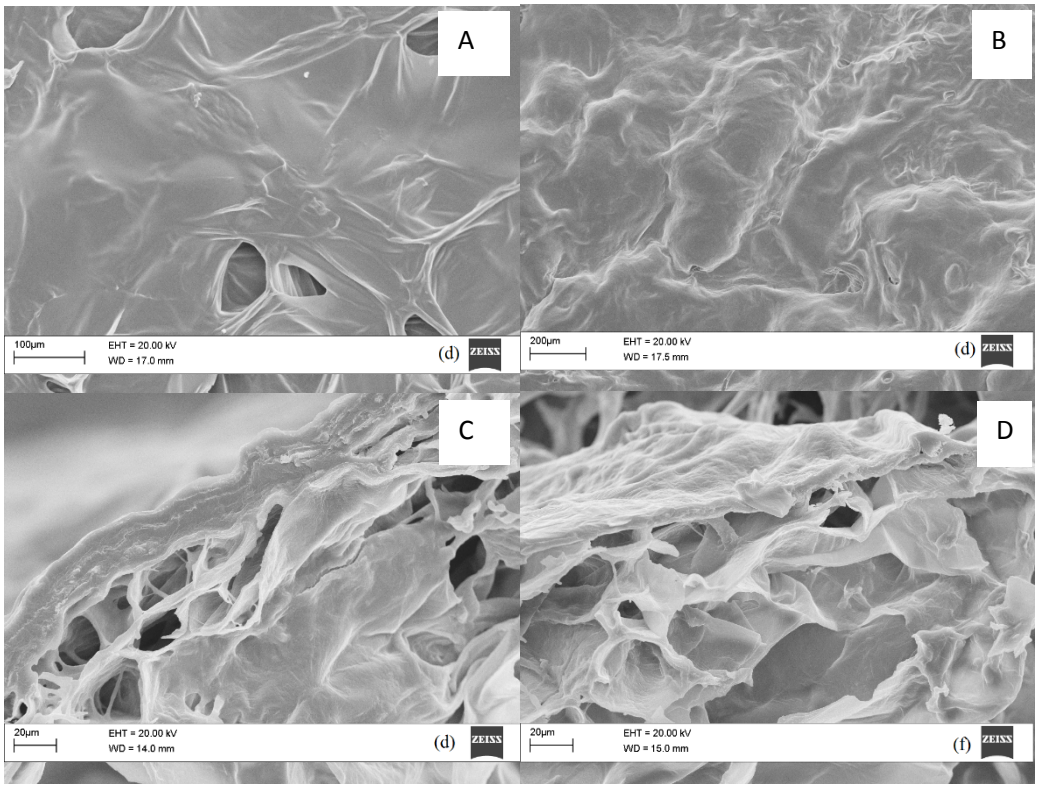

Figure 1. Surface (A, B) and section (C, D) of minimally processed apples coated with CH 2% and SC/CH blend, respectively.

At all studied temperatures the coating reduced the respiration rate of 50% or more as function of coating composition. The best results were obtained by using the SC/CH (Fig. 2). The WVR was not affected by coating, whatever the coating composition (data not shown). Samples SC/CH was the only one that did not show any difference from control samples in term of color parameter. The others coatings showed a negative impact on it. For these reasons, sample SC/CH has been chosen as coating for minimally processed apples. Preliminary results showed that the limiting factors for the product shelf life were appearance and texture properties. During storage time, relative humidity did not change

and the effect of coating was not statistically significant. The RH assumed an average value of 86%. The coating was able to reduce the color variation during the first seven days, whereas no differences were observed between coated and uncoated samples at 11 and 14 days. After 11 days samples were still acceptable in terms of color. Moreover, the increment of pH of minimally processed apples was reduced by using the coating up to 12 days (data not shown). The hardness of samples coated with SC/CH was constant during the 14 days of storage, whereas for uncoated samples a big reduction of hardness has been observed after 7 days of storage (Fig. 3).

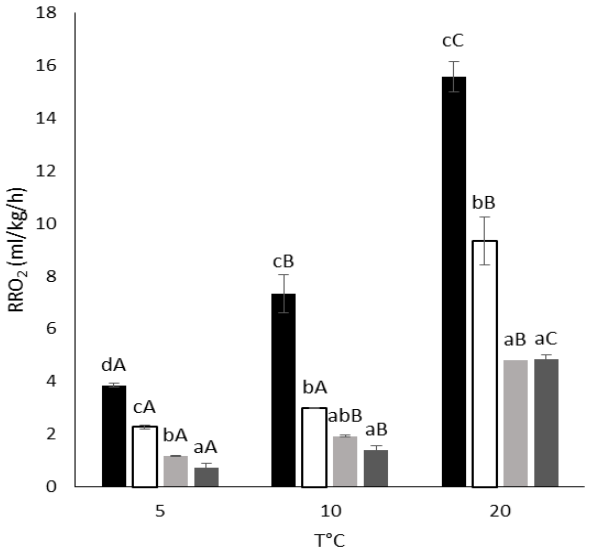

Figure 2. Respiration rate (RR_{O2}) of minimally processed apples as function of temperature.
■ control, □ CH1%, □ CH2%, □ SC/CH.

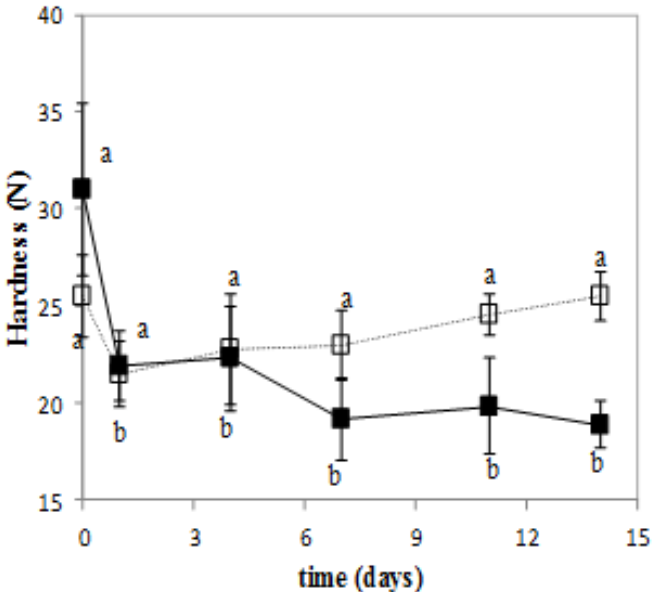

Figure 3. Hardness (N) of minimally processed apples during storage at 5°C.
□ control ■ SC/CH blend.

4. CONCLUSIONS

All coating tests did not performed very well as water vapor barriers. Nevertheless, the relative humidity of apples was constant during storage. All coatings were able to reduce the respiration rate of minimally processed samples, but the SC/CH coating was chosen due to its ability to preserve also the color of the product. Results showed that the coating was able to prolong the minimally processed apple shelf life to 11 days at 5°C.

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REFERENCES

Pan S.Y., Chen C.H. and Lai L.S. 2013. Effect of tapioca starch/decolorized hsian-tsao leaf gum-based active coatings on the qualities of fresh-cut apples. Food Bioproc. Technol. 6:2059-206.

Qi H., Hu W., Jiang A., Tian M. and Li Y. 2011. Extending shelf-life of Fresh-cut 'Fuji' apples with chitosan-coatings. Innov. Food Sci. & Emerg. Technol. 12: 62–66.

Rojas-Grau M.A., Tapia M.S. and Martin-Belloso O. 2008. Using polysaccharide-based edible coatings to maintain quality of fresh-cut fuji apples. Food Sci. Technol. 41:139-147.

Saba M. K. and Sogvar O. B. 2016. Combination of carboxymethyl cellulose-based coatings with calcium and ascorbic acid impacts in browning and quality of fresh-cut apples. LWT - Food Sci. Technol. 66:165-171.

Schou M., Longares A., Montesinos-Herrero C., Monahan F.J., O' Riordan D. and O'Sullivan M. 2005. Properties of edible sodium caseinate film and their application as food wrapping. LWT-Food Sci. Technol. 3:605-610.

Torrieri E., Perone N., Cavella S. and Masi P. 2010. Modelling the respiration rate of minimally processed broccoli (*Brassica rapa var. sylvestris*) for modified atmosphere package design. Int. J. Food Sci. Technol. 45(10):2186-2193.

PROBIOTIC ENCAPSULATION BY SPRAY DRYING USING KONJAC GLUCOMANNAN HYDROLYSATE AS WALL MATERIAL AND ITS APPLICATION IN ICE CREAM

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ABSTRACT

This research aims to study encapsulation efficiency of cell probiotics by using Konjac glucomannan hydrolysate (KGMH) under spray drying. KGMH was prepared from Konjac glucomannan solution (KGMS) at different concentrations. *Lactobacillus casei* was used in this study as it is commonly used in food industry and resistance to heat. The effect of wall material (HGMH) concentration on encapsulation efficiency of the cell probiotic was evaluated. Probiotics culture was prepared by inoculating in sterile MRS broth and incubated at 37°C for 48 hours, the cells were harvested in the early stationary phase. Konjac glucomannan solution was hydrolysed by mannanase at 40°C for 4 hours. Then, probiotic solution (10^8 log CFU/ml) was mixed with KGMH at concentration of 15%, 20% and 25% (w/w). Spray drying condition was kept at constant inlet air temperature of 170°C, outlet air temperature of 85°C and feed flow rate of 10 mL min^{-1}. Encapsulated probiotic with KGMH at concentration of 25% (w/w) exhibited the highest encapsulation efficiency (95.4 %), water activity of 0.198 and moisture content of 4.38 % (w.b.). For application in ice cream, microcapsules of probiotic encapsulated with KGMH at concentration of 25% with and without 10% (w/w) trehalose were then mixed into milk ice cream separately. The ice cream was stored at -18°C for one month. The survival rate of microencapsulated probiotic cells and free cells probiotics in the ice cream were measured. It was found that microcapsule of KGMH at concentration of 25% (w/w) with 10% (w/w) trehalose gave higher survival rate of probiotics cell (> 90%) in ice cream under freezing temperature than KGMH at concentration of 25% (w/w) and free cell probiotics during storage, respectively.

Keywords: encapsulation, glucomannan hydrolysate, probiotics, spray drying, trehalose

1. INTRODUCTION

Probiotics are live microorganisms that help in maintaining a good balance and composition of intestinal microflora and increase the resistance against the invasion of pathogens (KUMAR and SALMINEN, 2016). Ice creams is a well-accepted food product worldwide and an ideal food carrier for delivering probiotics to humans (CRUZ *et al*., 2009). However, the development of probiotic ice creams requires the overcoming of freezing process, which is unsuitable environment for probiotic microorganism. Encapsulation is one of alternative method that can be used to maintain viability of microorganism through unsuitable environment of ice cream (AHMADI *et al*., 2014; HOMAYOUNI *et al*., 2008). Konjac Glucomannan (KGM) is neutral polysaccharides which is high viscosity can be acting as stabilizer and entrapment of bioactive compounds (BEHERA and RAY, 2016; YI *et al*., 2017) as same as glucomannan hydrolysate (KGMH) was studied as wall material for antimicrobial, flavor and nutraceutical powder (ADAMIEC, 2012; WATTANAPRASERT *et al*., 2016; YANG *et al*., 2009). Moreover, spray drying is frequently applying in food powdering but its high temperature leads to low survival rate of probiotic microorganism. Therefore, this study focused on encapsulation efficiency of microcapsule from KGMH on survival probiotic bacteria after spray drying and application in food system as ice cream.

2. MATERIALS AND METHODS

2.1. Preparation of *Lactobacillus casei*-01

A frozen stock of *Lactobacillus casei*-01® (Christen- Hansen, Horsholm, Denmark) was used in this study. For activation, 0.4 mL from frozen stock was sub-cultured in 40 mL of MRS broth (Difco, USA), incubated at 37°C for 48 hours. Cell suspension was centrifuged at 2500 rpm for 15 min and washed twice with peptone. After that, one gram of cell suspension in peptone water was double-layer-pour-plated for oxygen preventing and incubated at 37°C for 72 hours aim to confirming the numbers of viable cell.

2.2. Preparation of microcapsule

Konjac glucomannan solution (KGMS) is used for hydrolysis reaction by mannanase enzyme (activity of 30,000 unit/g) by using concentration of 125 units per gram KGMS. KGMS concentration was varied at 15, 20 and 25% (w/w). Reaction was done in 4 h at 40°C along with stirring by overhead stirrer at 200 rpm. After that, heated at 70°C for 20 min to inactivate enzyme. Trehalose (T) was only added to 25% glucomannan hydrolysate solutions in the amount of 10% (w/w). Cell suspension of *Lactobacillus casei* (10^8 log CFU/ml) was added to the mixture at 10 g for each treatment (200g).

2.3. Spray drying

Cell suspension with different treatment of microcapsule were spray dried in co-current Mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland) by using inlet air temperature at 170°C, outlet air temperature at 85°C, feeding rate at 10 ml/min. Before spray drying, sterile water was sprayed for preventing contamination from equipment. Loaded samples were stirred with magnetic bar during drying process. Spray dried microcapsules were then analyzed as followed: Water activity (a_w) was measured by using Water Activity Meters (model 3TE, Aqua Lab, USA), Moisture content (%) was measured

by using moisture tester (Mettler Toledo HR43-S), encapsulation efficiency (%) was estimated as the ratio of cell viable from in microcapsule to theoretical cell viable in the preparation and yield (%) was measured and calculated according to formula (1);

$$\%\text{Yield} = \frac{\text{weight of powder after drying (g)} \times 100}{\text{weight of total solid content in the feed before drying (g)}} \quad (1)$$

2.4. Microbiological determination in microcapsule

One gram of spray dried microcapsules was re-suspended in 9 mL peptone water and vortex until all sample dissolve. Cell suspension was diluted and double-layer-pour-plated on MRS agar and incubated at 37°C for 72 hours. Number of cell viable were calculated (cfu/g). Survival rate was calculated according to formula (2):

$$\%\ \text{Survival rate} = \frac{\text{log cfu/mL viable cells after spray drying} \times 100}{\text{log cfu/mL viable cells before spray drying}} \quad (2)$$

2.5. Preparation of probiotic ice cream

The ice cream mix was formulated as follows: 21.2% milk powder, 11.35% butter, 6% sugar, 0.75% egg yolk, 0.05% salt, 0.55% gelatin and 60% water. In the production of probiotic ice cream, water was heated at 45°C for 5 min and then powder mixtures were added. All mixture was homogenized by homogenizer (ULTRA-TURRAX® T25, IKA, Germany) for 10 minute and pasteurized at 80°C for 30 second. Afterwards, ice cream was mixed with different probiotic inoculums include free cell culture, KGMH microcapsule and KGMH with 10% trehalose microcapsule and then blended them in ice cream maker for 30 minute. The frozen ice cream in plastic cups (200 mL in volume) was transferred to a freezer at -18°C for hardening and storage for 28 days. Microbiological quality in ice cream was determined on 0 and 28 days. Ten gram of ice cream was re-suspended in 90 mL peptone water. Cell suspension was diluted and double layer pour plated on MRS agar and incubated at 37°C for 72 hours. Number of cell viable was calculated as log CFU/g.

2.6. Statistical analysis

All analyses were performed in triplicate. Statistical analyses were carried out with Duncan's multiple range test ($P<0.05$) using SPSS statistical software (SPSS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

Physical properties of probiotic microcapsules were measured and are showed in Table 1. The microcapsules obtained in white powder form and their moisture content is in range of 3.91-4.54 % (w.b) while the water activity is ranged from 0.145-0.198.

Concentration of KGMS before hydrolysis has influenced the encapsulation efficiency and yield. Increasing the concentration of KGMH increased encapsulation efficiency while adding 10% trehalose decreases the encapsulation efficiency and yield. Trehalose may attract some free water during preparation and spray drying that resulted in osmotic stress of probiotic and decreases its viability. KGMH 25 gave a highest encapsulation efficiency of probiotic viability cell (95.4%) and acceptable yield (28.5%) is selected with

KGMH 25 +10T to apply in ice cream for 28 days storage at -18°C and the survival rates of the probiotic are shown in Table 2.

Table 1. Physical properties of probiotic microcapsules.

Wall materials[1]	% Moisture content (w.b.)	Water activity	Encapsulation efficiency (%)	% Yield
KGMH 15	4.54±0.02^{d}	0.185±0.01^{b}	79.6 ±7.49^{bc}	31.5±2.05^{a}
KGMH 20	4.32±0.28^{b}	0.198±0.02^{a}	95.4±3.25^{a}	20.1±0.02^{d}
KGMH 25	4.38±0.22^{c}	0.198±0.03^{a}	86.8±1.25^{b}	28.5±0.74^{b}
KGMH 25 + 10T	3.91±0.22^{a}	0.145±0.00^{c}	78.1±4.49^{c}	22.9±7.64^{c}

[1]KGMH 15, 20, 25 is prepared from KGMS at 15, 20, 25% (w/w), respectively.

Table 2. Cell viability in ice cream after storage for 28 days.

Storage (day)	Cell viability (log CFU/g)		
	Control	KGMH 25	KGMH 25 + 10 T
0	7.24±0.23^{a}	7.04±0.28^{a}	6.64±0.04^{a}
28	7.14±0.22^{a}	6.92±0.11^{b}	6.56±0.04^{b}
% Survival	98.6^{AB}	98.3^{B}	98.8^{A}

The results show that KGMH 25 can protect the probiotic in ice cream during frozen stage. The survival rate is more than 90%. However, adding 10% trehalose may reduce the survival rate of the probiotic after spray drying but during storage, it can maintain the survival rate of the probiotic slightly more than KGMH 25. Moreover, all of conditions in this study still have viable cell number more than 10^{6} CFU/g which followed by International Dairy Federation (IDF) declared that food contained probiotic shall be viable probiotic cell not less than 10^{6} CFU/g at the end of its shelf life (BANSAL *et al*., 2015). HOMAYOUNI *et al*. (2008) investigated the effect of microencapsulation by resistant starch on the survival of *Lactobacillus casei* added in a synbiotic ice cream for 30 and 180 days. Percentages of survival of encapsulated cells found only 53% at 30 days which less than present study and encapsulated cells required longer time to decrease one log cycle in viable counts. Moreover, *L. casei* is large cell, which is much more less sensitive to freezing damage. Therefore, extended ice cream storage should be further studied for obtaining more clear trend of probiotic survival rate.

4. CONCLUSIONS

KGMH 25 is able to protect probiotic from heat treatment during spray drying and be able to maintain cell viability during freezing for 28 days. However, when adding 10% Trehalose to KGMH 25, survival rate is dropped significantly during spray drying due to osmotic stress, but it can maintain survival rate of probiotic when applying the probiotic powder into ice cream that kept at -18°C for 28 days.

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REFERENCES

Adamiec J., Borompichaichartkul C., Srzednicki G., Panket W., Piriyapunsakul S. and Zhao J. 2012. Microencapsulation of Kaffir Lime Oil and Its Functional Properties. Drying Technol. 30:914-20.

Ahmadi A., Milani E., Madadlou A., Mortazavi S.A., Mokarram R.R. and Salarbashi D. 2014. Synbiotic yogurt-ice cream produced via incorporation of microencapsulated lactobacillus acidophilus (la-5) and fructooligosaccharide. J. Food Sci. Technol. 51(8):1568-1574.

Bansal S., Mangal M., Sharma S.K. and Gupta R.K. 2016. Non-dairy based probiotics: A healthy treat for intestine. Crit. Rev. Food Sci. Nutr. 56(11):1856-67.

Behera S.S. and Ray R.C. 2016. Konjac glucomannan, a promising polysaccharide of *Amorphophallus konjac* K. Koch in health care. Int. J. Biol. Macromol. 92:942-56.

Cruz A.G., Antunes A.E., Sousa A.L.O., Faria J.A. and Saad S.M. 2009. Ice-cream as a probiotic food carrier. Food Res. Int. 42(9):1233-1239.

Homayouni A., Azizi A., Ehsani M.R., Yarmand M.S. and Razavi S. H. 2008. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chem. 111(1):50-55.

Kumar, H. and Salminen, S. 2016. Probiotics In: "Encyclopedia of Food and Health". B. Caballero, P.M. Finglas and F. Toldrá, (Eds), p. 510-515. Academic Press, Oxford.

Wattanaprasert S., Borompichaichartkul C., Vaithanomsat P. and Srzednicki G. 2016. Konjac glucomannan hydrolysate: A potential natural coating material for bioactive compounds in spray drying encapsulation. Eng. Life Sci. 17:145-52.

Yang J., Xiao J.X. and Ding L.Z. 2009. An investigation into the application of konjac glucomannan as a flavor encapsulant. Eur. Food Res. Technol. 229:467-74.

Yi,Y., Lin W., Jie P., Xin H., Ruo-Jun M., Wei-Hai W. and Bing-Qing X. 2017. A review of the development of properties and structures based on konjac glucomannan as functional materials. Chinese J. Struct. Chem. 36(2):346-60.

SESSION II

Shelf-life Assessment and Modeling

SOUS-VIDE PACKAGING: SENSORY CHARACTERISTICS OF POTATO SLICES TREATED WITH ROSEMARY ESSENTIAL OIL

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ABSTRACT

Fresh-cut potatoes, already peeled, cut and packed, meet a growing favour of consumers, thanks to their high added value simplifying their use and consumption, both at home and in the food service industry. *Sous vide* is a combined technique used for raw or partially cooked food sources, packaged under vacuum. The request from consumers to reduce or eliminate the synthetic chemical additives, suggested to try natural alternatives such as essential oils (EOs), nowadays these compounds are frequently used, often through dipping techniques. On the basis of studies carried out, it was considered that the rosemary essential oil (EO) was the most appropriate and kindred with the preparation of fresh-cut sliced potatoes. The objective of this study was to evaluate the preserving effect of 0.5 % (w/v) rosemary (*Rosmarinus officinalis*) EO on physical, sensory and microbiological properties in fresh-cut sliced potatoes packaged in *sous vide* bags and stored for 12 days at +4 ± 2°C. A sample treated equally, but without EO, was packed in sous vide and stored at the same conditions and kept as control. Color change was measured through Image-Pro® Plus 7.0 software; sensory and microbiological analysis was carried out during the shelf life test. EO addition significantly reduced mesophilic bacteria and *Enterobacteriaceae* counts over the considered period. The sensory profile method UNI 10957 (2003) was used to measure any change in the sensory characters of samples, as a result of rosemary EO treatment. The best sensory results were for samples treated with EO, statistically significant results were observed on the descriptors sour and floury. HSI (hue, saturation, intensity) describes colors as like as they are perceived by human eyes, suggesting changes in color as browning index. The Hue value did not change during the 12 days of storage, for control slices as for slices treated with EO. Joint use of dipping in EO, sous-vide technology and refrigerated condition allowed to obtain a value-added product, safe, ready to cook, with appreciable characteristics for consumers.

Keywords: essential oil, fresh-cut potatoes, image analysis

1. INTRODUCTION

The higher convenience of fresh-cut vegetables corresponds, generally, to a higher proneness to perish, compared to the unprocessed product, due to the physiological implications of the technologies used. Despite the difficult moment for the Italian potato market, there are margins for growth and new strategies to improve the tuber consumption trends. Stresses such as temperature or wounding, affect the physiology of fresh produce by triggering responses that could induce the accumulation of phenolic compounds or other secondary metabolites. Moreover, recent research has shown that wounding increases the antioxidant capacity of some vegetables including potatoes (REYES *et al.,* 2007). Enzymatic browning is one of the most limiting factors for the shelf life of fresh-cut products, and its prevention has always been considered a challenge from food scientist (GARCIA and BARRETT, 2002).
To respond to the consumer's request to reduce or eliminate the synthetic chemical additives, natural alternatives, such as essential oils (EOs), are available, which can be applied through dipping or, more recently, by inclusion within the most common plastic materials. The antimicrobial action of EOs in model food systems or in real food is well documented in literature; although the majority of the essential oils are classified as Generally Recognized As Safe (GRAS), their use in foods as preservatives is often limited due to flavour considerations, since effective antimicrobial doses may exceed organoleptically acceptable levels (LAMBERT *et al.,* 2001). There are some reports available in the recent literature on the radical scavenging activity and the antimicrobial activities of *Rosmarinus officinalis* essential oil (HUSSAIN *et al*., 2010). The use of suitable packaging methods combined with the presence of natural preserving substances, could ensure the food safety and increase the shelf life. The aim of this work is to evaluate the attitude of an early potato cultivar, 'Marabel', to be processed with rosemary EO and packaged under vacuum in *sous vide* bags.

2. MATERIALS AND METHODS

2.1. Sample preparation

Potatoes were washed, peeled and cut. Slices (5 kg) were treated with a mixture of peanut seed oil and 0.5% (v/v) rosemary essential oil (E.O.) and a second aliquot (5 kg) were subjected to dipping in a peanut oil and used as control. Slices were placed in *sous vide* cooking bags, containing about 200 g of potatoes. The bags were sealed employing a vacuum packaging machine (Delta Vacuum DELTA 30). Bags were stored under refrigerated conditions at +4±2°C until analyses.

2.2. Image analysis

In order to quantify the real browning of potato cut zone, images were acquired by a digital camera and they were processed through Image-Pro® Plus 7.0 software (Media Cybernetics Inc., Rockville, USA). After opening each bag, the potato slices were placed in a darkroom and pictures were taken at the same distance.

2.3. Microbiological analysis

The total mesophilic bacteria (TMB), the yeast and mold (YM) count and the *Enterobacteriaceae* (TEB) were determined at the suitable incubation time and temperature.

All microbiological counts were performed in triplicate and expressed as average $\log_{10}$ cfu g^{-1} sample.

2.4. Sensory analysis

The sensory profile method UNI 10957 (2003) was used to measure any change in the sensory characters of samples during the cold storage. Ten judges (six females and four males) were trained in 4 sessions to develop a common vocabulary for the description of sensory attributes and to familiarize with scales and procedures. During their training, the judges produced a list of attributes to define the sensory profile. On the basis of the citation frequency (>50%), seventeen descriptors were selected for control samples and nineteen for samples with essential oil. The evaluation sessions, performed at 1, 5, 8 and 12 days of storage, were conducted in a sensory laboratory (UNI EN ISO 8589, 2010) from 11:00AM to 12:00AM in individual booths illuminated with white light. Randomized samples were evaluated by assigning a score between 1 (absence of sensation) and 9 (extremely intense) in the sensory laboratory of the Di3A. The potato samples were served on plates, coded with three-digit numbers, and water was provided to judges for rinsing between samples. All data were acquired by a direct computerized registration system (FIZZ Byosistemes. ver. 2.00 M, Couternon, France). The sensory data for each attribute were submitted to one-way ANOVA by the software package Statgraphics® Centurion XVI (Statpoint Technologies, INC.) using samples as factors. The significance was tested by means of the F-test. To differentiate the samples, the mean values were submitted to the multiple comparison tests using the least significant difference (LSD) procedure for $p \leq 0.05$.

4. RESULTS AND DISCUSSIONS

Potato slices exhibited changes in color and browning of the cut surface during storage. The study of the browning index is useful to assess shelf life. Image analysis performed during storage through the model of HSI, changes color image into black/white images and elaborate them in grey level (Fig. 1).

(A) (B)

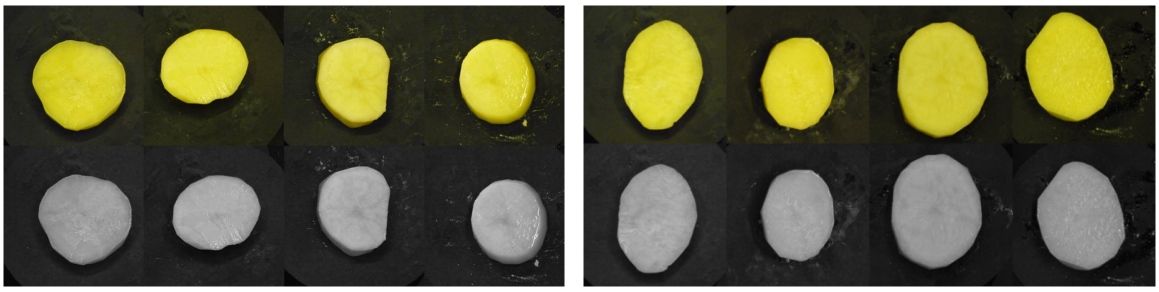

Figure 1. Image analysis as HSI (hue, saturation, intensity) on Marabel slices (A) and on Marabel slices treated with EO (B) at 1, 5, 8 and 12 days (from left to right).

Changes captured by image analysis software (Fig. 1) are numerical expressed in Fig. 2. As reported, the Hue value did not change during 12 days of storage in both samples for control slices as for slices treated with EO; saturation decreased in control samples and

showed a slight increase in slices with EO at 12 d. Also, intensity did not change significantly.

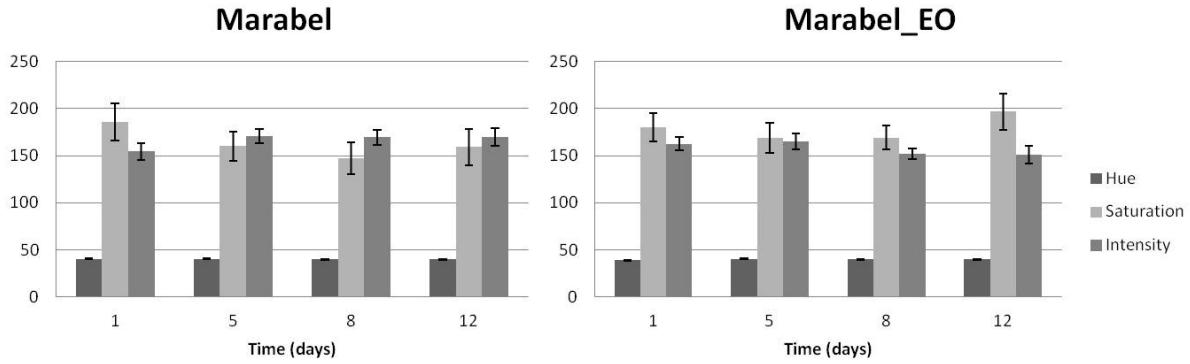

Figure 2. Image analysis in HSI (hue, saturation, intensity) on potato slices during storage.

The addition of rosemary EO to potato slices significantly controlled, over the storage period, the growth of mesophilic bacteria and *Enterobacteriaceae* when compared to the relative control samples. Differently, the yeast and mold population was not affected over time by the EO (Fig. 3).

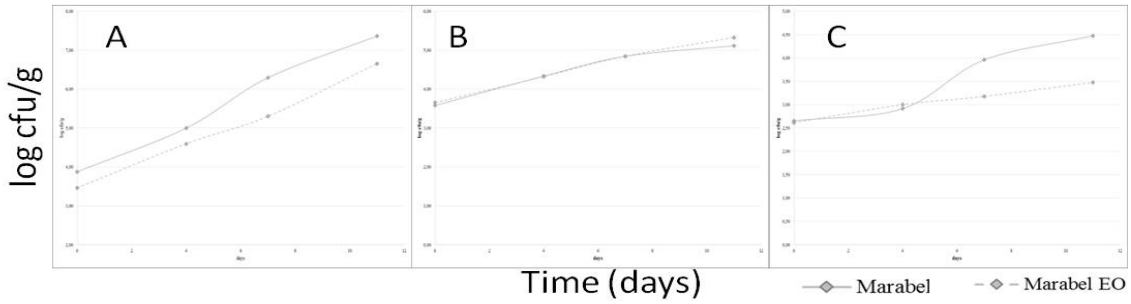

Figure 3. Microbiological traits of fresh-cut potato slices as affected by 'essential oil (EO) treatment × storage time' interaction.
[A]: TMB (total mesophilic bacteria); [B]: YM (yeasts and molds); [C]: TEB (*Enterobacteriaceae*).

The F values of sensory data Anova results were not shown. Table 1 reports the significant sensory attributes ($p \leq 0.05$) at each sampling time. The intensity (average value of ten judge's evaluations) was reported only for the significantly different attributes. The attributes sour and floury (Table 2) significantly differentiated ($p \leq 0.05$) for the control samples during the cold storage. In particular, the attribute sour was absent until 5 d and it increased from 8 d remaining constant after 12 d. Only the attribute sour significantly differentiated the EO-treated samples. For these samples, the attribute sour had increased after 8 days of storage.
Table 2 reports the comparison between control and EO-treated samples at 1 and 12 d. Four attributes differentiated significantly ($p \leq 0.05$) the samples: odor and flavor of rosemary, sour and floury. Only the samples at 12 d were characterized by the attribute sour, while the attribute floury was highest in the control at 12 d.

Table 1. Mean scores of the significant sensory attributes.

Sample	Attribute	Storage time (days) 1	5	8	12
Without EO	Sour	1.0±0^{a*}	1.0±0^{a}	1.5±0.92^{ab}	2.1±1.58^{b}
	Floury	2.6±1.69^{a}	4.0±1.48^{ab}	4.6±2.37^{b}	4.5±2.01^{b}
With EO	Sour	1.0±0^{a}	1.0±0^{a}	3.0±2.00^{b}	2.7±1.79^{b}

*Values marked with different letters in the same row are significantly different ($p \leq 0.05$) according to the LSD multiple comparison test.

Table 2. Mean scores of the significant sensory attributes (comparison during storage).

Attribute	Marabel 1 d	Marabel+EO 1 d	Marabel 12 d	Marabel+EO 12 d
Rosemary odor	1.0±0^{a*}	4.4±2.65^{b}	1.0±0^{a}	3.4±1.74^{b}
Sour	1.0±0^{a}	1.0±0^{a}	2.1±1.58^{ab}	2.7±1.79^{b}
Floury	2.6±1.78^{a}	3.4±1.96^{ab}	4.5±2.01^{b}	3.9±1.81^{ab}
Rosemary flavor	1.0±0^{a}	5.8±1.99^{b}	1.0±0^{a}	4.9±1.45^{b}

*Values marked with different letters in the same row are significantly different ($p \leq 0.05$) according to the LSD multiple comparison test.

4. CONCLUSIONS

Joint use of dipping in EO, sous-vide technology and refrigerated condition allowed to obtain a value-added product, safe and ready to cook. The attitude of the early potato cultivar, 'Marabel' to be processed has been defined, confirmed by analyses performed with appreciable characteristics for consumers.

REFERENCES

Garcia E.L. and Barrett D.M. 2002. Preservative treatments for fresh-cut fruits and vegetables. In: O. Lamikanra ed., FreshCut Fruits and Vegetables. Science, Technology and Market, CRC Press, 267-303.

Hussain, A.I., Anwar F., Chatha S.A.S., Jabbar A., Mahboob S. and Nigam P.S. 2010. *Rosmarinus Officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. Braz. J. Microbiol., 41:1070-1078.

Lambert R.J.W., Skandamis P.N., Coote P. and Nychas G.J.E. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91:453-462.

Reyes L.F., Villarreal J.E. and Cisneros-Zavallos L. 2007. The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. Food Chem. 101: 1254–1262.

UNI 10957 2003. "Analisi sensoriale – Metodo per la definizione del profilo sensoriale degli alimenti e bevande", Ente Nazionale Italiano di Unificazione, Milano, Italia.

UNI EN ISO 8589 2010. "Analisi sensoriale – Guida generale per la progettazione di locali di prova", Ente Nazionale Italiano di Unificazione, Milano, Italia.

ANTIBACTERIAL ACTIVITIES OF GREEN MANGO PEEL EXTRACTS AND ITS APPLICATION IN FISH FILLET

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ABSTRACT

The green mango peel extract **(**GMPE; cv. Keaw Ka-min**)** was dried at 40°C for 10 h and then extracted at 50°C for 1 h using different ethanol concentrations (50%, 70%, 95% v/v). The bioactive compounds of GMPE were determined for gallic acid and mangiferin using HPLC. GMPE-70%E had the highest amount of both gallic acid and mangiferin. Hence, it was applied to an un-innoculated and cocktail-inoculated fish fillet at 0%, 2%, 4% and 6% **(**v/w**)** for further antimicrobial analysis. Enumeration of total plate counts for *S. aureus*, *S. typhimurium* and *E. coli* were examined. The results indicated that 6% **(**v/w**)** of extract had the highest antibacterial activity on the fish sample after refrigerated storage for 5 days. In summary, this research work suggested that Thai green mango peel not only contained bioactive compounds, but its extract also showed antimicrobial activities on some foodborne pathogens which could help prolong shelf life of the fish fillet. This finding suggests a remarkable model on agricultural waste transformation into a value added product for solving microbial contamination in food industries.

Keywords: antibacterial, fish fillet, mango peel, polyphenolic

1. INTRODUCTION

Mango (*Mangifera Indica*) waste is considered as a source of phenolic compounds. Though type and content of the phenolic compounds are varied by natural factors, mangiferin, quercetin and gallic acid are predominantly occurred. These phenolic compounds were reported the inhibition properties on both Gram-positive and Gram-negative bacteria (ABDULLAH *et al.,* 2012). Many studies reported the polyphenolic contents and antioxidant properties from mango waste (MASIBO and HE, 2008; ZHI-QUAN *et al.,* 2011; ABDULLAH *et al.,* 2011; HASANI and HASANI, 2014), however, the study on peel was still limited.

Extraction method is a factor affecting phenolic contents. Drying and grinding process are widely used for inhibition of hydrolysis and enzymatic reaction in raw material (MASIBO and HE, 2009). Increasing temperature can promote solubility and mass transfer rate, which led to an increasing amount of bioactive compounds in crude extract. On the other hand, the over increasing time and temperature can deteriorate polyphenolic compounds by hydrolyzing and oxidizing mechanism (DAI and MUMPER, 2010).

Fish products have common problems on microbial spoilage, and pathogen contamination (OLGUNOGLU, 2012; FSANZ, 2013). With this regard, searching new natural compounds for using instead of the synthetic antimicrobial agents to retard fish deterioration is worthy. In this research, the effect of antimicrobial from mango peel extract was performed on fish fillet as a representative of food matrix system.

2. MATERIALS AND METHODS

2.1. Mango peel preparation and extraction

Mango peels (*Mangiferin indica* L. cv. Kaew Ka-min) was selected only green to greenish peel. The peel was dried by using a hot air oven at 40°C for 10 h, then ground with blender, and hammer mill, respectively (applied from ABDULLAH *et al.,* 2011). Two grams of mango peel powder was mixed with 50 ml of 50%, 70% or 95% ethanol solution. The extract was shaken in water bath shaker at 50°C 150 rpm for 1 h, and the supernatant was stored as aliquot at -18°C until used (applied from DORTA *et al.,* 2012b).

2.2. Bacteria strains

All bacterial strains were purchased from the Thailand Institute of Scientific and Technological Research (Pathumthani, Thailand) including *S. typhimurium* ATCC 13311, *E. coli* ATCC 25922, and *S. aureus* ATCC 13565.

2.3. Determination of polyphenolic compounds in mango peel extract

Gallic acid and mangiferin in the GMPE were determined by HPLC analysis. The polyphenolic compounds were calculated from standard curves and reported as $\mu g/g$ fresh weight (Applied from BERARDINI *et al.,* 2005).

2.4. Application to fish products

Fish fillet, Nile tilapia (*Oreochromis niloticus*) was cut to small size, the experiment was divided into 2 sections: 1) non-inoculation fish fillet by spraying the fillet with GMPE-

70%E, in 3 concentration levels: 2%, 4%, 6% (v/w); 2) inoculation the fillet with cocktail culture including *S. aureus*, *E. coli*, and *S. typhimurium*. The number of total plate count of all samples were examined by using pour plate technique in TSA and incubated for 48 h, the number of *S. aureus*, *S. typhimurium* and *E. coli* was examined using a spread plate technique in selective media MSA, XLD and MacConkey agar respectively, and incubated for 24-48 h. The samples are stored in a non-vacuum sealed plastic bag at 4°C throughout 5 days. The results were reported as log CFU/g. All conditions were repeated 3 times with duplication.

2.5. Statistical Analysis

Data were compared by using one-way ANOVA followed by Tukey's test ($p \leq 0.05$), SPSS software (version 16.0, SPSS Inc., Chicago, USA).

3. RESULTS AND DISCUSSION

3.1. Polyphenolic contents in GMPE

In our previous study on the effect of antibacterial properties by varying the temperature levels of drying, extraction and the concentrations of ethanol, the result indicated that only the concentration of extraction solvent affected the antibacterial activities. The higher antimicrobial activities were observed in the higher percentage of ethanol as extraction solvent (CHERDVORAPONG and TONGKHAO, 2015). The consequence agreed with the other researches that the increasing of ethanol ratio in the extraction solvent resulted in higher content of phenolic compounds in the crude extract (TEWTRAKUL *et al.*, 2008; ZOU *et al.*, 2014). The effect of ethanol on bacteria survival could be negligible since the final concentration of ethanol during antimicrobial examination was lower than the effective range, which is between 60%-90% (v/v) **(**RUTALA and WEBER, 2008**).**
On this research, the polyphenolic compounds of GMPE extracted with various ethanol concentrations were further determined. The HPLC result showed that GMPE-70%E had the highest amount of gallic acid and mangiferin (Table 1).

Table 1. Phenolic compounds contents **(**μg/g fresh weight**)** in mango peel extract.

Ethanol concentration (%)	Gallic acid	Mangiferin
95	0.144±0.005 ^{b}	0.500±0.011 ^{c}
70	0.715±0.015 ^{a}	2.472±0.011 ^{a}
50	0.135±0.036 ^{b}	1.342±0.184 ^{b}

Different letters indicate significant differences ($p \leq 0.05$) among polyphenol types.

This result was comparable with previous study that the best condition for extract phenolic compounds from mango peels was using 70% ethanol and drying mango peel for 10 h (PALMEIRA *et al.*, 2012). Therefore, it can be concluded that ethanol concentration is the main effect on polyphenolic compounds extraction in this study. Mangiferin was previously reported as the antimicrobial agent in Gram-positive, Gram-negative, yeast and virus (WAUTHOZ *et al.*, 2007; ZHI-QUAN *et al.*, 2011). Furthermore, gallic acid was also

mentioned as the most effective antibacterial property among polyphenol compound (HASANI and HASANI, 2014). From this result, the extract with the highest polyphenolic compounds was selected to apply in Nile tilapia fillet.

3.2. Application in fish fillet

Comparing with the untreated sample (control), the total bacteria number of all treated samples were significant lower ($p \leq 0.05$) with 1.50 log CFU/g difference in 1 h storage. However, only the samples treated with 4% and 6% GMPE showed significant lower ($p<0.05$) total bacteria count after a period of 3 days, with 2.00 and 1.30 log CFU/g differences. In the end of 5 days storage, only the sample treated with 6% GMPE had the total bacteria number significant lower ($p<0.05$) with 2.50 log CFU/g difference. Considering each inhibition activity on each bacterial strain, the number of *S. typhimurium* in the samples treated with 4% and 6% GMPE were significant lower ($p<0.05$) with 2.00 log CFU/g difference after a period of 1 day, whereas, there were not significantly ($p>0.05$) different between the number in *S. aureus* and *E. coli* in untreated and treated samples.

4. CONCLUSIONS

The ethanol concentration presented the effect on polyphenolic contents. The results indicated that 70% GMPE had the highest amount of mangiferin and gallic acid, which is relatable with the previous study on antibacterial property. For the application, 6% (v/w) GMPE-70%E could inhibit bacterial growth in term of total bacteria number and *S. typhymurium* in Nile tilapia fillet throughout a period of 5 days. It was obviously that Thai green mango peel had polyphenolic contents which those kinds and concentrations had potential to be used as a natural antibacterial agent in fish fillet. This research was the noteworthy initiative model which represented on agricultural waste utilization as a valuable material for solving the problem in food industries effectively.

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REFERENCES

Abdullah A.S.H., Mirghani M.E.S. and Jamal P. 2011. Antibacterial activity of Malaysian mango kernel. Afr. J. Biotechnol. 10 (81):18739-18748.

Abdullah N., Zulkifli K.S., Abdullah A., Aziman N. and Kamarudin W.S.S.W. 2012. Assessment on the antioxidant and antibacterial activities of selected fruit peels. Int. J. Chem. Tech. Res. 4 (4):1534-1542.

Berardini N., Knodler M., Schieber A. and Carle R. 2005. Utilization of mango peels as a source of pectin and polyphenolics. Innov. Food Sci. Emerging Tech. 6:442-452.

Cherdvorapong V. and Tongkhao K. 2015. Effect of drying and extraction temperature of green mango peel on antibacterial activity. P 938-945, present at 53rd Kasetsart University Annual conference, Bangkok, February 3-6.

Dai J. and Mumper R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 15:7313-7352.

Dorta E., Lobo M.G. and Gonzalez M. 2012. Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties. J. Food Sci. 71: C80-C88.

Food Standards Australia and New Zealand [FSANZ]. 2013. "Agents of Foodborne Illness" 2nd ed. Food Standards Australia and New Zealand (FSANZ), Canberra, Australia.

Hasani, S. and Hasani M. 2014. Antimicrobial properties of grape extract on common carp (*Cyprinus carpio*) fillet during storage at 4 ºC. Int. J. Fish. Aquat. Stud. 1 (3):130-136.

Masibo M. and He Q. 2008. Major mango polyphenols and their potential significance to human health. Compr. Rev. Food Sci. Food Saf. 7:309-319.

Masibo M. and He Q. 2009. Mango bioactive compounds and related nutraceutical properties-A Review. Food Rev. Int. 25:346-370.

Olgunoglu I.A. 2012. "Salmonella in Fish and Fishery Products" InTech, Croatia.

Palmeira S.M.V., Gois L.M. and Souza L.D. 2012. Extraction of phenolic compounds from mango peels. Lat. Am. Appl. Res. 42:77-81.

Rutala, W.A. and Weber D.J. 2008. "Guideline for disinfection and sterilization in healthcare facilities" Department of health and services, New York.

Tewtrakul S., Itharat A., Thammaratwasik P. and Ooraikul B. 2008. Anti-allergic and anti-microbial activities of some Thai crops. Songklanakarin J. Sci. Technol. 30 (4):467-473.

Wauthoz, N., Balde A., Balde E.S., Damme M.V. and Duez P. 2007. Ethnopharmacology of *Mangifera indica* L. Bark and pharmacological studies of its main C-glucosylxanthone, mangiferin. Int. J. Biomed. Pharmaceut. Sci. 1 (2):112-119.

Zhi-quan W., Jia-gang D. and Li Y. 2011. Pharmacological effects of mangiferin. Chinese Herbal Med. 3 (4):266-271.

Zou T.B., Xia E.Q., He T.P., Huang M.Y., Jia Q. and Li H.W. 2014. Ultrasound-assisted extraction of mangiferin from mango (*Mangifera indica* L.) leaves using response surface methodology. Molecules. 19:1411-1421

EFFECT OF PACKAGING FILM AND OXYGEN ABSORBER ON SHELF LIFE EXTENSION OF CHINESE PASTRY (KHA-NOM PIA)

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ABSTRACT

Chinese pastry (Kha-nom Pia) is a bakery product composed of crust and filling that has a short shelf life, approximately 5-7 days at room temperature. Mold is an important role on the spoilage. The objective of this research was to determine the effect of packaging film and oxygen absorber on shelf life of Chinese pastry. Preservative-free Chinese pastry samples, with a filling of mung bean and salted egg yolk, were packed in a plastic film either a nylon/linear low density polyethylene (Nylon/LLDPE) or a nylon/cast polypropylene (Nylon/CPP), and oxygen absorber. Control sample was packed in polypropylene bag without oxygen absorber. Microbial counts, a_w and texture analysis were examined during storage at room temperature. Both Nylon/LLDPE and Nylon/CPP in combination with oxygen absorber effectively extended shelf life of the Chinese pastry from 5 days (Control) to more than 30 days. However, Nylon/CPP retarded microbial growth better than Nylon/LLDPE. For texture analysis, hardness of crust and firmness of filling tended to decrease during storage. The samples stored in Nylon/CPP showed higher values of the hardness and the firmness than Nylon/LLDPE. Therefore, Nylon/CPP and oxygen absorber was suitable for prolong shelf life of Chinese pastry.

Keywords: bakery product, nylon, oxygen absorber, pastry, shelf life

1. INTRODUCTION

Chinese pastry, Kha-nom Pia, is a bakery product composed of crust and a variety of filling. Shelf life of preservative-free pastry is short due to moisture migration from filling to the crust and affecting to physical property, sensory property and microbial stability (LABUZA and HYMAN, 1998; SMITH *et al*., 2004). Foodborne microorganisms associated with pastry and filling are mainly mold, *S. aureus* and *B. cereus* (TCPS0115/2555). Spoilage microorganisms of pastry with filling are mainly mold. The most critical factors controlling the growth of undesirable mold on food are oxygen, temperature, pH, and water activity (a_w). Controlling the growth of post baking contaminants in the packaged products is approaches. Modified atmospheric packaging (MAP) including active packaging system is one of the successful hurdle technology to extend shelf life of mold-free bakery products (JANJARASSAKUL *et al*., 2016; SMITH *et al*., 2004). One approach to overcome this problem is to use oxygen absorber inside the package. Oxygen absorber have been used inhibit mold growth in high water activity product (BERENZON and SAGUY, 1998). Thus, the objective of this research is to investigate the effect of high barrier packaging and O_2 absorber to prolong shelf life and retain quality of preservative-free Chinese pastry.

2. MATERIALS AND METHODS

2.1. Samples and packaging materials

Chinese pastries with mung bean and salted egg yolk filling were received from Ban Kha-nom Sommas manufacturer (Nakhon Ratchasima, Thailand). After baking, samples were packed in three types of packaging materials: (1) a polypropylene (PP) bag used as control to demonstrate the traditional condition packaging as the outlet normally used, (2) a nylon/linear low density polyethylene (Nylon/LLDPE) and (3) a nylon/cast polypropylene (Nylon/CPP). The water vapor transmission rate (WVTR) of PP, Nylon/LLDPE and Nylon/CPP bags were 6.65, 2.17 and 3.92 g/m^2.day, respectively. The oxygen transmission rate (OTR) of PP, Nylon/LLDPE and Nylon/CPP bags were 2172.9, 2.5 and 5.4 cc/m^2.day, respectively. Thickness of PP, Nylon/LLDPE and Nylon/CPP film were 0.013±0.014, 0.075±0.009 and 0.052±0.013 mm, respectively. Both of Nylon/LLDPE and Nylon/CPP were combined with oxygen absorber (BestKept®; Alpine Foods Co., Ltd., Bangkok, Thailand) with the capacity of 100 ml. Microbial count, a_w, pH and texture analysis were evaluated at 0, 1, 3, 5 and 5- day interval until 30 days of storage at ambient temperature (27±3^{0}C), or until the sample spoiled with visible microbial growth. Experiments were done in 2 replications.

2.2. Microbial analysis

Samples were not inoculated. Chinese pastries were examined the growth of natural microorganisms. *B. cereus*, *S. aureus*, anaerobic bacteria, total aerobic bacteria count (TAC) and yeast and mold count (YM) were determined according to APHA (2001). Experiments were done in 2 replications.

2.3. Determination of a_w, pH and texture profile

The a_w value was measured on crust, filling and whole piece of Chinese pastries by a water activity meter 4TE (Aqualab, Pullman, USA) according to machine manual. pH value was

measured by using pH meter Lab 850 (Schott Instrument, Deutschland, Germany) according to APHA (2001). The texture of Chinese pastry was carried out by a texture analyzer (TA-XT Plus, Stable Micro System, Surrey, UK) for examining hardness of crust and firmness of filling using TPA mode with 6.0 cm cylindrical probe (P/6). The crust of Chinese pastries was measured first, then the crust was cut out and fillings were measured after that. Samples were measured 5 times.

3. RESULTS AND DISCUSSION

High barrier packaging and O_2 absorber effectively inhibited the growth of TAC and YM in Chinese pastries (Fig. 1). The appearance of mold was detected at day 5 of storage with using PP bag, and numbers of YM increased to *ca* 4.0 Log CFU/g (Fig. 1A). Whereas, Nylon/CPP and Nylon/LLDPE effectively increased mold free shelf life to 30 days. Even detectable numbers of YM increased to *ca* 3 Log CFU/g, there was no visible mold in the sample. The oxygen permeability of PP bag allowed the growth of mold. Conversely, Nylon/CPP and Nylon/LLDPE had lower oxygen permeability compared with PP bag, resulting in delayed the mold growth as shown in Fig 1.

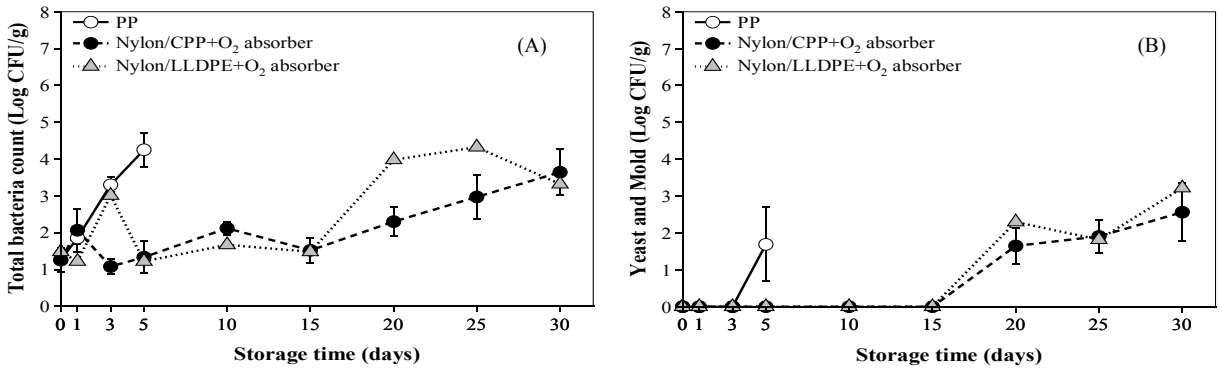

Figure 1. Total microbial count (A) and yeast and mold (B) of Chinese pastries packed in polypropylene (PP), Nylon/CPP with O_2 absorber and Nylon/LLDPE with O_2 absorber and stored at ambient temperature (27±3°C).

According to guideline for assessing microbiological standard in bakery products with filling, total microbial count must present below 10^4 CFU/g, yeast and mold as well as *B. cereus* are below 100 CFU/g and *S. aureus* is less than 10 CFU/g (DEPARTMENT OF MEDICAL SCIENCE, 2016). Shelf life of Chinese pastry in PP bag was less 5 days, regarding to over limit of TAC and YM (Fig. 1B), as well as *B. cereus* and *S. aureus* (Data not shown). Conversely, Nylon/CPP and Nylon/LLDPE could retard the growth of aerobic microorganism, count remain below 10^4 CFU/g, up to 25 and 20 days of storage, respectively. Similarly to TAC, Nylon/CPP and Nylon/LLDPE inhibited the growth of *B. cereus* and *S. aureus* to below the level of microbiological criteria (Data not shown). Furthermore, anaerobic bacteria were not detected on both of packaging during storage period. The rapidly increased of TAC in control correlated to OTR of packaging types, PP had the highest OTR compared with Nylon/CPP and Nylon/LLDPE. The OTR of Nylon/CPP and Nylon/LLDPE were hardly different. The combination of high barrier packaging, both Nylon/CPP and Nylon/LLDPE, with O_2 absorber had potential to controlled aerobic microbial growth, correspondingly with the O_2 absorber may prevent

headspace of O_2 from harboring aerobic microbial activities or may reduce the O_2 permeation rate through container wall (ROBERTSON, 2006).

3.2. a_w, pH and texture analysis

Initial a_w value of filling was *ca* 0.91 and a_w value of crust was ca 0.86 (Fig. 2). After storage, filling a_w value of all samples gradually reduced throughout the period of storage until 0.90 on day 30. Conversely, a_w values of crust in all packaging slowly increased to reach 0.89 on day 30. The phenomenon of moisture migration in multi-domain foods, such as pie with filling, causes an increasing of water content in the crust resulting in the growth of mold on surface (LABUZA and HYMAN, 1998).

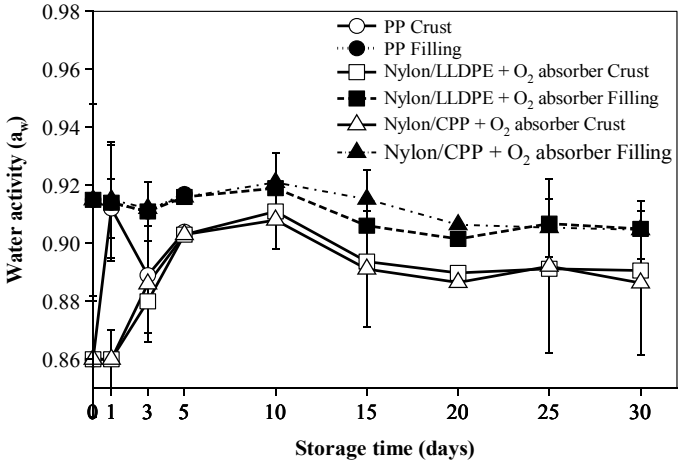

Figure 2. Water activity of Chinese pastries packed in polypropylene (PP), Nylon/CPP with O_2 absorber and Nylon/LLDPE with O_2 absorber and stored at ambient temperature (27±3°C).

The pH values of Chinese pastries in all packaging were neutral (in range of 6.52-7.69) throughout a period of storage. The hardness of crust and firmness of filling in all samples gradually decreased during storage time (Figs. 3A and 3B) due to the effect of moisture migration.

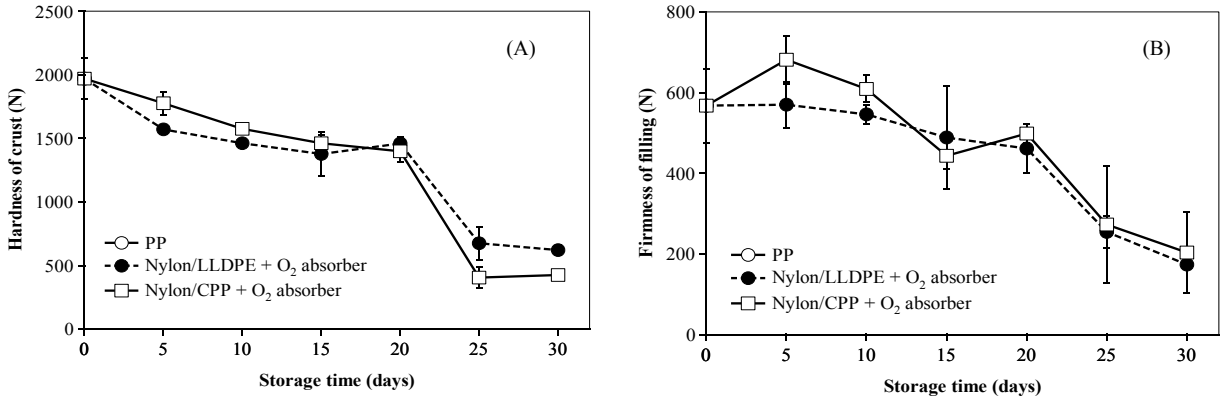

Figure 3. Crust hardness and filling firmness of Chinese pastries packed in polypropylene (PP), Nylon/CPP with O_2 absorber and Nylon/LLDPE with O_2 absorber and stored at ambient temperature (27±3°C).

Nylon/LLDPE has very low permeability to water vapor (WV). WV strongly affects the molds growth and texture of pastry. As expected, Nylon/LLDPE provides lower hardness. The change in texture quality of cake during storage was presumably caused by moisture migration (GUY, 1983). GAIKWAD (2015) reported water vapor is very important for food packaging, moisture may permeate plastic packaging material during storage, resulting in loss of food quality. High barrier package efficiently prevents moisture loss, leading to slight change in moisture content all of cake during storage (JANJARRASSKUL *et al*., 2016).

4. CONCLUSIONS

Active packaging, a high barrier of packaging material in combination with O_2 absorber, was evaluated for prolong shelf life of Chinese pastry. Nylon/CPP and Nylon/LLDPE were able to extend shelf life from 5 days to 30 days with no observed mold growth compared with samples in PP bag (control) were detected mold growth on day 5 of storage. However, Nylon/CPP retarded microbial growth greater than Nylon/LLDPE. According to the microbiological criteria, shelf life of a preservative-free Chinese pastry packed in Nylon/CPP with O_2 absorber, was extended to 25 days. Therefore, Nylon/CPP in combination with O_2 absorber can be applied as active packaging to prolong shelf life of other types of bakery products.

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REFERENCES

APHA. 2001. "Compendium of Methods for the Microbiological Examination of Foods" 4th edn. Washington, DC: American Public Health Association.

Berenzon S. and Sam I.S.1998. Oxygen absorbers for extension of crackers shelf-life. Lebensmittel Wissenschaft und Technologies 31:1-5.

Cruz R.S., Soares N.F.F., Andrade N.J. 2006. Evaluation of oxygen absorber on antimicrobial preservation of lasagna–type fresh pasta under vacuum packed. Ciênc Agrotec 30(6):1135-1138.

Department of Medical Science. 2017. "Microbiological Criteria for Food and Food Contact Containers" Available Source: http://www.dmsc.moph.go.th/dmscnew/userfiles/files/1S__1286203.pdf, 1 July 2017.

Gaikwad K.K. 2015. Water Vapour Permeability of Packaging Materials (LDPE) and Fabricated Package Systems, MS: Packaging, School of Packaging Michigan State University, East Lansing, MI, USA.

Guy R.C.E. 1983. Factors affecting the staling of madeira slab cake. Journal of the Science of Food and Agriculture 34: 477.

Janjarasskul T., Tananuwong K., Kongpensook V., Tantratian S. and Kokpol S. 2016. Shelf life extension of sponge cake by active packaging as an alternative to direct addition of chemical preservatives. LWT - Food Science and Technology 72: 166-174.

Labuza T.P. and Hyman C.R. 1998. Moisture migration and control in multidomain foods. Trends in Food Science and Technology 9:47-55.

Robertson G.L. 2006. "Food Packaging" 2nd ed. CRC Press, Boca Raton.

Smith J.P., Daifas D.P., El-Khoury W., Koukotsis J. and EL-Khouri A. 2004. Shelf life and safety concerns of bakery products – A review. Critical Reviews in Food Science and Nutrition 44:19-55.

Thai Industrial Standards Institute Ministry of Industry. 2012. "Thai Community Product Standard" Available Source: http://tcps.tisi.go.th/pub/tcps0115_55(ขนมเปี๊ยะ).pdf, 15 August 2017.

EFFECT OF PAPAYA PEEL EXTRACT ON BROWNING INHIBITION IN VEGETABLE AND FRUIT SLICES

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ABSTRACT

Enzymatic browning is a major problem in fruits and vegetables. It causes undesirable quality change, shortened shelf life and reduced market value of food. Enzymatic browning can be controlled by using chemicals especially sulfites. However, it has been reported to be harmful to consumer health. Papaya peel is a good source of bioactive phenolic compounds which can be used for browning inhibition. Therefore, the objective of this study was to investigate the effect of papaya peel crude extract (PPE) on the browning inhibition in fruit and vegetable slices. Papaya peel crude extract was extracted by using peel to distilled water at a ratio of 1 to 1(w/v) at 25 ºC. It was found that L* value of potato slices treated with PPE were higher (70.21±0.05) than that treated with distilled water (DW) (68.10±0.19) through 6 h storage ($p \leq 0.05$) at 25 ºC. The L* values of apple slices treated with PPE were lower (49.28±0.03) than treated with DW (66.50±0.09) through 6 h storage ($p \leq 0.05$). The L* values of the banana slices treated with PPE were higher than that treated with DW through 2-5 h storage, respectively ($p \leq 0.05$). The results of % browning inhibition of potato, apple and banana calculated from total color changes between sample treated with DW and PPE indicated that PPE could inhibit browning in potato slices (59.87±0.77%) more effective than apple (21.08±1.94%) and banana slices (12.47±2.86%) through 6 h of storage ($p \leq 0.05$). Total phenolic content in the papaya peel extract was 11.835mg of GAE/100g of dry weight basis. Its inhibitory effect might be due to the phenolic compounds in the PPE. These results suggested that PPE has a potential to be used as natural antibrowning agent of fruits and vegetables, especially in potatoes.

Keywords: apple, banana, browning inhibition, papaya peel extract, potato

1. INTRODUCTION

Papaya (*Carica papaya* L.) is a tropical fruit available throughout the year. Papaya peels are a good source of valuable compounds, including proteins and phenolic compounds (PARNIAKOV *et al.*, 2014). Enzymatic browning in vegetables and fruits is often an undesirable reaction, which affects not only appearance, but also nutritional quality of vegetable and fruit products (LE BOURVELLEC *et al.*, 2004). Sulphites are effectively used for the inhibition of enzymatic browning. However, there are several negative attributes associated with their use in food and beverages. Therefore, numerous natural compounds such as plant phenolic compounds have been investigated to reduce the enzymatic browning in fruits and vegetables. These included phenolic compounds from rice bran (KAEWKA *et al.*, 2009; SUKHONTHARA and THEERAKULKAIT, 2012; SUKHONTHARA *et al.*, 2016), pineapple juice (CHAISAKDANUGULL *et al.*, 2007), pineapple shell (SAISUNG and THEERAKULKAIT, 2011) and honey (DE LA ROSA *et al.*, 2011). Papaya peel is the waste of the papaya fruit processing industry. Therefore, the objective of this study was to investigate the effect of papaya peel crude extract (PPE) on the browning inhibition in potato, banana and apple slices.

2. MATERIALS AND METHODS

2.1. Materials

Potato (*Solanum tuberosum* L.), banana (*Musa* (AAA Group) '*Gros Michel*'), apple (*Malus pumila* cv. Fuji) were purchased from a local market in Bangkok, Thailand. Papaya (*Carica papaya* Linn.) peel (var. Khaek Dam) was obtained from the Siam Preserved Foods Co., Ltd. Thailand.

2.2. Preparation of papaya peel extract

Papaya peel extract was prepared by using peel to distilled water as 1:1(w/v). The mixture was stirred with an overhead stirrer at 700 rpm at room temperature (25 ºC) for 45 min, centrifuged at 8,000 xg at 20ºC for 30 min. The obtained supernatant was papaya peel crude extract (PPE) and was evaluated for browning color inhibitory effect on potato, apple and banana slices (modified from BOONSIRIPIPHAT and THEERAKULKAIT, 2009).
Concentration of all the tested PPE was expressed as equivalent weight (2.44 mg, dry weight basis) of papaya peel per mL of solvent (distilled water).

2.3. Sample treatment and dipping

Potato, apple and banana were peeled and cut into 0.5 cm slices and were dipped in 200 mL of PPE (without dilution) or distilled water (control) for 5 minutes at room temperature (25ºC) (modified from THEERAKULKAIT and BOONSIRIPIPHAT, 2007). After dipping the excess of extract or water was drained off and the samples were stored in covered crystalizing dishes at room temperature for 6 h. The color of samples were measured at 0 h and every h during storage.

2.4. Color measurement

A colorimeter (CR-310, Konica Minolta, Osaka, Japan) in the CIE L^*, a^*, b^* color scale was used. Percentage browning inhibition was calculated from equation 1 (GIRELLI *et al.*, 2004).

$$\% \text{ Browning inhibition} = (\Delta E^*_{control} - \Delta E^*_{inhibitor}) \times 100 / \Delta E^*_{control} \quad (1)$$

where ΔE^* was calculated from $(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2)^{1/2}$

2.5. Total phenolic content

Total phenolic content was determined by the modified method of SINGLETON and LAMUELA-RAVENTOS (1999). Briefly 5 μL of papaya peel extract or standard gallic acid solutions (31.25, 62.5, 125, 250, 500, and 1000 μg/mL) were mixed with 195 μL of distilled water in test tubes; 25 μL of Folin Ciocalteu reagent solution was then added. After 6 min, 75 μL of 7% Na_2CO_3 was added and mixed gently. The reaction mixture was kept in the dark for 2 h, and its absorbance was measured at 765 nm against a blank solution, which was prepared according to the procedure described above except that extract solution was substituted by 5 μL of water, using the spectrophotometer. TPC was expressed as milligrams of gallic acid equivalents (mg GAE/100g).

2.6. Statistical analysis

All the determinations were done in triplicate and the data were analyzed statistically using standard statistical software package to perform ANOVA. Significant difference ($p \leq 0.05$) among treatments was detected using Least Significant Difference (LSD) tests.

3. RESULTS AND DISCUSSION

L* value is the lightness. The decrease of L* value means darker color that related with a higher browning formation (SAPERS and JR DOUGLAS, 1987). Fig. 1 shows the L* values of potato, banana and apple slices during storage. It was found that L* value of potato slices treated with PPE were higher than treated with DW through 2-6 h ($p \leq 0.05$) (Fig. 1). The L* values of the banana slices treated with PPE were higher than treated with DW through 3-5 h ($p \leq 0.05$) (Fig. 1). However, the L* values of apple slices treated with PPE were lower than treated with DW through 1-6 h ($p \leq 0.05$) (Fig. 1). This might be due to the specific inhibition of PPE on potato and banana PPO more than apple PPO. The PPO isoenzymes in each plant were reported to be different (LABUZA *et al.*, 1990).

The results of % browning inhibition of potato, apple and banana calculated from total color changes between samples treated with DW and PPE are shown in Fig. 2.

The result showed that PPE had % browning inhibition in potato slices higher than banana and apple slices ($p \leq 0.05$), respectively at 1 and 3 h storage. This might be due to the inhibition of PPE on potato PPO by phenolic compounds in PPE. Total phenolic content of 11.835mg/100 g of dry weight basis were found in the PPE. ROHN *et al.* (2001) reported that phenolic compounds from plants could prevent browning reaction. SUKHONTHARA *et al.* (2016) also found that phenolic compounds from rice bran extract had higher inhibitory effect on potato than apple. Pineapple shell extract contained phenolic compounds that could inhibit enzymatic browning in banana slices (SAISUNG and THEERAKULKAIT, 2011). SANCHO *et al.* (2011) found that ferulic acid and *p*-coumaric

acid are the most abundant phenolic compounds in the papaya peel. These phenolic compounds were also found in rice bran extract and these compounds could inhibit browning in potato and apple (SUKHONTHARA *et al.*, 2016). Therefore, these phenolic compounds in PPE might be responsible for browning inhibition in fruits and vegetables.

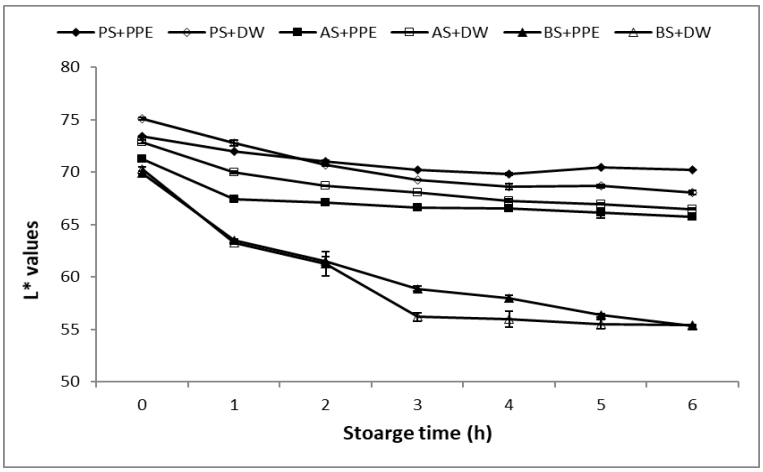

Figure 1. L* values of vegetable and fruit slices treated with papaya peel extract.
PS+PPE = Potatoes slice + papaya peel extract, PS+DW= Potato slice + distilled water AS+PPE = Apple slice + papaya peel extract AS+DW= Apple slice + distilled water; BS+PPE = Banana slice + papaya peel extract BS+DW= Banana slice+ distilled water PS+PPE = Potatoes slices + papaya peel extract and DW store at room temperature for 6 h.

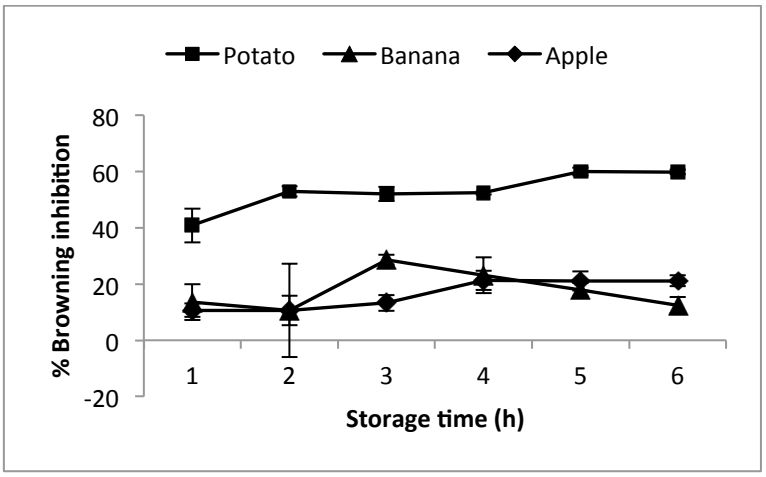

Figure 2. Browning inhibition (%) of papaya peel extract (PPE) in vegetable and fruit slices during storage at room temperature for 6 h.

4. CONCLUSIONS

PPE could inhibit browning in potato slices more effective than banana and apple slices. It has potential to be used as a natural antibrowning agent for shelf life extension of fruits and vegetables, especially potato slices.

REFERENCES

Boonsiripiphat K. and Theerakulkait C. 2009. Extraction of rice bran extract and some factors affecting its inhibition of polyphenol oxidase activity and browning in potato. Prep. Biochem. Biotechnol. 39:147-158.

Chaisakdanugull C., Theerakulkait, C. and Wrolstad R. E. 2007. Pineapple juice and its fractions in enzymatic browning inhibition of banana [*Musa* (AAA group) Gros Michel]. J. Agric. Food Chem. 55:4252-4257.

Girelli A. M., Mattei E., Messina A. and Tarola A. M. 2004. Inhibition of polyphenol oxidases activity by various dipeptides. J. Agric. Food Chem. 52:2741-2745.

Kaewka K., Portongkum K. and Theerakulkait C. 2009. Preparation of ultrafiltered rice bran extract and some factors affecting its browning inhibition in potato puree. Prep. Biochem. Biotechnol. 39:360-371.

Labuza T., Lillemo J. and Taoukis. P. 1990. Inhibition of polyphenol oxidase by proteolytic enzymes "Killer Enzymes", 8. In. Lamkin, W. 1981. Polyphenol oxidase activities of hard red winter, soft red winter, hard red spring, white common, club, and durum wheat cultivars. Cereal Chem. 58:27-31.

Le-Bourvellec C.L., Quere J-M.L., Sanoner P., Drilleau J.F. and Guyot S. 2004. Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. J. Agric. Food Chem. 52 (1):122-130.

Parniakov O., Francisco J., Barba C., Grimi N., Lebovka N. and Vorobiev E. 2014. Impact of pulsed electric fields and high voltage electrical discharges on extraction of high-added value compounds from papaya peels. Food Res. Int. 65:337-343.

Rohn S., Rawel H. and Kroll J. 2001. The influence of plant phenols on the biological activity of selected enzymes. Symposium on the 20th International Federation of Fruits Juice Producers. Paris, France.

de la Rosa L.A., Alvarez-Parrilla E., Moyers-Montoya E., Villegas-Ochoa M., Ayala-Zavala J.F., Hernández J., Ruiz-Cruz S. and González-Aguilar G.A. 2011. Mechanism for the inhibition of apple juice enzymatic browning by Palo Fierro (desert ironweed) honey extract and other natural compounds. Food Sci. Technol. 44:269-276.

Saisung P. and Theerakulkait C. 2011. Inhibitory effect of pineapple shell extract and its ultrafiltered fractions on polyphenol oxidase activity and browning in fresh-cut banana slices. CyTA- J. Food 9(1):37-42.

Sancho L.E.G-G., Yahia E.M. and Gonzalez-Aguilar G.A. 2011. Identification and quantification of phenols, carotenoids, and vitamin C from papaya (*Carica papaya* L., cv. Maradol) fruit determined by HPLC-DAD-MS/MS-ESI. Food Res. Int. 44:1284-1291.

Sapers G. M. and Jr Douglas F.W. 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. J. Food Sci. 52:1258-1285.

Sukhonthara S. and Theerakulkait C. 2012. Inhibitory effect of rice bran extract on polyphenol oxidase of potato and banana. Int. J. Food Sci. Tech. 47:482-487.

Sukhonthara S., Kaewka K. and Theerakulkait. C. 2016. Inhibitory effect of rice bran extracts and its phenolic compounds on polyphenol oxidase activity and browning in potato and apple puree. Food Chem. 190:922-927.

Theerakulkait C. and Boonsiripiphat K. 2007. Effects of rice bran extract on browning and polyphenol oxidase activity in vegetable and fruit. Kasetsart J. (Nat. Sci.) 41:272-278.

EFFECT OF HEAT TREATMENT AND STORAGE ON VOLATILE COMPOUNDS OF COCONUT MILK

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ABSTRACT

Coconut milk is a popular ingredient used for many Thai and Asian foods. Thermal process is used in order to preserve quality and extend shelf life of coconut milk products. Ultra high temperature (UHT) process and canning are widely used heat treatments for commercial coconut milk for world markets. Therefore, the aim of this work was to study effect of different heat treatments on volatile compound profiles of coconut milk during storage. Coconut milk was processed with two different heat treatments. It was heated at 140ºC for 4 sec by UHT/HTST processors and filled in sterilized glass jar or it was heated at 121ºC for 35 min in can. Coconut milk samples were stored at room temperature (30-32ºC) for 6 months. Changes of volatile compounds of coconut milk were evaluated in fresh sample and heat-treated sample after 2, 4 and 6 months of storage. Volatile compounds were extracted with Headspace-Solid Phase Microextraction (HS-SPME) and analyzed with gas chromatography-time-of-flight mass spectrometry (GC-TOFMS). Five groups of compounds consisting of alcohol, aldehyde, ketone, ester and lactone were identified in fresh and heated coconut milk. Some alcohols, esters, ketones, aldehydes and lactones increased during storage. Lactone such as delta decalactone, one of coconut-like aroma compounds, was not found in fresh coconut milk but it was presented during storage. These results indicated that changes of some key volatile compounds of coconut milk were affected by heat treatment and storage time.

Keywords: aroma compounds, coconut milk, heat treatment, storage volatile, profiles

1. INTRODUCTION

Coconut milk is a popular ingredient used for many Thai and Asian foods. It is made from grated coconut kernel with or without the addition of water. Fresh coconut milk is easy to spoil after extraction. Therefore, thermal process is used in order to preserve quality and extend shelf life when compared with fresh coconut milk (SEOW and GWEE, 1997). Ultrahigh temperature (UHT) and canning processes are most widely used heat treatments for production of coconut milk commercially worldwide. Heat treatment has been reported to affect volatile compound profile of products. Moreover, chemical reactions taking place during storage may also lead to changes in aroma and flavor such as lactones, the major volatile compounds for coconut-like, creamy and sweet odors (TINCHAN *et al.*, 2015). Thus, the aim of this work was to study effect of different heat treatments on volatile compound profiles of coconut milk during storage.

2. MATERIALS AND METHODS

2.1. Sample preparation

Coconut meat was purchased from a local market. Water was added in grated coconut meat and pressed with a hydraulic machine. The fat content of coconut milk was in the range of 12-13 % analyzed by Gerber method. Coconut milk was treated with two different heat treatments (ultrahigh temperature (UHT) and canning). UHT treatment was done by heating coconut milk at 140 ºC for 4 sec by UHT/HTST processors and then it was filled into sterilized glass jars, while canning was performed at 121 ºC for 35 min. Heated samples by two different methods were stored at room temperature (30-32 ◦C) for 6 months. Samples were analyzed every two months with two replications.

2.2. Extraction of volatile compounds

Volatile compounds of fresh and heated coconut milk were extracted by Headspace-Solid Phase Microextraction (HS-SPME) technique. The extraction methods were modified from PRADES *et al.* (2012). 5 mL of sample with 2-methyl-3-heptanone as the internal standard and 1 g NaCl were put in a headspace vial. The vial was equilibrated at 45 ◦C for 30 min. After that, SPME fiber was inserted into the vial and extraction at headspace for 20 min at similar temperature. The fiber was then desorbed in the GC inlet at 250 ◦C for 15 min.

2.3. Gas chromatography analysis

Volatile compounds were analyzed by gas chromatography (7890A; Agilent Technology, USA) equipped with a time-of-flight mass spectrometry (Pegasus 4D, LECO Corp., USA). The column was Stabilwax® (30 m × 0.25 mm i.d., 0.25 μm). Helium was used as a carrier gas at flow rate of 1 mL/min. Oven temperature was at 35 ºC and raised to 225 ºC at 4 ºC/min and held for 10 min. Volatile compounds were identified by comparing mass spectra with the NIST Mass Spectral Library and retention indices (RI) were calculated from alkane series of C6-C30 and compared with literatures. Relative concentration was calculated by internal standard.

3. RESULTS AND DISCUSSION

3.1. Effect of heat treatment on volatile compounds in coconut milk

Volatile compounds in fresh and heated coconut milk samples after processes were extracted by Headspace-Solid Phase Microextraction (HS-SPME) technique (Table 1). Four groups of volatile compounds consisting of alcohol, ketone, ester and lactone groups were analyzed in fresh coconut milk. The most abundant group of compounds was alcohols followed by ketones and esters. In alcohol group, ethanol was the most abundant compound found in coconut milk followed by 2-methyl-1-butanol. TINCHAN *et al*. (2015) reported more type of volatiles (27 compounds in total) in fresh coconut milk by using solvent extraction technique. However, ethanol was not listed in their report since it is a small molecule that appears early in the chromatogram; thus, it gets co-eluted with solvent peak in solvent extraction method.

Table 1. Relative concentration of aroma compounds in coconut milk for fresh, UHT and canning treatments.

Compound	Aroma description	Relative concentration (ppb)		
		fresh	UHT	canning
alcohol				
ethanol	sweet, ethereal[c]	130.97	521.55	446.47
2-methyl-1-propanol	sweet, whiskey-like[c]	1.23	2.99	3.40
1-butanol	fruity[a]	0.77	5.85	8.29
2-methyl-1-butanol	alcoholic[c]	6.61	13.31	10.54
1-pentanol	-	n.d.	1.79	1.54
2-heptanol	-	0.64	1.77	0.88
1-hexanol	fatty-fruity[c]	0.03	9.13	8.70
1-heptanol	-	n.d.	3.30	3.82
aldehydes				
hexanal	grass, tallow, fatty, leaf[a]	n.d.	1.65	0.10
heptanal	fat, almond[c]	n.d.	1.89	5.42
octanal	lemon, green, fatty[a]	n.d.	0.79	2.93
nonanal	-	n.d.	0.29	0.71
ketones				
2-butanone	-	31.18	44.28	45.53
2-heptanone	-	n.d.	1.03	1.10
acetoin	yogurt-like, buttery[a]	1.24	n.d.	n.d.
2-nonanone	-	n.d.	n.d.	0.67
esters				
ethyl acetate	-	4.99	12.63	38.53
ethyl octanoate	fruity, apple, sweet[a]	0.78	2.52	1.79
lactones				
δ-octalactone	cream, coconut-like, minty[b]	0.46	1.23	12.40
δ-decalactone	coconut-like, cream, peach[b]	n.d.	n.d.	0.29

[a]Aroma description from TINCHAN *et al.* (2015).

[b]Aroma description from PRADES *et al.* (2012).

[c]Aroma description from LEFFINGWELL (2004).

n.d. = not detected.

For heat treated coconut milk, concentration of some alcohols, esters and lactones rapidly increased compared to fresh sample. Heat treatment could cause degradation of lipids, which leads to lipid oxidation resulting in formation of ketones, aldehydes, alcohols and lactones (NAWAR, 1969; TINCHAN *et al.*, 2015). Interestingly, aldehydes were not found in fresh coconut milk but they were presented in both UHT and canned coconut milk samples. Some aldehyde compounds are products of autooxidation of fatty acids. Hexanal, the most abundant aldehyde identified in this study, might come from oxidation of polyunsaturated linoleic and linolenic acids by enzyme activity. The reaction might take place during preparation of sample and early part of heating (PRADES *et al.*, 2012).
Acetoin was presented in fresh coconut milk but it was not detected after heat treatments. Some researchers studied possible change of acetoin into 2,3-butanediol through enzymatic reaction (YANG *et al.*, 2014).
Lactones are the major volatile compounds in coconut products and responsible for coconut-like, creamy and sweet odors (TINCHAN *et al.*, 2015). *δ*-Octalactone, which presented in all samples, was stated to be the characteristic compound in products from coconut of all variety (PRADES *et al.*, 2012). The δ-octalactone concentration of canning treatment was higher than that of UHT treatment. Meanwhile, *δ*-decalactone was found only in canned sample. Longer processing time in canning, compared to UHT process, might give more opportunity for lipid degradation and oxidation to happen, thus leading to formation of secondary product such as lactones.

3.2. Effect of storage time on selected volatile compounds in coconut milk

Changes in selected volatile compound concentrations of coconut milk during storage are shown in Fig. 1.

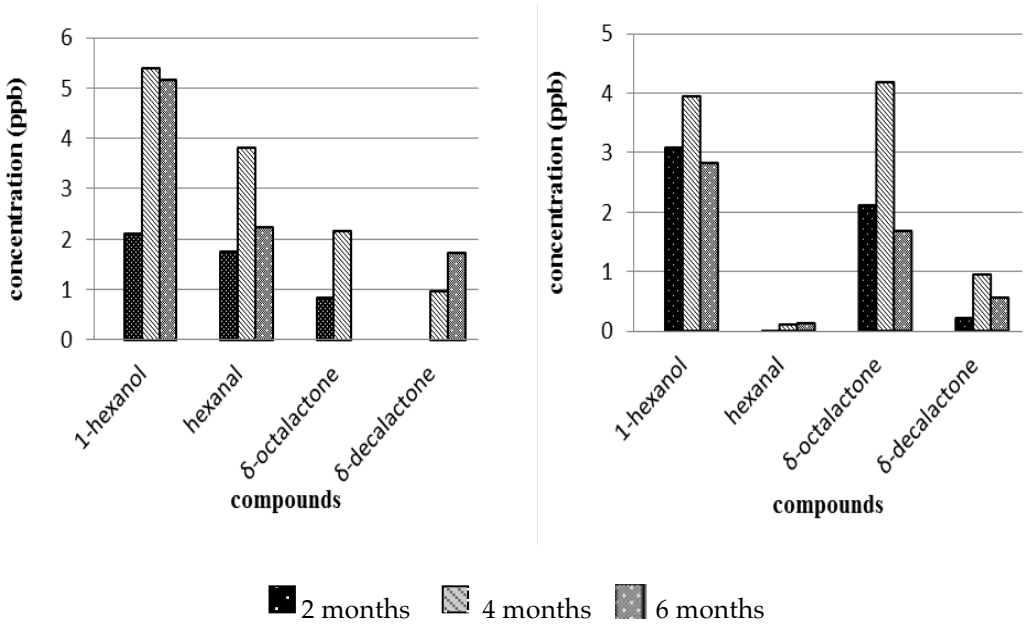

Figure 1. Relative concentration of selected volatile compounds in UHT and canning treated coconut milk during storage: UHT (A), Canning (B).

These four selected compounds have been reported to be important for aroma properties of coconut milk (TINCHAN *et al.*, 2015). Increased concentration of some alcohol,

aldehyde and lactone during storage showed that those compounds might be secondary products of oxidation. In general, there was increase in aldehyde compounds; therefore, these might indicate lipid oxidation of heated coconut milk during storage. Aldehyde, such as hexanal, is one of products indicating lipid oxidation (LI *et al.*, 2012). However canned sample received longer period of heating time that might cause some loss of volatile compounds including aldehydes (FABIOLA *et al.*, 2015). Lactones were increased at 4 months of storage. The formation of these compounds during storage might from fatty acids and hydroxyl fatty acids produced from autooxidation of lipids (PRADES *et al.*, 2012).

1-Hexanol also showed increasing trend during storage. This alcohol seemed to be responsible for the typical coconut aroma when it was presented together with lactones (JIROVETZ *et al.*, 2003; PRADES *et al.*, 2012). Thus, inclining presence of both 1-hexanol and δ-lactones might give desirable effect of coconut-like aroma to heated coconut milk.

In most compounds, the increasing trend could still be observed even at longer storage period. However, in 6 months of storage, some compounds started to decline in their concentrations. It might indicate that oxidation reaction started to slow down, due to a lowering availability of oxygen in the headspace of the containers (TINCHAN *et al.*, 2015).

4. CONCLUSIONS

Heat treatment caused changes in volatile compound profile of coconut milk. Longer processing time in canning provided more opportunity for reaction to happen, thus resulting in formation of δ-decalactone as compared to UHT samples. Interestingly acetoin, yogurt-like aroma, was presented in only fresh non-heated coconut milk but it was not detected in heated as well as stored UHT and canned coconut milk. These results indicated that changes of some key volatile compounds of coconut milk were differently affected by heat treatment and storage time.

REFERENCES

Fabiola D.M., Aprea E., Endrizz I., Charles M., Betta E., Corollaro M.L., Cappelletti M., Ferrentino G., Spilimbergo S. and Gasperi F. 2015. Effects of pasteurization on volatile compounds and sensory properties of coconut (*Cocos nucifera* L.) water: thermal vs. high-pressure carbon dioxide pasteurization. Food Bioprocess Technology 8:1393-1404.

Jirovetz L., Buchbauer G., and Ngassoum M.B. 2003. Solid-phase-microextraction headspace aroma compounds of coconut (*Cocos nucifera* L.) milk and meat from cameroon. Nutrition 27:300-303.

Leffingwell J.C. 2004. Flavor-Base Database, Version Date July 1, 2004.

Li Yanhua., Zhang L. and Wang W. 2012. Formation of aldehyde and ketone compounds during production and storage of milk powder. Molecules 17:9900-9911.

Nawar WW. 1969. Thermal degradation of lipids. Journal of agricultural and food chemistry 17:18-21.

Prades A., Assa R.R.A., Dornier M., Pain J-P. and Boulanger R. 2012. Characterization of the volatile profile of coconut water from five varieties using an optimized HS-SPME-GC analysis. Journal of the science of food and agriculture 92:2471-2478.

Seow C.C. and Gwee C.N. 1997. Coconut milk: chemistry and technology. International journal of food science and technology 32:189-201.

Tinchan P., Lorjaroenphon Y., Cadwallader K.R. and Chaiseri S. 2015. Changes in the profile of volatiles of canned coconut milk during storage. Journal of food science 80:49-54.

Yang T.H., Rathnasingh C., Lee H.J. and Seung D. 2014. Identification of acetoin reductases involved in 2,3-butanediol pathway in *Klebsiella oxytoca*. Journal of biotechnology 172:59-66.

EFFECT OF ANTIOXIDANT-CONTAINING EDIBLE COATING ON STABILITY OF BANANA CONSERVE STICK

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ABSTRACT

The objective of this study was to investigate the effect of agar coating fortified with antioxidant on physical and chemical stability of banana conserve stick. Agar coating containing 0-200 ppm of tertiary butylhydroquinone (TBHQ) was applied to banana conserve stick and quality changes of the sample were monitored during 5-week storage as compared to conserve stick wrapped with cellophane sheet and the unwrapped/uncoated control. Upon storage, the control exhibited the greatest increase in moisture content (35.0% wb at Week 0 vs. 38.6% wb at Week 3). Water activity (a_w), on the other hand, did not change significantly ($p>0.05$), with all samples exhibiting a_w within the range of 0.80-0.86. Regarding texture, the control demonstrated a decrease in hardness over time and this was significantly lower than the wrapped or coated samples ($p\leq0.05$). In terms of appearance, coating and wrapping seemed to pose a minimal effect on CIE L^*, a^* and b^* of the conserve stick. Thiobarbituric acid reactive substances (TBARs) content was determined in order to monitor lipid oxidation in the conserve samples. The control demonstrated the greatest TBARs value (1.3 μg/g at Week 5) while those samples with TBHQ-added coating showed a slower increase in TBARs. A decrease in lipid oxidation was noticed upon increasing TBHQ concentration (0.9 μg/g at Week 5 for the sample with 200 ppm TBHQ). From this study, edible agar coating was shown to be effective in retarding moisture uptake of banana conserve stick. Addition of TBHQ to the coating further increased oxidative stability of the sample. Active edible coating can thus be used to impose storage stability of conserve stick.

Keywords: antioxidant, banana conserve stick, edible coating

1. INTRODUCTION

Fresh as well as processed food undergoes various changes during storage and distribution. To maintain quality and safety of the food, packaging became indispensable to modern food industry. Films from petroleum-based polymers have been widely used as food packaging due to their superior barrier and mechanical properties. In spite of that, they create non-biodegradable waste. Due to the global awareness of the importance of waste management, edible films and coatings are thus considered potential replacement for conventional plastic counterparts to provide stability, integrity, as well as convenience to a food product. Besides those passive roles, edible films and coating can be formulated in such a way that they also perform an active role such as carrier of food additives like flavor, antioxidant and antimicrobial agent (MARTÍN-BELLOSO *et al.*, 2009; QUEZADA-GALLO, 2009).

Banana conserve stick or banana candy is made from crushed ripe banana with added sugar and coconut milk, and cooked to evaporate water until obtaining high enough consistency to be able to form into a stick. Being high in sugar and fat content, it is prone to quality changes during storage such as moisture migration and lipid oxidation. The product sold in Southeast Asia is conventionally wrapped with cellophane or low-density polyethylene (LDPE) sheet, which could be replaced by an edible coating.

The objective of this study was therefore to investigate the effect of edible agar coating fortified with tertiary butylhydroquinone (TBHQ) as an antioxidant on physical and chemical stability of banana conserve stick.

2. MATERIALS AND METHODS

2.1. Materials

Materials for making banana conserve stick, i.e. ripe banana, cane sugar, and UHT coconut milk, were purchased from local stores. Food-grade agar-agar powder was a product of Patanasin Enterprise (Bangkok, Thailand). TBHQ was kindly provided by White Group Public Co., Ltd. (Bangkok, Thailand). All analytical chemicals were of AR grade.

2.2. Preparation of banana conserve stick

Banana conserve stick was prepared in the laboratory. Ripe banana (4000 g) was blended with 500 g coconut milk. The puree was then added with 1250 g coconut milk and 500 g sugar. Calcium propionate (0.2%) was used as mold inhibitor. After heating for 6 hr to evaporate water, the resulting paste was transferred to an aluminum square pan and cooled down before being cut into 13×13×20 mm sticks.

A total of six treatments were prepared in order to investigate the effect of edible coating on stability of banana conserve stick: (1) conserve stick without wrapping or coating (Control), (2) conserve stick twist-wrapped using clear cellophane sheet (Cellophane), (3) conserve stick with agar coating (Agar), (4) conserve stick with agar coating containing 50 ppm TBHQ (Agar+TBHQ50), (5) conserve stick with agar coating containing 100 ppm TBHQ (Agar+TBHQ100), and (6) conserve stick with agar coating containing 200 ppm TBHQ (Agar+TBHQ200).

Coating solution (2%) was prepared by dispersing agar-agar powder (10 g) in 490 g water. The dispersion was then heated at 80°C until the agar was completely dissolved. For those antioxidant-containing coating, TBHQ was added at concentration of 50-200 ppm. To apply coating on the conserve stick, the stick was dipped in coating solution for 3 s, then

removed and let dry at room temperature. The conserve stick samples were stored at room temperature for a period of 5 weeks and monitored for changes in chemical and physical properties.

2.3. Determination of conserve stick properties

Moisture content was determined according to AOAC (2000) and a_w was measured using AquaLab® water activity meter (model 3TE, Decagon Devices, Pullman, WA, USA).
Texture profile analysis (TPA) was carried out using Texture Analyzer (model TA.XT2i, Stable Micro System, Surrey, UK) equipped with P/100 stainless steel cylindrical probe. A test speed of 5 mm/s and 50% deformation was used. Hardness was extracted from the TPA curve.
Color was measured in CIELAB system using a chroma meter (model CR-400, Konica Minolta Sensing, Osaka, Japan) under D65 illuminant. To monitor lipid oxidation, TBARs content was determined according to the method described by HODGES *et al.* (1999).

2.4. Statistical analysis

A completely randomized design was used for the experiment, which was carried out in three replicates. Data were analyzed using Analysis of Variance. A Duncan's new multiple range test was used to determine the difference among sample means at p=0.05.

3. RESULTS AND DISCUSSION

3.1. Moisture content and water activity

Moisture content of the conserve samples is shown in Fig. 1. Being without any kind of barrier, the control exhibited a greater increase in moisture content as compared to other samples. This was due to that the conserve contained large amount of sugar, which readily absorbed moisture from the environment. After 3 weeks of storage, the moisture content started to decrease. This could be due to the changes of ambient humidity during storage. Moisture content of those samples with agar coating was, in general, similar to that wrapped with cellophane sheet. Even though polysaccharides are of hydrophilic nature, those with gel-forming ability were reported to demonstrate moisture barrier capability, particularly when being stored for a limited time period (KESTER and FENNEMA, 1986). Water activity, on the other hand, were similar in all samples ($p>0.05$), with a value in the 0.80-0.86 a_w range (data not shown).

3.2. Texture

After being stored for one week, it was evident that the control was lower in hardness than the sample wrapped in cellophane sheet and those with agar coating ($p\leq0.05$) (Fig. 2). This was consistent with the increase in moisture content of the control upon storage. The samples with agar coating exhibited similar hardness to that wrapped with cellophane sheet.

3.3. Color

Wrapping and coating posed a minimal effect on CIE L^*, a^*, b^* of the samples (data not shown). For example, at Week 0, all samples exhibited L^*, a^*, b^* within a narrow range

(L^*=43.0-45.0, a^*=5.6-6.1, b^*=7.8-8.5). Upon storage, all samples demonstrated a slight decrease in L^* and b^* and a slight increase in a^*. At the end of the storage (Week 5), the samples had L^*, a^*, b^* in the range of 37.3-38.9, 8.0-8.4, and 5.3-6.2, respectively.

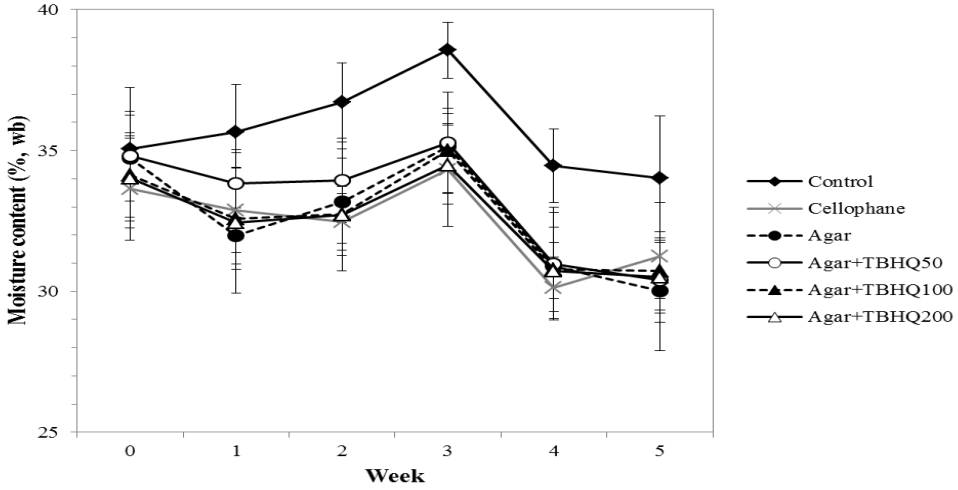

Figure 1. Moisture content of banana conserve stick samples during 5-week storage.

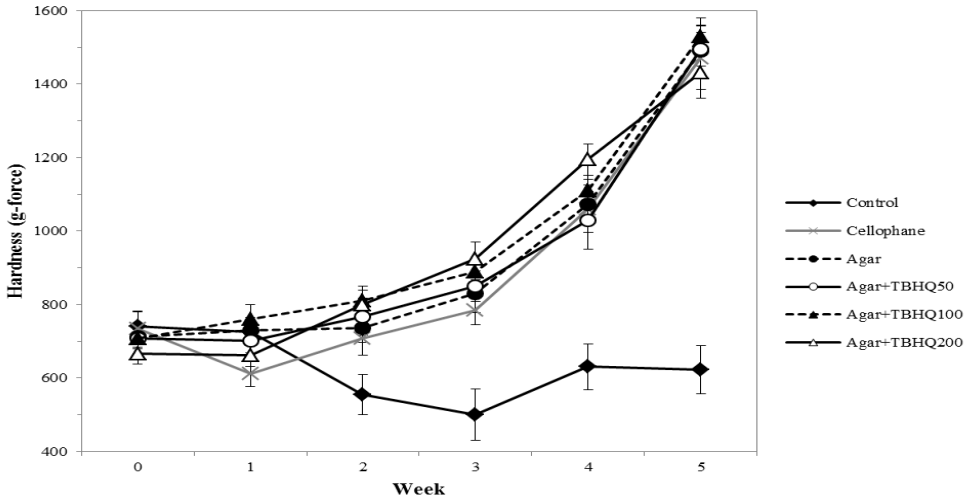

Figure 2. Hardness of banana conserve stick samples during 5-week storage.

3.4. TBARs

Lipid oxidation was monitored by determining TBARs content (Fig. 3). TBARs was found to increase with increasing storage time, with the control demonstrated the most striking increase in TBARs value, particularly after 2 weeks of storage. For instance, at Week 5, the control had TBARs of 1.3 μg/g, which was 1.6 times greater than that at Week 0. In contrast, the samples with TBHQ-added coating showed a slower increase in TBARs. A decrease in lipid oxidation was noticed upon increasing TBHQ concentration (0.9 μg/g at Week 5 for the sample with 200 ppm TBHQ).

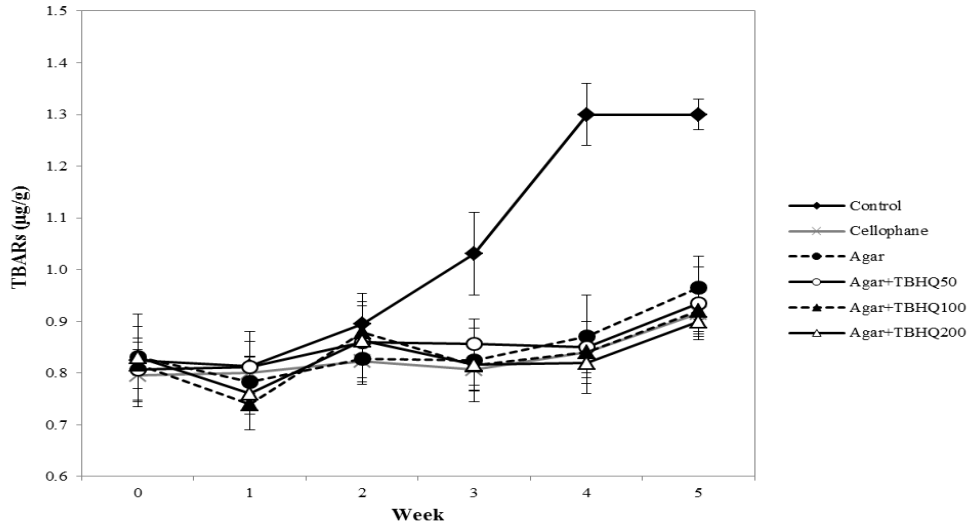

Figure 3. TBARs of banana conserve stick samples during 5-week storage.

4. CONCLUSIONS

Agar coating has a potential to be used as a substitute to cellophane wrap for banana conserve stick. Apart from its ability in terms of protecting the product inside from moisture migration, edible agar coating also provides convenience to consumers. With addition of antioxidant, agar coating could be formulated in a way that it possesses an active role in retarding lipid oxidation.

ACKNOWLEDGEMENTS

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REFERENCES

AOAC. 2000. "Official Methods of Analysis" 17th ed. The Association of Analytical Chemists, Washington, D.C.

Hodges D.M., Delong J.M., Forney C.F. and Prange R.K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 207:604-11.

Kester J.J. and Fennema O.R. 1986. Edible films and coatings: a review. Food Technol. 40:47-59.

Martín-Belloso O., Rojas-Graü M.A. and Soliva-Fortuny R. 2009. Delivery of flavor and active ingredients using edible films and coatings. In: "Edible Films and Coatings for Food Applications". M.E. Embuscado, and K.C. Huber (Eds.), p. 295-313. Springer, New York, NY.

Quezada-Gallo J.A. 2009. Delivery of food additives and antimicrobials using edible films and coatings. In: "Edible Films and Coatings for Food Applications". M.E. Embuscado, and K.C. Huber (Eds.), p. 315-333. Springer, New York, NY.

EXTENDING THE SHELF LIFE OF DOCOSAHEXAENOIC ACID (DHA) INCORPORATED CHEVON PATTIES USING BIOACTIVE BLOOD PEPTIDES AND DIFFERENT PACKAGING METHODS

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ABSTRACT

Functional chevon patties were developed with standardized level of 1% algal docosahexaenoic acid (DHA) oil, an omega-3 fatty acid source. The patties were added with 0.09% of <1 kDa bioactive peptide fractions of papain hydrolysates of goat blood with pre-evaluated antioxidant and antimicrobial efficacy (results not included) as an alternative to chemical preservatives. The control (CON) and treated (with bioactive blood peptides (BBP)) products were packaged aerobically (AP) and in modified atmosphere (MAP), stored in refrigeration (4±1°C) and analysed on 0th, 7th, 14th, 21st, 28th and 35th day. Quality attributes viz. physico-chemical (pH, a_w, titratable acidity (TA), instrumental colour and textural profile) lipid peroxidation (peroxide value (PV), TBARS and free fatty acid (FFA)), sensory and microbiological were evaluated. There was significant ($P<0.05$) increase in pH, TBARS, PV and FFA with storage days in all the samples, however, BBP-AP and BBP-MAP exhibited lower values than their respective CON. The microbiological quality was significantly ($P<0.05$) improved in the BBP incorporated products than CON and in MAP packed than AP. Standard Plate Count was 3.5 log cycle lower in BBP-MAP than CON-AP on 35th day of storage. Coliforms and yeast and molds were not detected in BBP-MAP throughout the storage period. The sensory quality attributes were better maintained in MAP products than in AP. The sensory panellists rated BBP-MAP as 'Very Good to Excellent' on 35th day of storage. Results concluded that DHA enriched functional chevon patties can be successfully stored at refrigeration for 35 days with incorporation of BBP under modified atmospheric (MAP) conditions.

Keywords: aerobic packaging, modified atmospheric packaging, omega-3 fatty acid, papain hydrolysates

1. INTRODUCTION

Nutrition experts recommend diets composed of omega-6/omega-3 fatty acids in a ratio of less than 5 : 1 (MOYNIHAN, 2000), but the modern diets have high concentration of omega-6 fatty acids leading this ratio to more than 15:1 (SIMOPOULOS, 2000). This balanced fatty acid ratio has many health benefits such as decreased blood cholesterol level. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are reported to have anti-inflammatory (FURUHJELM *et al.,* 2009) cardiovascular protective (DAWCZYNSKI *et al.,* 2010) and cancer reducing properties (SEO *et al.,* 2005). European Food Safety Agency (EFSA) has recommended an intake of at least 250mg/day of combined dose of EPA and DHA for adults (TETENS, 2008). Omega-3 fatty acids are essential fatty acids and are not generated by human body and need to be taken through diets. DHA and EPA are primarily obtained through marine resources (fish and algae). Algae oil has been described as one of the best natural sources of DHA, containing up to 45% of its total fatty acids. However, fortification of DHA oil into food products imparts characteristic fishy flavour leading to the compromise of consumer preference (KROFA *et al.,* 2017). Moreover omega-3 fatty acids are highly susceptible to oxidative deteriorative changes. Lipid oxidation and microbial growth can be controlled with the use of known synthetic antioxidants (BHA & BHT etc.) and antimicrobials (tetracyclines, antibiotics etc.). However, the synthetic compounds have debatable health hazards on consumers such as carcinogenicity, antibiotic resitsant etc. This has lead to increase in the demand of natural preservatives. (SHAN *et al.,* 2009).
Hence, the study was conducted to explore selected bioactive peptides generated from animal blood as a preservative to extend the storage stability of DHA incorporated chevon patties at refrigeration temperature under different packaging conditions.

2. MATERIALS AND METHODS

Male Beetal goat aged one year procured from University Goat Farm was slaughtered by humane method approved by ethical committee. Hygienically collected blood and goat meat (chevon) were packed in presterilized low-density polyethylene (200 gauge LDPE) bags and stored at -18°C till the analysis. Algal DHA oil (35%) procured from V.B. Medicare Pvt. Ltd., Hosor, India. Functional chevon patties were developed with 1% DHA oil and evaluated for various sensory and nutritive quality attributes (results not included). Selected papain hydrolysed ultra-filtered bioactive peptide fractions of <1 kDa (0.09%) of goat blood with measured antioxidant and antimicrobial efficacy in comparison to synthetic antioxidant (BHT; 0.02%) and against food associated microorganisms (results not included). DHA fortified patties were grouped as; CON-AP (control, packed aerobically), BBP-AP (blood peptide fraction & packed aerobically), CON-MAP (control & MAP) and BBP-MAP (blood peptide fraction & MAP). LDPE 200 gauge was used for aerobic packing, whereas, 70% N_2 and 30% CO_2 in double layered laminated plastic pouches (Polyester/ Polyethylene 100/100µ) was used for modified atmospheric packaging (MAP). Samples were stored at refrigeration (4±1°C) temperature and analysed on 0th, 7th, 14th, 21st, 28th and 35th day. Quality attributes viz. physico-chemical (pH, a_w, titratable acidity, ERV), instrumental colour (Lovibond Tintometer Model: RT-300, The Tintometer Limited, Amesbury, UK) and textural profile (TMS-PRO, Food Technology Corporation, Maries Road, Suite 120 Sterling, VA, USA) lipid peroxidation (peroxide value; PV, TBARS and free fatty acid; FFA), sensory (8-point descriptive scale) and microbiological (SPC, Psychrophilic, Coliform, Staphylococcus and Yeast and Mould counts) were evaluated.

Free fatty acids and peroxide value were evaluated as per KONIECKO (1979) and statistical analysis (SPSS 22, IL USA) was conducted at 5% level of significance.

3. RESULTS AND DISCUSSION

The pH followed an increasing trend during storage in all the products, irrespective of addition of peptides or packaging conditions (Fig. 1).

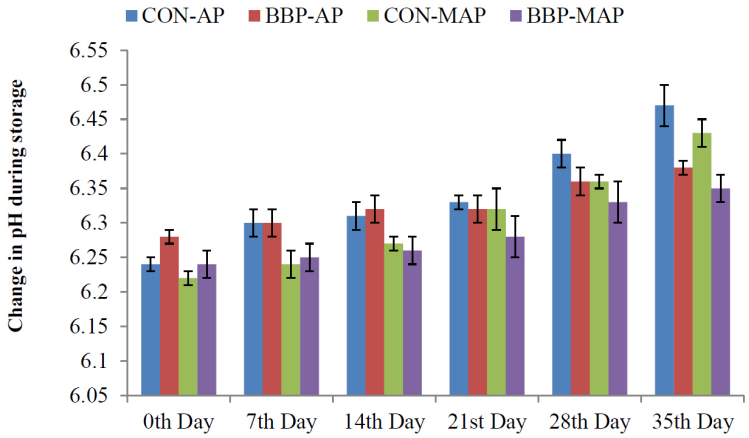

Figure 1. Effect of incorporation of goat blood hydrolysate fraction (<1 kDa) on pH of functional chevon patties during refrigeration storage (4±1°C).

The increase in pH might be due to deamination of proteins caused by bacterial growth. The antimicrobial effects of peptide fraction in treated groups might have caused decline in the rate of increase in pH during storage. JIN *et al.* (2015) also observed increase in pH of refrigerated chicken meat hydrolysates incorporated sausages. Formation of carbonic acid due to the presence of CO_2 might have caused the lower rate of increase in the pH of products under MAP. Water activity (a_w) decreased during storage, however the rate of decrease was significantly ($P<0.05$) higher in AP than in MAP. It might be attributed to better barrier properties of laminate packaging material used for MAP. TA dropped significantly ($P<0.05$) in all the groups as a reflux of pH of the products (THOMAS *et al.*, 2006)

PV, TBARS and FFA increased significantly ($P<0.05$) with the progress of storage and AP products exhibited significantly ($P<0.05$) higher values than MAP due to presence of O_2 in aerobic package. BBP samples were better than CON confirms antioxidant effect of the selected peptides. LI *et al.* (2015) as well as DEY and DORA (2014) also documented lower values for oxidative deteriorative indicators in minced fish muscles and croaker fillets incorporated with fish protein and shrimp hydrolysates, respectively.

Redness (a^*) is considered as most sought out factor for freshness by the consumers. The values of a^* increased, whereas yellowness (b^*) decreased in the treated products however both followed a decreasing trend during storage. This might be due to breakdown of proteins and oxidation of the fats with storage time. The a* and b* values remain comparable in the treated products throughout storage attributed to antioxidant and antimicrobial properties of added peptides. MAP maintained a^* and b^* values better then AP during storage due to superior gas barrier properties of the packaging material of

MAP. Our results are in accordance with those of JIN *et al.* (2015) who reported that the colour parameters were better in the sausages added with chicken meat hydrolysates.
Texture profile analysis (TPA) values of springiness, stringiness, cohesiveness, chewiness, gumminess and resilience were significantly (P<0.05) lower in treated groups compared to control, however, these increased with storage whereas MAP kept the values more stable compared to AP. CAVALHEIRO *et al.* (2014) also reported better maintenance of texture quality attributes with introduction of chicken meat hydrolysate in sausages.
Standard plate count (SPC) counts were significantly (P<0.05) lower in the treated and MAP products (Table 1).

Table 1. Effect of incorporation of goat blood hydrolysates on SPC (log cfu/g) of chevon patties during refrigeration storage (4±1°C).

Groups	0 Day	7 Day	14 Day	21 Day	28 Day	35 Day
CON-AP	2.40 ± 0.07^{Ba}	3.10 ± 0.08^{Bb}	4.13 ± 0.12^{Dc}	5.17 ± 0.15^{Dd}	6.15 ± 0.09^{De}	6.24 ± 0.08^{Cf}
BBP-AP	2.16 ± 0.14^{ABa}	2.53 ± 0.09^{Ab}	2.90 ± 0.07^{ABc}	3.73 ± 0.14^{Bd}	4.14 ± 0.08^{Be}	4.81 ± 0.16^{Bf}
CON-MAP	2.32 ± 0.08^{ABa}	2.97 ± 0.12^{Bb}	3.86 ± 0.14^{Cc}	4.67 ± 0.11^{Cd}	5.05 ± 0.15^{Ce}	5.75 ± 0.11^{Cf}
BBP-MAP	2.08 ± 0.14^{Aa}	2.40 ± 0.11^{Ab}	2.75 ± 0.08^{Ac}	3.16 ± 0.14^{Ad}	3.80 ± 0.14^{Ae}	4.15 ± 0.08^{Af}

Means values bearing different superscripts in (small letters in the same row and capital letters in the same column) differ significantly (P<0.05) n=6; CON-AP: goat meat patties without bioactive peptides and aerobic packaged, BBP-AP: goat meat patties incorporated with bioactive peptide from goat blood protein and aerobic packaged, CON-MAP: goat meat patties without bioactive peptides and MAP packaged), BBP-MAP: goat meat patties incorporated with bioactive peptide from goat blood protein and MAP packaged.
The lower values of SPC for treated groups might be due to antimicrobial properties of peptides in addition to inhibition of bacterial growth in MAP (ASHIE *et al.*, 1996). Coliforms were first detected on the 14 day in CON-AP and on 35th day in BBP-MAP. DAOUD *et al.* (2005) also postulated that the blood hydrolysates are an excellent source of antimicrobial peptides. Yeast and molds appeared in CON-AP on 14th day, while it was observed after 21 days in all the samples except BBP-MAP, where it continued to inhibit it upto 28 days. This might be due to the superior barrier properties of MAP synergized by antimicrobial efficacy of blood hydrolysates. LIU *et al.* (2008) also documented that the enzymatic hydrolysates from oyster exhibited anti-fungal properties.
Sensory scores of colour and appearance declined irrespective of treatment or packaging method throughout the storage period, however, the rate of decline in the scores of AP was faster than MAP. These scores were in agreement with instrumental colour profile. Decrease in scores might be due to moisture loss leading to concentration of pigments and in turn darker product. These changes were higher in AP than MAP and in CON than in treated products. Better colour in BBP-AP and BBP-MAP than in CON-AP and CON-MAP reflects the protective effect of peptide fraction and MAP. Improvement in the scores of flavor in treated products might be due to the influence of taste active peptides in the fraction.

4. CONCLUSIONS

The results concluded that the papain hydrolysed goat blood can be successfully used as a source of generation of bioactive peptides that can be used as natural preservative to check

the oxidation of fortified DHA and microbial deterioration of chevon patties packed under MAP, up to 35 days, without compromising its quality attributes.

REFERENCES

Ashie I.N., Smith J.P. and Simpson B.K. 1996. Spoilage and shelf-life extension of fresh fish and shellfish. Crit. Rev. Food Sci. Nutr. 36:87-21.

Cavalheiro C.P., Ludtke F.L., Stefanello F.S., Kubota F.H., Terran N., Fries L.L.M. 2014. Replacement of mechanically deboned chicken meat with its protein hydrolysate in mortadella-type sausages. Food Sci. Technol. 34:478-84.

Daoud R., Dubois V., Bors-Dodita L., Nedjar-Arroume N., Krier F. and Chihib N.E. 2005. New antibacterial peptide derived from bovine hemoglobin. Peptides. 26:713-19.

Dawczynski C., Martin L., Wagner A. and Jahreis G. 2010. n-3 LC-PUFA-enriched dairy products are able to reduce cardiovascular risk factors: a double-blind, cross-over study. Clin. Nutr. 29:592-99.

Dey S.S. and Dora K.C. 2014. Antioxidative activity of protein hydrolysate produced by alcalase hydrolysis from shrimp waste (*Penaeus monodon* and *Penaeus indicus*). J. Food Sci. Technol. 51:449-57.

Furuhjelm C., Warstedt K., Larsson J., Fredriksson M., Bottcher M.F. and Falth-Magnusson K. 2009. Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. Acta Paediatrica. 98:1461-67.

Jin S.K., Choi J.S., Choi Y.J., Lee S.J., Lee S.Y., Hur S.J.. 2015. Development of sausages containing mechanically deboned chicken meat hydrolysates. J. Food Sci. 80:S1563-S67.

Koniecko R. 1979. "Hand Book for Meat Chemists" Avery Publishing Group, Inc. Wayne, New Jersy, pp. 53-55.

Krofa D., Chatli M.K., Singh V.P. and Mehta N. 2017. Economic evaluation of functional goat meat patties to supplement farm returns. Indian J. Econ. Dev. 13:543-50.

Li X., Luo Y., You J. and Shen H. 2015. Stability of papain-treated grass carp (*Ctenopharyngodon idellus*) protein hydrolysate during food processing and its ability to inhibit lipid oxidation in frozen fish mince. J. Food Sci. Technol. 52:542-48.

Liu Z.Y., Dong S.Y., Xu J., Zeng M.Y., Song H.X. and Zhao Y.H. 2008. Production of cysteine-rich antimicrobial peptide by digestion of oyster (*Crassostrea gigas*) with alcalase and bromelin. Food Control 19:231-35.

Moynihan P. 2000. Nutrition: the British nutrition foundation oral task force report - issues relevant to dental health professionals. Br. Dent. J. 188: 308–12.

Seo T., Blaner W.S. and Deckelbaum R.J. 2005. Omega-3 fatty acids: molecular approaches to optimal biological outcomes. Curr. Opin. Lipidol. 16:11-18.

Shan B., Cai Y. Z., Brooks J. D. and Corke H. 2009. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. J. Sci. Food Aagril. 89(11):1879-85.

Simopoulos A. P. 2000. Human requirement for N-3 polyunsaturated fatty acids. Poult. Sci. 79:961–70.

Tetens I. 2008. "Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a Request from the: Question No EFSA-Q-2008-269" European Food Safety Authority, 2008.

Thomas R., Anjaneyulu A. S. R. and Kondaiah N. 2006. Quality and shelf life evaluation of emulsion and restructured buffalo meat nuggets at cold storage (4±1°C). Meat Sci. 72:373-79.

EFFECT OF ETHYLENE CONCENTRATIONS ON THE QUALITY AND ENZYME ACTIVITY OF DRAGON FRUIT *(HYLOCEREUS UNDATUS)*

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ABSTRACT

Dragon fruit (*Hylocereus undatus*) is one of the important tropical fruits in many countries such as Vietnam, Thailand, and Malaysia. Postharvest life of dragon fruits is usually limited by yellowing and bract wilting. Exposure to ethylene during mix load transportation may hasten senescence of dragon fruits. The objective of this research was to study the effect of ethylene on the quality and enzyme activity of dragon fruit. Dragon fruits harvested at 30-34 days after anthesis were dipped in ethephon at concentrations of 0, 100, 200, 400 and 800 ppm for 2 min, and stored at room temperature (27±2°C) for 8 days. Color (L*, a*, and b*), texture (compression test), total soluble solids (refractometer), respiration rate and ethylene production (gas chromatograph TCD and FID, respectively), peroxidase (POD), and phenylalanine ammonia-lyase (PAL) activities, phenolic content, and reducing sugar content (spectrophotometer) of samples were determined during storage. On day 4 and day 6 of storage, total color difference (△E) of fruit peel increased as ethylene concentration. The results showed that firmness of fruit pulp significantly decreased as the concentration of ethylene increased. Generally, respiration rate of dragon fruits increased with storage time and decreased after 6 days of storage. The results also showed that ethylene treatment had no significant effect on ethylene production of dragon fruits ($p \geq 0.05$). POD and PAL activities of dragon fruits in all treatments were significantly increased after storage. Ethylene treatment (100-800 ppm) had significant effect on color, firmness, respiration rate, and enzyme activity when compared to control.

Keywords: dragon fruit, enzyme activity, ethylene, quality, postharvest life

1. INTRODUCTION

Dragon fruit is one of the important tropical fruits in many countries such as Vietnam, Thailand, and Malaysia. The dragon fruit is the good nutritious fruit. Several studies showed that dragon fruit is a good source of minerals, glucose, fructose, dietary fiber, and vitamins (BERBEU, 1990; WU and CHEN, 1997). Ethylene has been used commercially to hasten ripening and promote a uniform appearance of fruit (REID, 1992). However, ethylene is not always beneficial for postharvest shelf life (FRESH, 2000). Exposure to ethylene could shorten shelf life of produce due to hasten senescence, loss of chlorophyll and increased susceptibility of product to microbial (FRESH, 2000). For dragon fruits, the main postharvest problem is senescence and yellowing in bract. Therefore, the objective of this research was to study the effect of ethylene concentrations on the quality and enzyme activity of dragon fruit during postharvest period.

2. MATERIALS AND METHODS

2.1. Raw material

Dragon fruits were harvested at 30-34 days after anthesis from the grower in Patumthani, Thailand. The fruits were selected for uniformity of size and no mechanical damage condition. The prepared fruits were separated into 5 treatments as follows: 1) Distilled water (control), 2) 100 ppm of ethephon solution, 3) 200 ppm of ethephon, 4) 400 ppm of ethephon, and 5) 800 ppm of ethephon. 45 dragon fruits per each treatment were put into ethephon solution in 2 minutes, stored at room temperature (27±2°C), and observed at 0, 2, 4, 6 and 8 days after storage.

2.2. Quality assessment

Changes in the color of peel were determined using an Ultra Scan Pro (Hunter Lab, Reston, VA, USA) by the method of GUI *et al.*, 2006. The pulp firmness was evaluated with a Texture Analyzer (TA.XT. Plus, Stable Micro System, UK) (ZHOU *et al.*, 2007). The total phenolic content in dragon fruit was determined by the method of JOUBERT *et al.*, 2008. Total soluble solids (TSS) were measured using a hand refractometer (Model PR-1, Japan). Reducing sugar content was assayed as described by SOMOGYI (1952). Respiration rate and ethylene production were measured with a gas chromatograph (GC, Model 6890 N, Agilent, USA with TCD, and FID, respectively). Peroxidase (POD) and phenylalanine ammonia-lyase (PAL) activities were detected as described by LIU *et al.* (2007), and the method of ZUCKER (1965), respectively.

2.3. Statistical analysis

The experiment was designed using a Completely Randomized Design (CRD). Each treatment was comprised of two replicates. Statistical comparisons were made by analysis of variance (ANOVA) followed by a Duncan multiple range test using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL). For all statistics, $p \leq 0.05$ was considered to be the statistically significant difference.

3. RESULTS AND DISCUSSION

On day 4 and day 6 of storage, total color difference (△E) of fruit peel increased as ethylene concentration (Fig. 1a). Similar results were shown in yellow pitahaya fruits by DEAQUIZ *et al.* (2014). Treatment with ethylene accelerated chlorophyll degradation and increased an appearance of yellow or orange color of carotenoid pigments (SALTVEIT, 1999). The firmness of fruit pulp significantly decreased as the concentration of ethylene increased (Fig. 1b). The firmness of the control fruit slightly declined, whereas the firmness of ethylene treated fruits had greater decreased from the initial date to day 6 of storage. Loss of firmness may be related to the action of hydrolase enzymes (polygalacturonase, pectin methyl esterase, pectate lyase) (GIOVANNONI, 2004; GOULAO *et al.*, 2007). Those enzymes have weakened the cell walls, reduced the tensile force, and accelerated the softening of the dragon fruit (DEAQUIZ *et al.*, 2014).

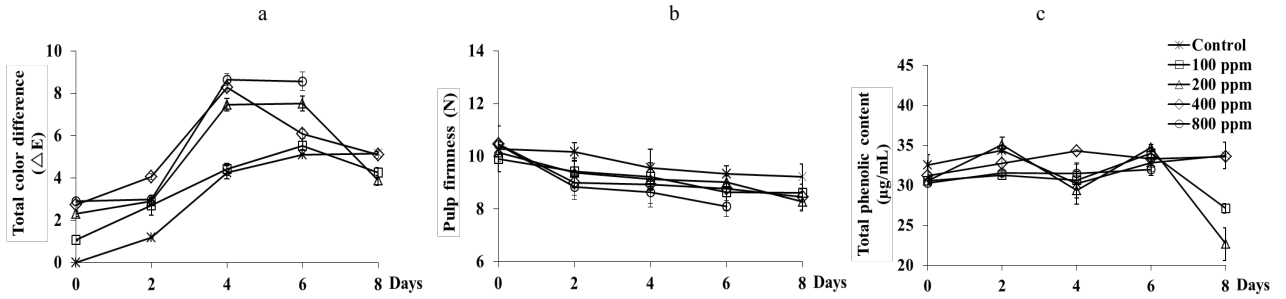

Figure 1. Total color difference (△E) of fruit peel (a), pulp texture (b) and total phenolic content (c) changes of dragon fruit stored at room temperature for 8 days.

Ethylene treatment had minimal changes total phenolic content, total soluble solids, and reducing sugar content of dragon fruits (Fig. 1c, 2a, and 2b, respectively).

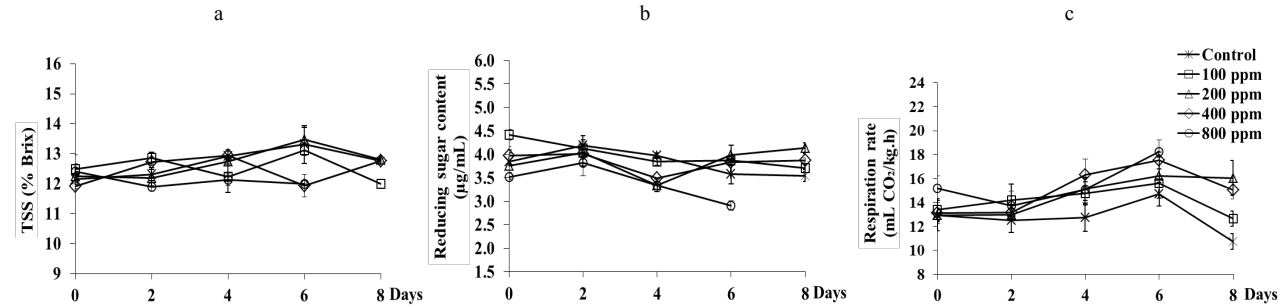

Figure 2. Total soluble solids (a), reducing sugar content (b) and respiration rate (c) changes of dragon fruit stored at room temperature for 8 days.

The respiration rates of dragon fruits tended to increase with storage time and then decreased after 6 days of storage at both treated and non-treated fruits (Fig. 2c). Respiration rate was higher in ethylene-treated samples when compared to control. In contrast, the ethylene production was not significantly affected by ethylene treatments (Fig. 3a). POD and PAL activities of dragon fruits in all treatments were significantly increased after storage (Fig. 3b, and 3c, respectively). However, fruits dipped in ethephon

(100- 800 ppm) had no significant differences in POD activity when compared to control. PAL activity of fruits dipped in ethephon (100- 800 ppm) was significantly higher than those of untreated samples ($p \geq 0.05$). The results were in agreement with those reported for Citrus fruit (LAFUENTE *et al.*, 2004), in which ethylene increased PAL activity.

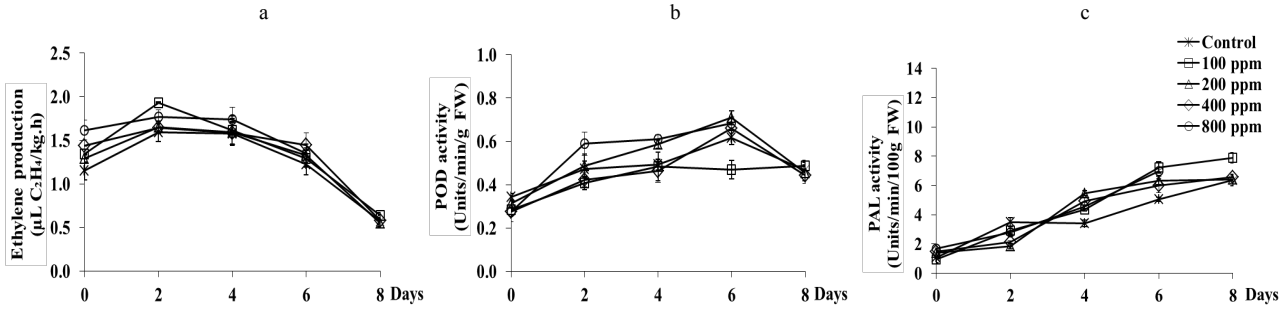

Figure 3. Ethylene production (a), peroxidase (b) and phenylalanine ammonia-lyase (c) activities changes of dragon fruit stored at room temperature for 8 days.

4. CONCLUSIONS

Ethylene treatment by dipping in ethephon (100-800 ppm) for 2 min had significant effects on color, firmness, respiration rate, and enzyme activity when compared to control. Minimal changes of total phenolic content, total soluble solids, and reducing sugar content were observed in ethylene-treated dragon fruits. Based on the results, dragon fruit should not be exposed to ethylene at a concentration of 100 ppm or more in order to maintain the quality and extend the shelf life of dragon fruit after harvesting.

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REFERENCES

Berbeu G. 1990. The strawberry pear, a new tropical fruit. Fruits 45:141-147.

Deaquiz Y.A, Alvarez-Herera J. and Fischer G. 2014. Ethylene and 1-MCP affect the postharvest behaviour of yellow pitahaya fruits (*Selenicereus megalanthus* Haw.). Posthar. Biol. and Technol., Agron. Colomb. 32:44-51.

Fresh O. 2000. The Fruit, Vegetable and Fresh Produce Storage Expert System. CSIRO Publishing, Collingwood Vic. Australia.Giovannoni J.J. 2004. Genetic regulation of fruit development and ripening. Plant Cell 16:170-180.

Goulao L.F., Santos J., Sousa L.D. and Oliveira C.M. 2007. Patterns of enzymatic activity of cell wall-modifying enzymes during growth and ripening of apples. Posthar. Biol. and Technol. 43:307-318.

Gui F., Wu J., Chen F., Liao X., Hu X., Zhang Z. and Wang Z. 2006. Change of polyphenol oxidase activity, color, and browning degree during storage of cloudy apple juice treated by supercritical carbon dioxide. Eur. Food Res. Technol. 223:427-432.

Joubert E., Richards E.S., Van der Merwe J.D., De Beer D., Manley M. and Gelderblom W.C.A. 2008. Effect of species variation and processing on phenolic composition and in vitro antioxidant activity of aqueous extracts of *Cyclopia spp. (honeybush tea*). J. Agric. Food Chem. 56:954-963.

Lafuente M.T., Sala J.M. and Zacarias L. 2004. Active oxygen detoxifying enzymes and phenylalanine ammonia-lyase in the ethylene-induced chilling tolerance in Citrus fruit. J. Agric. Food Chem. 52:3606-3611.

Liu J., Tian S., Meng X. and Xu Y. 2007. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. Posthar. Biol. Technol. 44:300-306.

Reid M. 1992. Ethylene in Postharvest Technology, pp. 97-108. In "Postharvest Technology of Horticultural Crops". University of California.

Saltveit M.E. 1999. Effect of ethylene on quality of fresh fruits and vegetables. Posthar. Biol. Technol. 15: 279-292.

Somogyi M. 1952. Notes on sugar determination. J. Biol. Chem. 195:19-23.

Wu M.C. and Chen S.C. 1997. Variation of sugar contents in various part of pitaya fruit. Proc. FI. State Hortic. Soc. 110:225-227.

Zhou H., Forest M.G. and Wang Q. 2007. Anchoring induced texture and shear banding of nematic polymers in shear cells. Discrete Cont. Dyn.- B 8:707-733.

Zucker M. 1965. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiol. 40:779-784.

EFFECT OF CHITOSAN ON PHYSICAL PROPERTIES, TEXTURE AND SHELF LIFE OF SUSHI RICE

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ABSTRACT

Sushi is a popular Japanese traditional food, which is composed of vinegar-flavored cold cooked rice and garnished with raw fish, vegetables, egg or other foods. However, the foodborne outbreaks from sushi have occurred frequently from inappropriate preparation and abuse temperature during storage. *Bacillus cereus* and *Staphylococcus aureus* were reported as the main pathogenic microorganisms found in sushi. Chitosan is an effective natural preservative, which can inhibit the growth of board spectrum of microorganisms. This work aimed to study the effect of crab chitosan on physical properties (moisture content, pH, whiteness, and a_w), texture and shelf life of sushi rice. The sushi rice was prepared by mixing cooked rice with a dressing containing rice vinegar (53.48% w/w), sugar (33.28%), salt (11.89%), and crab chitosan (1.35%). The final concentration of chitosan in sushi rice was 0.1%w/w. Sushi rice with chitosan and control (sushi rice without chitosan) were inoculated with *B. cereus* and *S. aureus* to obtain a final concentration approximately 3 log CFU/g. The inoculated sushi rice was packed in polypropylene bag and stored at 37°C for 2 days. Chitosan shows the significant antibacterial effect against *B. cereus* and *S. aureus* from the initial day, as a result of 1-log reduction or 90% reduction of the initial loads of total plate count, *B. cereus* and *S. aureus* ($p \leq 0.05$). In addition, the population of total plate count, *B. cereus* and *S. aureus* of sushi rice with chitosan were lower than control during storage for 1 day. Chitosan has no effect on moisture content, whiteness, and a_w of sushi rice during storage ($p > 0.05$). Hardness, adhesiveness, and pH of sushi rice with chitosan were significantly higher than the control at day 0 and during storage ($p \leq 0.05$). However, sensorial result (triangle test) showed that there was no significant difference between sushi rice with chitosan and control ($p > 0.05$). Therefore, chitosan has a potential to be used as food preservative for sushi rice with no negative effects on rice quality.

Keywords: antimicrobial activity, chitosan, rice, shelf life, texture

1. INTRODUCTION

Sushi, vinegar seasoned rice garnished with a variety of toppings such as vegetables, egg or others, is a popular ready-to-eat (RTE) Japanese food due to its convenience and being a nutritious meal. However, its safety and microbiological quality should be concerned as this RTE food do not undergo any additional treatment or cooking steps to ensure safety prior to consumption. The foodborne outbreaks from sushi have occurred frequently from inappropriate preparation and abuse temperature during storage. *B. cereus* and *S. aureus* were reported as the main pathogenic bacteria found in sushi (NSW Food Authority, 2008). Chitosan, a natural antimicrobial agent, has been approved as a food additive in many countries including South Korea, Japan and USA. It can be applied in many foods to improve the safety, quality and shelf life of food (RACHTANAPUN *et al.*, 2015). This research aims to investigate the potential of using chitosan as a natural preservative in sushi rice. Effect of chitosan on the antimicrobial activity, physical and textural properties of sushi rice was determined for ensuring the safety and quality of the sushi rice.

2. MATERIALS AND METHODS

2.1. Preparation of sushi dressing

Crab chitosan was purchased from Ta Ming Enterprises Co., Ltd., Samut Sakhon, Thailand. The sushi dressing was prepared by mixing vinegar, sugar, salt and chitosan at a concentration of 53.49%, w/w, 33.27% (w/w), 11.89% (w/w) and 1.35 % (w/w), respectively. The sushi dressing was pasteurized by heating the dressing until 85 ºC, hot-filled in the pasteurized bottle and hold for 3 min and then immediately cooled.

2.2. Preparation of sushi rice

Four hundred grams of raw japonica rice (C&A Rice Mill Co., Ltd., Pathum Thani, Thailand) was rinsed and cooked in a rice cooker (SR-G10; Panasonic Home Appliances Co., Chachoengsao, Thailand) with 500 g of water. The cooked rice was mixed with sushi seasoning at ratio 1:0.08 by weight. The final concentration of chitosan in sushi rice was 0.01%, w/w, as an effective dose against foodborne pathogens associated with cooked rice (RACHTANAPUN *et al.*, 2015). Sushi rice samples with a size of 2.5x2.7x1.7cm^3 per piece (approximately 15 g) were packed in polypropylene bags, sealed and stored at 37ºC for 2 days to demonstrate temperature abuse during food handling and storage; for example, sushi outlets displayed foods at room temperature.

2.3. Inoculation of *B. cereus* and *S. aureus* into sushi rice

After mixing cooked rice with chitosan dressing as mentioned in section 2.2, 5 mL of *B. cereus* TISTR 687 suspension and 5 ml of *S. aureus* TISTR 1466 suspension were inoculated into sushi rice (500 g) and mixed manually for 5 min to get a final concentration of approximately 3 log CFU/g. The mixture of sushi rice, mix with sushi seasoning without chitosan and with a cocktail culture of *B. cereus* and *S. aureus* was used as the control. Each inoculated sushi rice sample was aseptically packed in plastic bags (PP) and stored at 37°C for 2 days prior to enumerating bacteria numbers using the spread plate technique. This experiment was repeated in triplicate.

2.4. Microbial enumeration

B. cereus, *S. aureus*, total aerobic bacteria count (TAC) and yeast and mold count (YM) were determined according to APHA (2001).

2.5. Moisture content and pH

The moisture content and pH of the sushi rice samples at each day were measured according to AOAC methods 935.29 and 943.02, respectively (AOAC, 2000).

2.6. Color measurement

The color of the sushi rice at each day was measured using a colorimeter (MiniscanXE, Hunter Associates Laboratory, Inc., Virginia, USA) in CIE L*a*b* system. The whiteness index (WI) was calculated following RACHTANAPUN *et al.* (2015).

2.7. Textural measurement

The texture of sushi rice at each storage day was measured by using a TPA test with a texture analyser (TA-XT plus, Stable Micro System, Surrey, UK). Each sushi rice piece was compressed twice at a test speed of 1.0 mm/s with a P/100 probe at 50% strain. Hardness and adhesiveness were analyzed from the measured TPA curve.

2.8. Water activity

The water activity (a_w) of sushi rice samples at each storage day were measured using a water activity meter (AQUA LAB, 4) according to the manufacturing manual.

2.9. Sensory test

The difference between sushi rice prepared with or without chitosan at day 0 was evaluated by 30 untrained panelists using a triangle test. The results were analyzed using chi-square test at 95% confidence interval level.

2.10. Statistical analysis

Means of all results were compared using analysis of variance (ANOVA) and Duncan's multiple range test was used to determine significant differences among the treatment means ($p \leq 0.05$) using the SPSS version 12.0.

3. RESULTS AND DISCUSSION

3.1. Effect of chitosan on the shelf life of sushi rice

In sushi rice without chitosan (Control sample), the number of *B. cereus*, *S. aureus* and TAC (Figs. 1a-1c) tended to decrease, while the number of yeast and mold (Fig. 1d) was constant during storage for 2 days. Adding 0.1% chitosan into sushi rice significantly reduced the initial numbers of *B. cereus* and *S. aureus* by 1 Log or 90% at day 0 ($p \leq 0.05$) (Figs. 1a and 1b). After that, numbers of *B. cereus*, *S. aureus*, TAC and yeast and mold were constant during the storage time. Regarding to the ANZFA guideline for determining the

microbiological quality of RTE food (NSW FOOD AUTHORITY, 2008), the control sample containing *B. cereus* and *S. aureus* at level 3.3 and 3.9 Log CFU/g, respectively, categorized as unsatisfactory. After 1-log reduction of these pathogens in sushi rice with chitosan, this RTE food categorized as acceptable. From the results, it can be assumed that the lower numbers of *B. cereus* and *S. aureus* in sushi rice added with chitosan, at the initial time (Day 0), are due to the antibacterial effect of chitosan. Furthermore, the chitosan retarded increases of microbial counts in sushi rice stored at 37°C for 1 day. It is consistent with the result of RACHTANAPUN *et al.* (2015) that rice cooked with chitosan inhibited increases in the TAC and *B. cereus* during the initial 24 h of storage at 37°C.

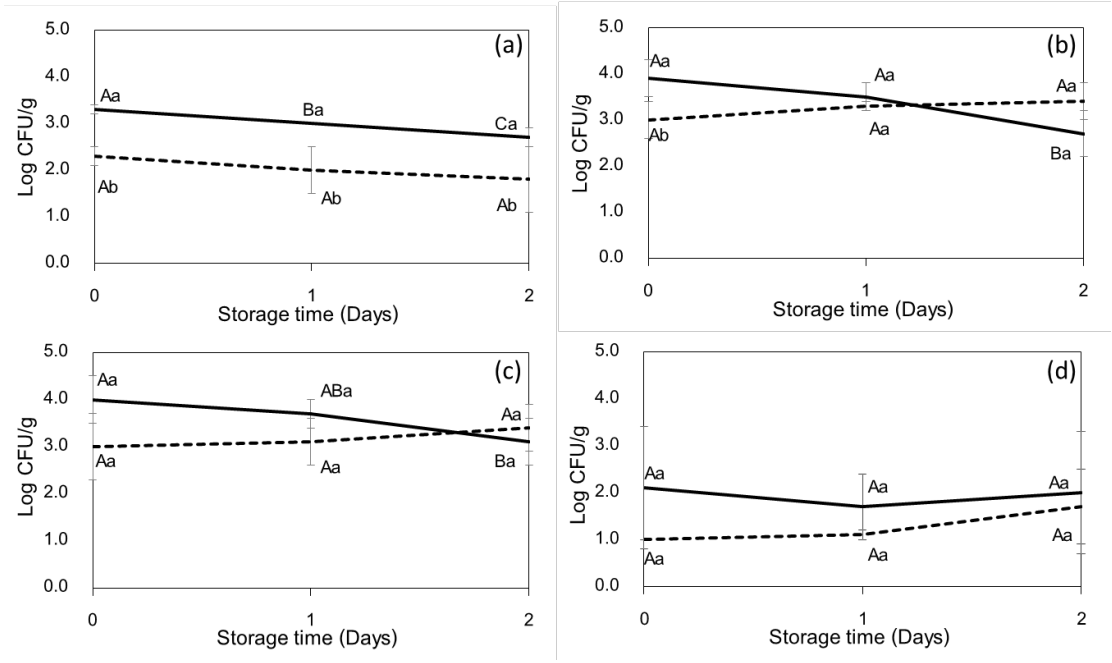

Figure 1. Antimicrobial effect of chitosan on *Bacillus cereus* (a), *Staphylococcus aureus* (b), total aerobic bacteria count (c), and yeast and mold (d) on sushi rice without (….) and with 0.1%w/w chitosan (——) during storage at 37°C for 2 days.

Different lowercase letters [a, b] for the same day are significantly different at $p \leq 0.05$.

Different capital letters [A, B] for the same treatment are significantly different at $p \leq 0.05$.

3.2. Effect of chitosan on physical and textural properties of sushi rice during storage

The moisture content, water activity and whiteness of samples with chitosan were not significantly different from that of the control samples ($p>0.05$) for day 0 and during storage. This is similar to the work of RACHTANAPUN *et al.* (2015), which reported that chitosan did not affect the whiteness of the cooked rice during storage. The pH of sample with chitosan was slightly higher than the control sample ($p \leq 0.05$) for day 0. The addition of chitosan increased the pH of sushi rice due to alkali residue from the chitosan preparation process. The alkali, mostly sodium hydroxide, was used in the deproteinization and deacetylation of chitin to obtain the chitosan (ABDOU *et al.*, 2008). However, the pH of both samples was constant during storage. The hardness and adhesiveness of the sample containing chitosan was significantly higher than the control sample ($p \leq 0.05$) at day 0. This might be attributed to the formation of chitosan film on the rice surface.

Table 1. Moisture content, pH, Whiteness, water activities (a_w) and textural properties of sushi rice with and without chitosan during storage at 37°C.

Parameter	Days	Treatment	
		Control	0.1 %chitosan
Moisture content (% wet basis)	0	59.96 ± 0.50^{Aa}	60.52 ± 0.66^{Aa}
	1	59.85 ± 0.89^{Aa}	59.79 ± 0.28^{Aa}
	2	60.40 ± 0.86^{Aa}	60.65 ± 0.63^{Aa}
pH	0	5.69 ± 0.11^{Aa}	6.08 ± 0.18^{Ab}
	1	5.68 ± 0.12^{Aa}	6.02 ± 0.05^{Ab}
	2	5.52 ± 0.02^{Aa}	5.97 ± 0.06^{Ab}
Whiteness	0	76.23 ± 0.23^{Aa}	76.43 ± 0.37^{Aa}
	1	76.62 ± 0.38^{Aa}	76.15 ± 0.44^{Aa}
	2	75.64 ± 0.88^{Aa}	75.45 ± 0.16^{Aa}
a_w	0	0.9660 ± 0.0035^{Aa}	0.9669 ± 0.0021^{Aa}
	1	0.9704 ± 0.0008^{Aa}	0.9708 ± 0.0019^{Aa}
	2	0.9658 ± 0.0058^{Aa}	0.9678 ± 0.0023^{Aa}
Hardness (g)	0	908.47 ± 45.83^{Aa}	1112.39 ± 52.66^{Ab}
	1	879.29 ± 21.10^{Aa}	1028.44 ± 37.67^{Ab}
	2	876.15 ± 26.45^{Aa}	1077.35 ± 31.05^{Ab}
Adhesiveness (g.sec)	0	-91.60 ± 48.95^{Aa}	-422.65 ± 15.82^{Ab}
	1	-67.99 ± 34.54^{Aa}	-137.38 ± 6.62^{Bb}
	2	-126.45 ± 6.75^{Aa}	-151.24 ± 9.88^{Bb}

Values expressed are mean ± standard deviation. Different lowercase letters ($^{a, b}$) in the same row are significantly different at $p \leq 0.05$. Different capital letters ($^{A, B}$) in the same column are significantly different at $p \leq 0.05$.

However, the triangle test results shown that 13 panelists were aware of the difference between sushi rice with and without chitosan and 17 panelists failed to recognize the difference. Statistical analysis by Chi-square calculation revealed that the addition of chitosan in sushi rice did not affect on the sensory characteristic ($p>0.05$). The hardness of sushi rice with and without chitosan was constant during storage. This may be due to the presence of acetic acid, which can retard the starch retrogradation. However, the adhesiveness of sushi rice with chitosan decreased after day 0. This indicates that the rice became less sticky.

4. CONCLUSIONS

Chitosan effectively reduced the initial load of *B. cereus* and *S. aureus* contaminated in sushi rice and retarded the growth of microorganisms for 2 days. Moreover, it had no effect on moisture content, a_w, and whiteness of sushi rice. Even though, it increased pH, hardness, and adhesiveness of sushi rice, there was no effect on sensory evaluation. Thus, chitosan has potential to be a food preservative for sushi rice with no negative effects on rice quality.

REFERENCES

Abdou E.S., Nagy K.S. and Elsabee M.Z. 2008. Extraction and characterization of chitin and chitosan from local sources. Bioresour. Technol. 99:1359-1367.

AOAC. 2000. Official Methods of Analysis of the Association of Official Analytical Chemists, 17th ed. Washington, DC: Association of Official Analytical Chemists.

APHA. 2001. Compendium of methods for the microbiological examination of foods, 4th ed. Washington, DC: American Public Health Association.

NSW Food Authority. 2008. Report on food handling practices and microbiological quality of sushi in Australia. Available Source: www.foodstandards.gov.au, 1 May 2017.

Rachtanapun C., Tantala J., Klinmalai P. and Ratanasumawong S. 2015. Effect of chitosan on *Bacillus cereus* inhibition and quality of cooked rice during storage. Int. J. Food Sci. Technol. 50:2419-2426.

EFFECT OF PACKAGES AND STORAGE TIME ON QUALITY OF JACKFRUIT SEED CRACKER

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ABSTRACT

This research tries to produce Jackfruit seed cracker in order to utilize byproduct and study effect of shelf life on product's qualities. The formula was contained pregelatinized Jackfruit seed, which prepared by steamed and blended. Jackfruit seed crackers were formulated by mixing, kneading, 45 min for steaming, 12 h for refrigeration, sliced into thin pieces and then dried in tray dry for 5 h and 5 min deep frying at 190°C. Qualities and shelf life of Jackfruit seed crackers kept in laminated aluminum foil bag (LAF) were statistically compared to those kept in the polypropylene bag (PP) at ambient condition (~30°C) for 13 weeks. Texture properties, color, water activity, moisture content, peroxide value (PV) and microbiological quality were studied. Sensory quality was monitored by trained panel with the quality descriptive analysis (QDA) method. Fresh fried Jackfruit seed cracker was used as control sample. Jackfruit seed crackers kept in the LAF appeared higher quality than those kept in PP. During storage time, moisture content, water activity, PV and some texture properties such as toughness and hardness increased due to permeation and accumulation of oxygen and water vapor. At 13 weeks storage, moisture content increased from 3.29% to 9.92% (PP) and 5.57% (LAF), water activity increased from 0.31 to 0.54 (PP) and 0.40 (LAF), PV increased from 3.78 meq/kg to 22.02 meq/kg (PP) and 17.74 meq/kg (LAF). On the other hand, crispness decreased at 13 weeks storage from 89.67 N to 32.33 N and 78.73 N for products kept in PP and LAF, respectively. The storage time slightly affected on color properties including lightness (L^*), redness (a^*) and yellowness (b^*) of products kept in PP and LAF which could be due to the oxidation accelerated by light. Sensory evaluation found that product kept in LAF for 5 weeks has higher rancid odor when comparing with control sample leading to the shelf life of 4 weeks for product kept in LAF. While, product kept in PP for 3 weeks has higher rancid odor and lower crispness when comparing with control sample leading to the shelf life of 2 weeks for product kept in PP.

Keywords: cracker, jack fruit seed, shelf-life

1. INTRODUCTION

Jackfruit is an exotic fruit grown in tropical climates including the Indian subcontinent, Southern china, South-eastern Asia, middle Africa and Latin American countries. Consumer like jackfruit for its sweet, freshy, fibrous, delicious and attractive golden yellow colored ripe bulbs which is the perianth portion of the fruit. It is rich source of carbohydrates, minerals, dietary fibers and vitamins such as ascorbic acid and thiamine (MAITY *et al.*, 2014). However, jackfruit seed is byproduct, which make up around 10 to 15% of the total fruit weight and have high carbohydrate, protein contents, dietary fiber, vitamins, minerals and phytonutrients. They are light brown in color, oval, or oblong ellipsoid or round shape, 2-3 cm (0.8-1.2 inch) in length and 1-1.5 cm (0.4-0.6 inch) in diameter (ABRAHAM and JAYAMUTHUNAGAI, 2014).
Storage stability of an oxygen sensitive product inside a package depends on package oxygen permeability, amount of oxygen consumed by the product, oxygen concentration in package headspace temperature and relative humidity. These environmental, product and package factor can be correlated to simulate the oxidative deterioration of the packaged product during storage (JENA and DAS, 2012). Commercially available polymer based food packaging material exhibit both oxygen and water vapor permeability and food processing industry demands a packaging material with less permeability and more bearing strength. Polypropylene (PP) has the lowest density and highest melting point of all the high volume usage thermoplastics and has a relatively low cost. This versatile plastic can be processed in many ways and has many food packaging applications in both flexible film and rigid form. It is a barrier to water vapor and has oil and fat resistance (MANIKANTAN *et al.*, 2012). Aluminium laminate protect product against light, as light acts as catalyst for oxidation (NAGI *et al.*, 2012). Laminated aluminum foil (LAF) could prevent the permeations of oxygen and water vapor from surrounding which lead to oxidative rancidity through lipid oxidation and soften textures through water vapor absorption, respectively
Generally, the main ingredient of Thai snacks is flour, such as white rice flour, white glutinous rice flour and black glutinous rice flour (CHITTAPALO and SONGSANANDE, 2014). Due to the large consumption of snacks in Thailand, this research was developed jackfruit seed cracker and the effect of package and storage time on the shelf life of jackfruit seed crackers was studied.

2. MATERIALS AND METHODS

2.1. Product preparation

The formula contained pregelatinized jackfruit seed which prepared by steaming and blending. Jackfruit seed crackers were formulated by mixing, kneading, 45 min for steaming, 12 h for refrigeration, sliced into thin pieces and then dried in tray dry for 5 h and 5 min deep frying at 190°C.

2.2. Storage

Prepared jackfruit seed crackers were packed in laminated aluminum foil bag (LAF) and polypropylene bag (PP) at ambient atmospheric condition (~30°C). Evaluated for shelf life by estimating water activity, moisture content, texture properties, color, peroxide value (PV), microbiological quality and sensory quality at the regular intervals of one week over the period of 13 weeks or until the sample degradation.

2.3. Moisture content analysis

Jackfruit seed crackers were ground with the mortar and pestle. Weighted samples (2 g) were dried in a hot air oven at 105°C and moisture content in percent was calculated from loss of weight (AOAC, 2000).

2.4. Water activity (a_w) measurement

The a_w of jackfruit seed crackers were determined using a Series3 TE, AQUA (USA). The samples were ground with the mortar and pestle and added to 50% of the cuvette volume. Precondition of the equipment was done before starting analysis as well as for sample container in cool temperature (25°C), for approximately 30 min until stable. The measurement was carried out ~30 min before the results could be displayed.

2.5. Texture measurement

The texture analysis was performed with a Stable Micro Systems model TA.TX *Plus* Texture Analyzer (USA) using A/OTC probe for crispness of jackfruit seed crackers.

2.6. Color measurement

The color of jackfruit seed crackers was determined using a Colorflex model 4510, Hunter Lab colorimeter (USA). The samples were ground with the mortar and pestle and added to 50% of the cuvette volume. The instrument was calibrated using the black and white standard tiles the came with the instrument. The operating conditions were 10° observer, D_{65} illuminant. L^*, a^* and b^* values were recorded.

2.7. Peroxide value (PV) analysis

Peroxide value was determined by extracting 4 g oil from jackfruit seed crackers using a soxtec™ 2045, Foss (Sweden). Peroxide value was quantified using a standard method (AOAC, 2000).

2.8. Sensory evaluation

The samples were analyzed by the Quantitative Descriptive Analysis (QDA) method of intensity scaling using 10 trained panelists with fresh fried jackfruit seed cracker was used as control sample. The measured qualities included the yellowness, rancid odor, salty, spicy, pepper flavor and crispness. The scorecard consisted of 15 cm scale at 1.25 cm was anchored at either ends as low and high, representing recognition threshold and saturation threshold.

3. RESULTS AND DISCUSSION

3.1. Effect of package and storage time on physical properties of Jackfruit seed cracker

Table 1 showed a_w of jackfruit seed crackers in range of 0.31 to 0.54. Increasing of a_w with storage time could be due to water vapor absorption. Jackfruit seed crackers kept in LAF appeared lower a_w than those kept in PP because LAF could prevent the permeations of oxygen and water vapor from surrounding which lead to oxidative rancidity through lipid

oxidation and soften textures through water vapor absorption, respectively. As the texture properties found that crispness decreased as storage time progress.

Table 1. Effect of package and storage time on physical properties and chemical qualities of Jackfruit seed cracker.

		Storage time (Weeks)						
		0	**1**	**2**	**3**	**4**	**5**	**6**
Crispness	PP	89.67 ± 3.05^{aA}	59.00 ± 2.00^{bA}	58.00 ± 2.00^{bA}	53.67 ± 2.63^{cdA}	55.33 ± 3.05^{bcA}	53.67 ± 4.16^{cdA}	49.67 ± 0.58^{deA}
	LAF	89.67 ± 3.05^{deA}	$100.33\pm4.73^{a}_{B}$	99.00 ± 4.73^{abB}	$96.67\pm2.52^{abc}_{B}$	$93.00\pm3.60^{bcd}_{B}$	92.00 ± 2.65^{cdB}	90.33 ± 3.51^{dB}
a_w	PP	0.31 ± 0.00^{aA}	0.38 ± 0.01^{bA}	0.38 ± 0.025^{bA}	0.42 ± 0.01^{cA}	0.44 ± 0.00^{dA}	0.46 ± 0.03^{eA}	0.48 ± 0.01^{efA}
	LAF	0.31 ± 0.00^{aA}	0.31 ± 0.01^{bB}	0.32 ± 0.01^{abB}	0.35 ± 0.01^{cB}	0.36 ± 0.00^{cdB}	0.38 ± 0.00^{deB}	0.38 ± 0.01^{efB}
*L**	PP	72.81 ± 0.30^{aA}	70.80 ± 0.51^{deA}	70.48 ± 0.03^{eA}	72.46 ± 0.36^{abA}	71.36 ± 0.37^{cdA}	$71.25\pm0.31^{cde}_{A}$	69.66 ± 0.17^{aA}
	LAF	72.81 ± 0.30^{aA}	69.62 ± 0.33^{fA}	70.25 ± 0.27^{deA}	70.73 ± 0.05^{cB}	70.12 ± 0.11^{deB}	70.39 ± 0.34^{cdB}	68.70 ± 0.19^{gB}
*a**	PP	3.87 ± 0.08^{aA}	3.81 ± 0.04^{aA}	3.81 ± 0.08^{aA}	3.77 ± 0.09^{abA}	3.64 ± 0.02^{bA}	3.43 ± 0.013^{cA}	3.40 ± 0.02^{cA}
	LAF	3.87 ± 0.08^{aA}	3.77 ± 0.08^{abA}	3.74 ± 0.07^{abA}	3.73 ± 0.02^{abA}	3.77 ± 0.04^{abA}	3.68 ± 0.02^{bcdB}	$3.63\pm0.03^{bcde}_{B}$
*b**	PP	24.37 ± 0.05^{gA}	26.65 ± 0.69^{aA}	26.77 ± 0.38^{aA}	26.47 ± 0.28^{aA}	25.79 ± 0.04^{bA}	25.39 ± 0.05^{cA}	25.59 ± 0.07^{bcA}
	LAF	24.37 ± 0.05^{eA}	26.33 ± 0.14^{bA}	26.34 ± 0.07^{bA}	26.95 ± 0.36^{aA}	26.28 ± 0.42^{bA}	26.14 ± 0.29^{bA}	26.51 ± 0.04^{bB}
Moisture content (%)	PP	3.29 ± 0.08^{aA}	4.36 ± 0.22^{bA}	4.82 ± 0.12^{cA}	4.894 ± 0.09^{cA}	5.16 ± 0.12^{cdA}	5.45 ± 0.45^{dA}	5.92 ± 0.68^{eA}
	LAF	3.29 ± 0.08^{aA}	3.57 ± 0.08^{bB}	3.79 ± 0.07^{cB}	3.86 ± 0.13^{cA}	3.97 ± 0.11^{cdB}	4.09 ± 0.01^{dB}	4.49 ± 0.03^{eB}
PV (meq/kg)	PP	3.78 ± 0.02^{aA}	6.16 ± 0.04^{bA}	7.17 ± 0.03^{cA}	7.74 ± 0.02^{dA}	7.99 ± 0.01^{eA}	9.23 ± 0.02^{fA}	11.74 ± 0.09^{gA}
	LAF	3.78 ± 0.02^{aA}	4.40 ± 0.00^{bB}	5.01 ± 0.01^{cB}	6.16 ± 0.01^{dB}	6.79 ± 0.04^{eB}	7.53 ± 0.02^{fB}	9.50 ± 0.01^{gB}
		Storage time (Weeks)						
		7	**8**	**9**	**10**	**11**	**12**	**13**
Crispness	PP	47.67 ± 2.08^{efA}	48.68 ± 1.53^{eA}	44.33 ± 2.52^{fgA}	42.67 ± 2.08^{ghA}	39.33 ± 0.58^{hiA}	36.67 ± 1.53^{iA}	32.33 ± 2.52^{jA}
	LAF	$88.00\pm3.65^{de}_{B}$	89.00 ± 2.00^{deB}	87.00 ± 2.00^{deB}	87.33 ± 4.73^{deB}	83.67 ± 2.08^{efB}	80.00 ± 5.57^{fB}	78.33 ± 4.04^{fB}
a_w	PP	0.48 ± 0.01^{fA}	0.49 ± 0.00^{fgA}	0.51 ± 0.01^{ghA}	0.52 ± 0.02^{hiA}	0.53 ± 0.01^{iA}	0.54 ± 0.00^{iA}	0.54 ± 0.00^{iA}
	LAF	0.39 ± 0.02^{fgB}	0.39 ± 0.01^{fgB}	0.39 ± 0.01^{fgB}	0.39 ± 0.01^{fgB}	0.39 ± 0.01^{fgB}	0.39 ± 0.00^{fgB}	0.40 ± 0.01^{gB}
*L**	PP	72.83 ± 0.33^{aA}	70.68 ± 0.32^{deA}	70.78 ± 0.37^{deA}	71.40 ± 0.33^{cdA}	69.04 ± 0.54^{aA}	71.73 ± 0.90^{bcA}	71.99 ± 0.79^{bcA}
	LAF	69.89 ± 0.22^{efB}	70.11 ± 0.11^{deB}	68.97 ± 0.08^{gB}	$70.02\pm0.16^{def}_{B}$	71.33 ± 0.14^{bB}	69.87 ± 0.46^{efB}	$70.01\pm0.77^{def}_{B}$
*a**	PP	3.36 ± 0.02^{bcA}	3.22 ± 0.02^{dA}	3.15 ± 0.06^{deA}	3.10 ± 0.04^{deA}	3.06 ± 0.03^{eA}	3.02 ± 0.01^{eA}	3.02 ± 0.01^{eA}
	LAF	3.71 ± 0.06^{cdB}	$3.62\pm0.04^{bcde}_{B}$	3.56 ± 0.03^{cdeB}	3.54 ± 0.36^{deB}	3.49 ± 0.03^{efB}	3.49 ± 0.03^{efB}	3.37 ± 0.05^{fB}
*b**	PP	25.22 ± 0.04^{bA}	24.99 ± 0.13^{deA}	$24.91\pm0.04^{def}_{A}$	$24.92\pm0.07^{def}_{A}$	24.77 ± 0.29^{efA}	$24.59\pm0.08^{efg}_{A}$	24.55 ± 0.32^{gfA}
	LAF	26.23 ± 0.22^{eB}	26.13 ± 0.23^{bB}	26.59 ± 0.25^{abB}	26.28 ± 0.22^{bB}	26.48 ± 0.29^{bB}	25.20 ± 0.05^{cB}	25.27 ± 0.13^{dB}
Moisture content (%)	PP	6.19 ± 0.61^{efA}	6.47 ± 0.08^{fA}	6.93 ± 0.12^{gA}	7.96 ± 0.23^{hA}	8.63 ± 0.35^{iA}	8.69 ± 0.40^{iA}	9.92 ± 0.34^{jA}
	LAF	4.74 ± 0.10^{gB}	4.73 ± 0.10^{gB}	4.89 ± 0.22^{fgB}	5.60 ± 0.05^{iB}	4.97 ± 0.09^{gB}	5.32 ± 0.17^{hB}	5.57 ± 0.76^{jB}
PV (meq/kg)	PP	13.17 ± 0.10^{hA}	14.95 ± 0.03^{iA}	16.18 ± 0.07^{jA}	18.80 ± 0.08^{kA}	19.00 ± 0.02^{lA}	19.93 ± 0.10^{mA}	22.02 ± 0.14^{nA}
	LAF	10.63 ± 0.04^{hB}	12.40 ± 0.01^{iB}	12.62 ± 0.12^{jB}	13.59 ± 0.10^{kB}	14.63 ± 0.06^{lB}	14.57 ± 0.01^{lB}	17.74 ± 0.03^{mB}

A, B, C showed the significant difference in column ($P<0.05$).
a-m showed the significant difference in row ($P<0.05$).

There were only slight changes in products kept in LAF. Baked crackers and fried snack lost crispness when their a_w exceeded 0.35 to 0.50 depending on the product. Physical structure is often altered by changes in a_w due to moisture gain resulting in a transition from glassy to the rubber state. The Lightness (L^*) redness (a^*) and yellowness (b^*) were slightly different during the storage time (Table 1) due to increasing a_w and headspace oxygen. Moreover, the discoloration might be due to the difference in transmittance of light of packages. PP are clear and more transparent than LAF, light is able to enter into the package and influence the color of product (MANIKANTAN *et al.*, 2012).

3.2. Effect of package and storage time on chemical quality of Jackfruit seed cracker

Moisture content of food product determines acceptability of the product by consumers, as well as determines shelf life of the product. Most reactions taken place in food materials is derived from moisture content whether it is coming from outside or from the food itself and will affect on quality changes of product. Free water plays an important role in deterioration process of food but water in other different form (bound water, capillary form etc.) does not (AGUSTINI *et al.*, 2009). Base on the experiment the moisture content of jackfruit seed crackers ranged between 3.29 to 9.92% (Table 1). Increasing of moisture content was proportional to storage time. The products kept in PP had the higher moisture content than the products kept in LAF due to the permeations of water vapor from atmosphere. The increasing of a_w influences chemical reactivity. Peroxide value (PV) of jackfruit seed crackers found to continuously increase throughout the storage period (Table 1). At 13 weeks storage, PV were increased from 3.78 meq/kg to 22.02 meq/kg (PP) and 17.74 meq/kg (LAF), which higher than the threshold limit for oxidative rancidity and standards for product cracker (>10 meq/kg) (MAZAHREH, 2011; JENA and DAS, 2012). The highest values at individual experimental day were found among the products kept in the clear PP bags. The LAF bags reduce accumulation of oxygen in the package headspace and protect products against light, which acts as a catalyst for oxidation. The PV is an indicator of oxidative rancidity, which creates an off-smell in the product (SIRIWONG *et al.*, 2012).

3.3. Effect of package and storage time on sensory quality of Jackfruit seed cracker

Sensory quality was monitored by trained panel with the quality descriptive analysis (QDA) method. Fresh fried Jackfruit seed cracker was used as control sample. Deterioration in organoleptic qualities was mainly due to the softening of product, loss of crispness, color change, tastes change, off flavor and development of rancidity. So, the testing attributes included the yellowness, rancid odor, salty, spicy, pepper flavor and crispness.

The results from Table 2 showed that there was no significant difference between the products stored in the LAF and the control sample ($P \geq 0.05$) until 5 weeks of storage that the product kept in the LAF had higher rancid odor when comparing with the control sample leading to the shelf life of 4 weeks for the products kept in the LAF. While, the product kept in the PP for 3 weeks had higher rancid odor and lower crispness when comparing with the control sample leading to the shelf life of 2 weeks for the products kept in the PP. The loss of quality during storage of jackfruit seed crackers are mainly due to lipid oxidation which can transform food flavor and change the nutritional value of food. The use of palm oil might contribute to lipid oxidation. And crispness loss caused by increased moistness of material (JAKUBCZYK *et al.*, 2008).

Table 2. Effect of package and storage time on sensory quality of Jackfruit seed cracker.

		Storage time (weeks)					
		1	2	3	4	5	6
	control	3.29±0.53^{A}	4.39±0.52^{A}	3.12±0.35^{A}	3.46±0.64^{A}	3.18±0.35^{A}	3.51±0.34^{A}
Yellowness	PP	3.30±0.52^{A}	4.42±0.61^{A}	3.62±0.22^{A}	3.59±0.78^{A}	3.06±0.42^{A}	2.97±0.51^{A}
	LAF	3.23±0.56^{A}	4.38±0.44^{A}	2.97±0.75^{A}	3.53±0.52^{A}	3.27±1.05^{A}	3.51±0.95^{A}
	control	4.36±0.54^{A}	2.95±0.36^{A}	4.18±0.70^{A}	4.08±0.59^{A}	4.61±0.46^{A}	4.35±0.47^{A}
Pepper flavor	PP	4.50±0.97^{A}	4.18±1.20^{A}	4.54±0.80^{A}	4.65±0.67^{A}	4.15±1.39^{A}	4.15±1.39^{A}
	LAF	4.22±0.39^{A}	2.72±0.30^{A}	4.46±0.55^{A}	4.51±0.62^{A}	4.89±0.90^{A}	4.74±1.45^{A}
	control	2.87±0.74^{A}	2.86±0.43^{A}	2.78±0.43^{A}	2.83±0.37^{A}	2.84±0.47^{A}	2.60±0.31^{A}
Rancid odor	PP	3.15±0.36^{A}	3.20±0.89^{A}	4.27±0.51^{B}	4.73±0.49^{B}	5.41±0.49^{C}	6.22±0.69^{C}
	LAF	2.84±0.27^{A}	2.87±0.52^{A}	2.98±0.35^{A}	3.14±0.45^{A}	3.23±0.34^{B}	3.36±0.54^{B}
	control	5.38±0.69^{A}	6.07±0.33^{A}	5.45±0.48^{A}	5.57±0.38^{A}	5.34±0.65^{A}	5.25±0.58^{A}
Salty	PP	5.48±0.57^{A}	6.78±1.02^{A}	5.43±1.18^{A}	5.17±0.63^{A}	4.87±2.29^{A}	4.36±2.29^{A}
	LAF	5.43±0.84^{A}	6.55±1.55^{A}	6.11±0.97^{A}	5.14±0.55^{A}	5.43±2.19^{A}	5.12±0.72^{A}
	control	6.39±0.84^{A}	6.95±0.58^{A}	5.88±0.37^{A}	5.94±0.52^{A}	6.31±0.68^{A}	6.23±0.67^{A}
Spicy	PP	6.09±0.59^{A}	6.31±0.76^{A}	6.20±0.54^{A}	6.07±0.31^{A}	6.36±2.29^{A}	6.76±0.45^{A}
	LAF	6.39±0.49^{A}	6.98±0.80^{A}	6.40±0.96^{A}	6.10±0.53^{A}	6.45±0.82^{A}	6.41±0.45^{A}
	control	9.97±0.65^{A}	9.39±0.52^{A}	10.11±0.78^{A}	9.95±0.54^{A}	9.96±0.51^{A}	9.93±0.67^{A}
Crispness	PP	9.53±0.76^{B}	9.42±0.61^{A}	8.18±0.54^{B}	7.60±0.51^{B}	7.19±0.44^{B}	6.96±0.45^{B}
	LAF	10.16±0.72^{A}	9.38±0.44^{A}	9.83±0.77^{A}	9.91±0.31^{A}	9.61±0.72^{A}	9.41±0.45^{A}

A, B, C in column showed the significant difference from control sample ($P<0.05$).

4. CONCLUSIONS

Types of package significantly affected shelf-life of Jackfruit seed cracker. The product kept in PP for 3 weeks has higher rancid odor and lower crispness when comparing with control sample leading to the shelf life of 2 weeks for the PP packed products. While, the product kept in LAF for 5 weeks has higher rancid odor when comparing with control sample leading to the shelf life of 4 weeks for the LAF packed products.

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REFERENCES

AOAC. 2000. Official methods of analysis of the association of agricultural. Chemist. A.O.A.C., Maryland.

Abraham A. and Jayamuthunagai J. 2014. An analytical study on jackfruit seed flour and its incorporation in pasta. Res. J. Pharm. Biol. and Chem. Sci. 5(2):1597-1610.

Agustini T.W., Darmanto Y.S. and Susanto E. 2009. Physicochemical properties of some dried fish products in Indonesia. J. Coast. Dev. 12(2):73-80.

Chittapalo T. and Songsanandr P. 2014. Product development of black glutinous rice cracker with Panag flavor and its quality changes. Int. Food Res. J. 21(5):2025-2029.

Jakubczyk E., Marzec A. and Lewicki P.P. 2008. Relationship between water activity of crisp bread and its mechanical properties and structure. Pol. J. Food Nutr. Sci. 58 (1):45-51.

Jena S. and Das H. 2012. Shelf life prediction of aluminum foil laminated polyethylene packed vacuum dried coconut milk powder. J. Food Eng. 108:135-142.

Maity T., Bawa A.S. and Raju P.S. 2014. Effect of vacuum frying on changes in quality attributes of jackfruit (Artocarpus heterophyllus) bulb slices. Int. J. food Sci. Nutr.1-8.

Manikantan M. R., Sharma R., Kasturi R. and Varadharaju N. 2012. Storage stability of banana chips in polypropylene based nanocomposite packaging films. J. Food Sci. Technol. 51(11):2990-3001.

Mazahreh A. S. 2011. Evaluation of vitamin E and the change in its quality during the storage period of some cracker product. J. Food Technol. 9(3): 95-98.

Nagi H.P.S., Kaur J., Dar B.N. and Sharma S. 2012. Effect of storage period and packaging on the shelf life of cereal bran incorporated biscuits. Am.J. Food Technol. 7 (5):301-310.

Siriwong N., Wangabklang O. and Utto W. 2012. Development of active modified atmosphere packaging systems for traditional Thai fried banana snacks. As. J. Food Ag-Ind. 5(4):292-298.

THE EFFECT OF PASTEURIZATION ON ENZYME ACTIVITY AND QUALITY OF AROMATIC COCONUT WATER

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ABSTRACT

Aromatic coconut water is an isotonic beverage with natural unique volatile compounds from green coconut. Enzyme activity usually causes changes of quality during storage of aromatic coconut water. The objective of this study was to investigate the effect of pasteurization on enzymatic activity of aromatic coconut water. Aromatic coconut water was pasteurized at 85°C and 90°C for 3, 5, 7 and 10 min. The samples were stored at 4±1°C for 14 days. Polyphenol oxidase (PPO) and peroxidase (POD) activity as well as quality (pH, total soluble solid and titratable acidity) were evaluated. The results showed that temperature and time significantly influenced on PPO and POD activities in aromatic coconut water. At 85°C or 90°C for 10 min, PPO activity was almost completely inhibited (95% inhibition). After storage at 4±1°C for 14 days, PPO activity of fresh coconut water significantly increased as storage time increased, whereas the PPO activity of samples pasteurized at 85°C for 10 min and 90°C for 5-10 min did not increase. All of heat treatments in this study could effectively inhibit POD activity. There was no significant difference in pH (5.0-5.4), total soluble solid (4.0-4.5 °Brix) and titratable acidity (0.056-0.067%) among all treatments during storage at 4°C for 14 days ($p>0.05$). Pasteurization aromatic coconut water at either 85°C or 90°C for 10 minutes was efficient for PPO and POD inactivation.

Keywords: aromatic coconut water, pasteurization, peroxidase, polyphenol oxidase

1. INTRODUCTION

Aromatic coconut water (*Cocos nucifera* L.) is largely consumed as a natural functional drink which is popular in global market. It contains a remarkable content of salts and minerals, such as potassium, sodium, chloride, magnesium and also of sugars (JAYASUNDERA and DHARMASENA, 2014). However, there is a challenge for developing processes to ensure that the product is available with safety and high nutritional and sensorial quality (AUGUSTO *et al*., 2011). The shelf life of coconut water depends on the physicochemical changes that take place after harvest, during processing and transport up to the point of sale. The most important problem related to the stability of coconut water during its shelf life is related to the activity of the polyphenol oxidase (PPO) and peroxidase (POD) enzymes. Such enzymes have relatively high thermal resistance and their activity leads to pink coloring during storage, even under refrigeration (PRADES *et al.,* 2012). Thermal processing is one of the most widely used preservation method in food industries for shelf life extension of coconut water. The commercial thermal processing used temperature at 70-100ºC for 5-20 min (MATSUI *et al.,* 2008). The objective of this study was to investigate the effect of pasteurization on enzymatic activity of aromatic coconut water.

2. MATERIALS AND METHODS

2.1. Raw material

Aromatic coconut (green) was obtained from a local market in Samut Sakhon province, Thailand. Ten coconut fruits were cleaned and sanitized. Then the mesocarp of coconuts was removed using a stainless steel knife and the coconut shell was cracked to obtain coconut water.

2.2. Effect of pasteurization on enzyme activity and quality of coconut water

Coconut water sample (350 mL) were pasteurized at 85°C or 90°C for 3, 5, 7 and 10 minutes. Samples were cooled in an ice bath at 4°C for 10 min. Fresh coconut water was used as control. All samples were kept in plastic bottles (PET) and storage at 4±1°C for 14 days. Physicochemical properties (pH, total soluble solids and titratable acidity) and enzyme activity of PPO and POD were analyzed every two days.

2.2.1. Enzyme activity

PPO activity was determined according to the method modified from TAN *et al*. (2014). A volume of 5.5 mL of 0.2M phosphate buffer (pH 6.0) and 1.5 mL of 0.2 M pyrocatechol solution were added into a test tube. The test tube was then immersed in a water bath at 25°C for 5 min. Two mL of coconut water was added to the mixture and mixed using vortex mixer (Genie II, USA). Changes in absorbance at wavelength of 425 nm were measured using UV–Vis spectrophotometer. POD was determined according to the method modified from CAMPOS *et al.* (1996). A volume of 2.1 mL of 0.1M phosphate buffer (pH 7.0), 0.5 mL of 1.5% (w/v) guaiacol and 0.2 mL of 1%(v/v) hydrogen peroxide were added into a test tube. The test tube was then immersed in a water bath at 35°C for 5 min. 0.2 mL of coconut water was added to the mixture and mixed using vortex mixer (Genie II, USA). Changes in absorbance at wavelength of 470 nm were measured using UV–Vis spectrophotometer. The absorbance value was acquired every 60 sec during 60

min for both enzymes. The absorbance values obtained were plotted against time and the initial slope value of the curve was used to calculate both PPO and POD activities (U/mL).

$$\text{Enzymatic activity (U/mL)} = \frac{A - A_0}{t}$$

Where, A is the average enzymatic activity after thermal processing and A_0 is the average initial enzyme activity before thermal processing.

2.2.2. Physicochemical properties

The pH of aromatic coconut water was determined using pH meter (Orion2 star benchtop, Beverly, Massachusetts, USA.). Total soluble solids was measured with a refractometer (ATAGO, Master-M, Japan) at 25°C. Titratable acidity was determined using titratable acidity in wine or juice method (AOAC, 2000).

2.2.3. Statistical analysis

All data was reported as means±SD from three replicates. The treatments were analyzed by analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD). The statistical significance level was measured by p-value ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

The results showed that pasteurization temperature and time (85 °C or 90 °C for 3, 5, 7 and 10 minutes) significantly influenced PPO and POD activities in aromatic coconut water. At 85 °C and 90 °C for 10 min, PPO activity was almost completely inhibited (95% inhibition) (Fig. 1A). After storage at 4±1°C for 14 days, PPO activity of fresh aromatic coconut water (control) significantly increased as storage time increased, whereas the PPO activity of samples pasteurized at 85°C for 10 min and 90 °C for 5, 7 and 10 min did not increase. At 85°C and 90°C for 3, 5, 7 and 10 min, POD activity was completely inhibited (100% inhibition) (Fig. 1B).

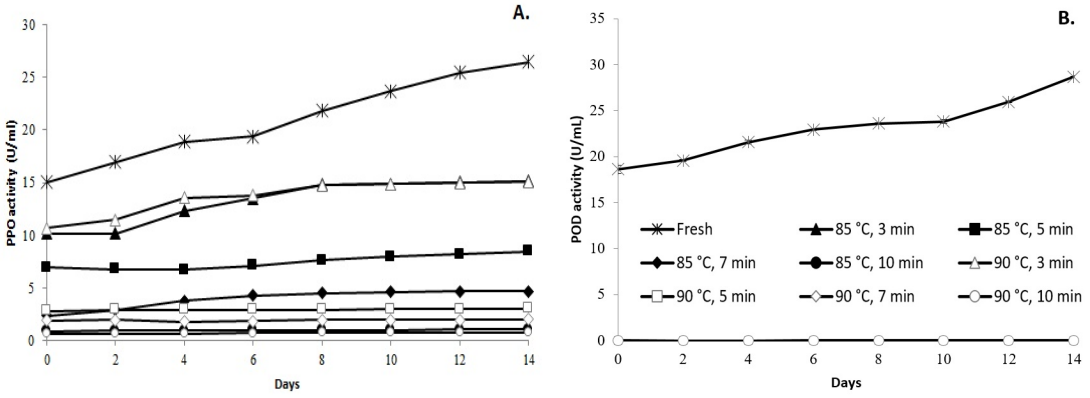

Figure 1. Effect of pasteurization temperatures and times on A) PPO activity and B) POD activity in aromatic coconut water during storage at 4±1 °C for 14 days.

The results also showed that POD activity of fresh aromatic coconut water significantly increased as storage time increased, whereas the POD activity of samples pasteurized at 85°C and 90°C for 3, 5, 7 and 10 min has been inactivated immediately after heating.
The results showed that PPO is more heat resistant than POD. Thus, PPO might be used as a heat treatment indicator for aromatic coconut water. Similar results were also reported by CAMPOS *et al.* (1996), MATSUI *et al.* (2008) and TAN *et al.* (2014), where PPO was reported to have higher heat resistance when compared to POD in coconut water. The temperature range of 80-95°C for 15 min could inactivate POD and PPO activities in coconut water. In Table 1, there was no significant difference in pH (5.0-5.4), total soluble solid (4.0-4.5°Brix) and titratable acidity (0.056-0.067%) among all treatments during storage at 4±1°C for 14 days ($p>0.05$).

Table 1. Physicochemical properties of fresh and pasteurized coconut water during storage at 4±1°C for 14 days.

Sample		pH	TSS (°Brix)	TA(%)
Fresh		5.33± 0.12^{a}	5.3± 0.11^{a}	0.058± 0.03^{a}
Pasteurization				
85°C	3 min	5.02±0.32^{b}	4.0±0.09^{e}	0.065±0.03^{a}
	5 min	5.21±0.19^{ab}	4.3±0.09^{bcd}	0.056±0.03^{b}
	7 min	5.19±0.12^{ab}	4.2±0.09^{d}	0.063±0.07^{a}
	10 min	5.29±0.09^{a}	4.5±0.05^{a}	0.067±0.03^{a}
90°C	3 min	5.36±0.12^{a}	4.4±0.04^{ab}	0.060±0.05^{ab}
	5 min	5.41±0.12^{a}	4.2±0.10^{cd}	0.066±0.03^{ab}
	7 min	5.33±0.09^{a}	4.4±0.07^{abc}	0.060±0.06^{a}
	10 min	5.35±0.04^{a}	4.4±0.12^{a}	0.064±0.07^{a}

Mean with same superscripts within the same column are not significantly different from each other at $p>0.05$ level.

4. CONCLUSIONS

Pasteurization of aromatic coconut water at either at 85°C or 90°C for 10 minutes could completely inhibit both PPO and POD activity during storage at 4±1°C for 14 days. Pasteurization temperature and time had slightly effect on quality of aromatic coconut water.

REFERENCES

AOAC. 2000. Official methods of analysis of AOAC international. 17th ed. Association of Official Analytical Chemists. Gaithersburg, Maryland.

Augusto P.E.D., Tribst A.A.L. and Cristianini M. 2011. Thermal inactivation of *Lactobacillus plantarum* in a model liquid food. J. Food Process Eng. 34:1013-1027.

Campos C.F., Souza P.E.A., Coelho J.V. and Gloria M.B.A. 1996. Chemical composition, enzyme activity and effect of enzyme inactivation of flavor quality of green coconut water. J. Food Process. Preserv. 20:487-500.

Jayasundera M. and Mathew A.G. 2014. Preservation of tender coconut water of Sri Lankan tall coconut variety. Annals. Food Sci and Technol. 15:111-114.

Matsui A.K.N., Wilhelms J.A., Oliveira P.V. and Tadini C.C. 2008. Inactivation kinetics of polyphenol oxidase and peroxidase in green coconut water by microwave process. J. Food Eng. 88:169-176.

Prades A., Dornier M., Diop N. and Pain J.P. 2012. Coconut water uses, composition and properties: A review. Fruits. 67:87-107.

Tan T.C., Cheng L.H., Bhat R., Rusul G. and Easa A.M. 2014. Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (*Cocos nucifera*) water obtained from immature, mature and overly-mature coconut. Food Chem. 142:121-128.

PHYSICOCHEMICAL PROPERTIES OF CHINESE-STYLE SAUSAGE WITH THE INCORPORATION OF MECHANICALLY DEBONED CHICKEN MEAT

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ABSTRACT

Mechanically deboned chicken meat (MDCM) is obtained from the skeletal bone tissue by forcing under high pressure through a sieve to separate the bone from the edible meat tissue. In our study, MDCM and hand-deboned chicken meat (HDCM) was formulated into Chinese-style sausage (0%, 30%, 50%, 70%, 100% MDCM) and kept in the vacuum packaging. The physicochemical properties were determined during storage at 25 ˚C for 56 days. Moisture contents of Chinese-style sausages incorporated with MDCM (21.4-25.9%) were significantly lower than that of control (29.7%) at any storage time. No significantly difference was found in a_w between Chinese-style sausages providing MDCM and control. The initial a_w of all sausages were approximately 0.85 and still maintained throughout storage time. The addition of MDCM higher than 30% in the Chinese-style sausages significantly decreased lightness (L*) and increased (a*) values after 14 days of storage which could have a negative effect on product acceptability. MDCM incorporated Chinese-style sausages were decreased in hardness properties as increased MDCM content in those sausages. However, the hardness value of sample with 30% MDCM was close to control. Therefore, overall, up to 30% MDCM could be incorporated into Chinese-style sausages without any major effects on its physicochemical properties.

Keywords: Chinese sausage, mechanically deboned chicken meat

1. INTRODUCTION

MDCM is obtained by application of mechanical force to chicken carcass from which all of the meat parts have been manually removed. In general, mechanical deboning process includes that meat is recovered from the skeletal bone tissue by forcing under high pressure through a sieve to separate the bone from the edible meat tissue. The proximate composition of MDCM has higher fat and lower moisture and protein contents than HDCM. MDCM is suitable to use for food industry to produce variety of processed meat products because of its good nutritional and functional properties and inexpensive cost (SAVADKOOHI *et al.*, 2013). Although the use of MDCM in emulsified meat products have been performed by several studies, no experiment appears to study the effect of adding MDCM on quality of Chinese sausage. Therefore, the purpose of our study was to investigate the physicochemical properties of Chinese-style sausages providing various contents of MDCM during storage at room temperature.

2. MATERIALS AND METHODS

2.1. Chinese sausage preparation

Chinese-style sausages samples were produced for five batches with different formula ratios mixing between MDCM and HDCM. Among five batches of sausage, four batches were formulated with different contents of MDCM (100%, 70%, 50%, 30% and 0%). The 0% MDCM (100% HDCM was considered as control sample. The meat mixture was manually stuffed into collagen casing (21.0 mm diameter) and linked into 13.0 cm units by manually. The sausages were dried in tray dryer with three steps of drying process: 50-55˚C for 1.5 hours, 60-65˚C for 5.5 hours and 65-70˚C for 3 hours, respectively. After drying process, products were cooled for about 20 min at room temperature, then the products were packed in polyethylene bags immediately by using vacuum sealing machine and kept at room temperature (25±3˚C) for 56 days and the samples were taken randomly every 14 days for laboratory test.

2.2. Determination of moisture, a_w and pH

Moisture content of Chinese-style sausages was determined by oven drying method. For determination of $\mathbf{a}_w$, ground Chinese-style sausages sample (4 g) was put into a holding cup. Water activity of each sample was determined at room temperature (25˚C) using automatic analyzer equipment. For determination of pH, ground Chinese sausage sample (10 g) was homogenized with 90 ml of distilled water and the homogenate was filtrated through a Whatman No.1 filter paper. The solution was measured by using pH meter.

2.3. Determination of hardness value

Hardness value of each sample was measured by texture analyzer. The condition of texture analyzer was followed the method by PERLO *et al*. (2006) with slight modification. A 50 kg load cell and p/36R adaptor with 2 mm/s test speed and 80% compression strain were used to determine hardness values.

2.4. Statistical analysis

The experiment was independently performed at least in duplicate. Difference of mean were determined in Duncan's test using the general linear model in the statistical analysis system program and means were considered significantly different at $P<0.05$. The statistical analysis was performed by using SPSS version 16.0 with 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Physical appearance

The appearance of all MDCM added samples were quite similar (Fig. 1). At the beginning, the samples containing MDCM had more bright red color due to the presence of heme pigments, particularly, myoglobin which was responsible for meat color. In the presence of nitrite in Chinese-style sausages, nitric oxide reacted with myoglobin to form the product with desirable bright red color.

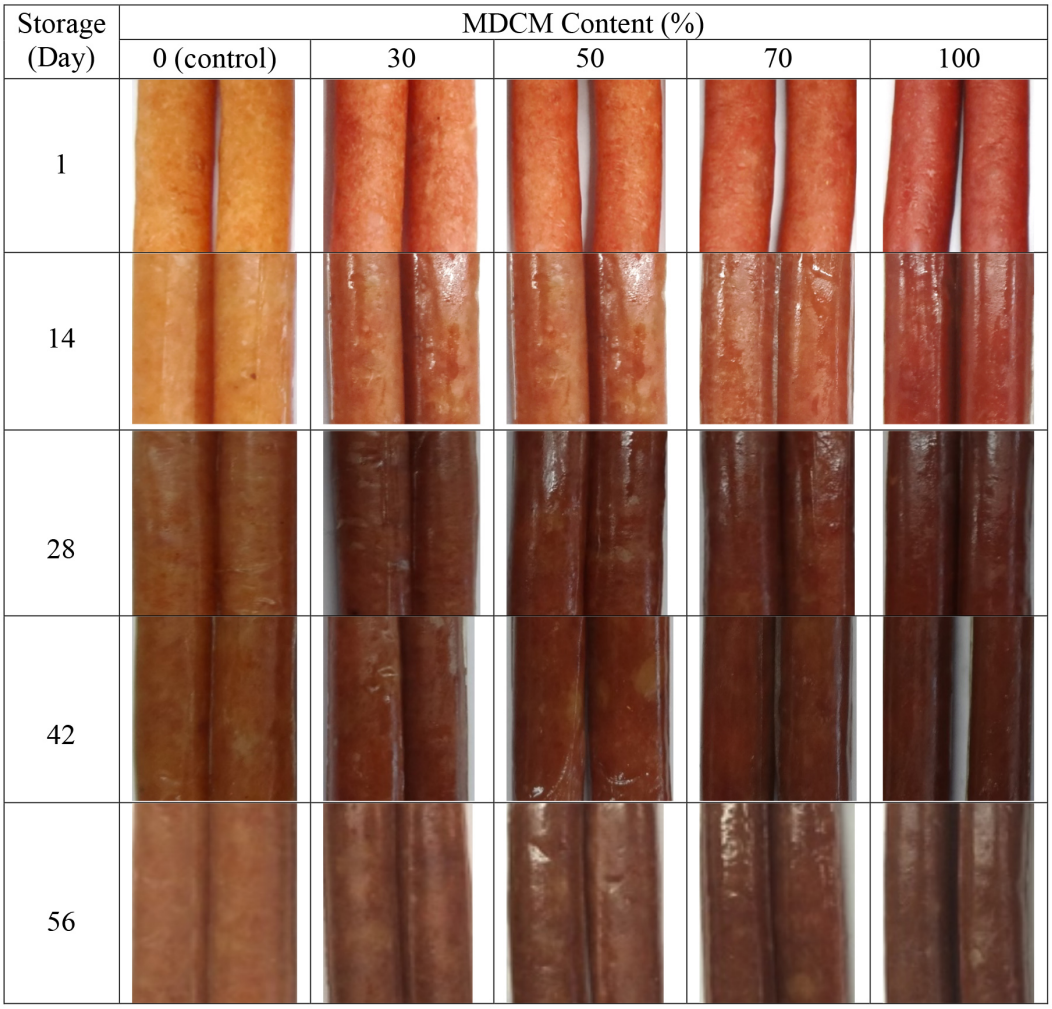

Figure 1. Physical appearance of Chinese-style sausages formulated with different contents of MDCM during storage at 25±3°C for 56 days.

The result for color changes of sausages during storage could be explained from higher amount of myoglobin which had a strong tendency to oxidize and also from higher pH value in MDCM. Although the addition of MDCM in samples had a great influence on the color values, 30% MDCM formulation in those sausage still showed the color close to control sample and had less changes in color values during storage.

3.2. Color, moisture, pH, a_w and hardness

Color (lightness, redness), moisture, a_w, pH and hardness of Chinese sausages formulated with difference contents of MDCM during storage at 25±3˚C for 56 days are shown in Fig. 2.

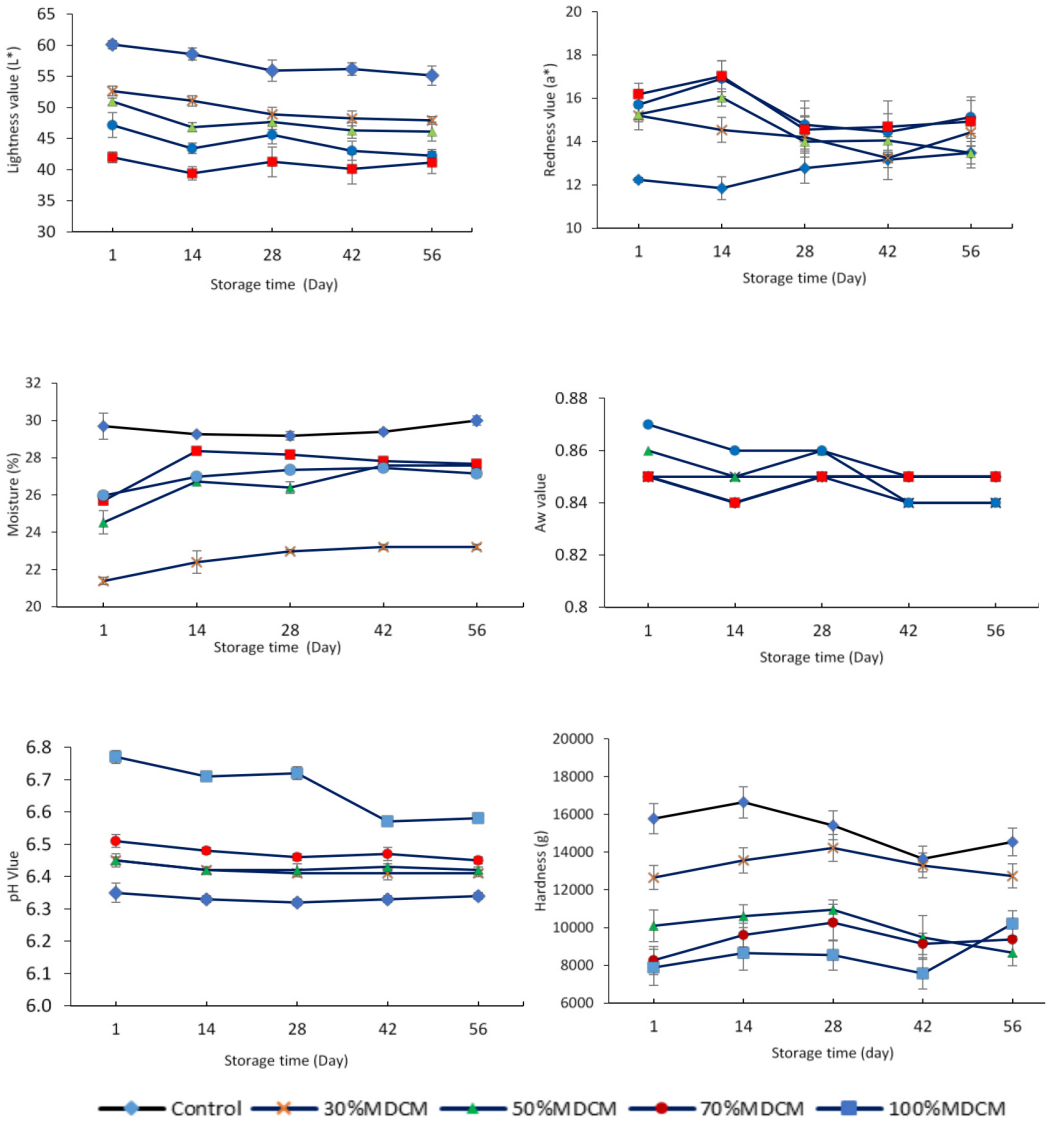

Figure 2. Color (lightness, redness), moisture, a_w, pH and hardness of Chinese-style sausages formulated with different contents of MDCM during storage at 25±3˚C for 56 days.

Higher levels of MDCM incorporated in the sample caused the lower in lightness and higher in redness values. When compared to control, Chinese-style sausages made from MDCM were significantly (P<0.05) lower in moisture content at any storage time. The moisture content of those sausages varied from 21.4 to 25.9%, while control sample was 29.7%. No significantly (P>0.05) difference was found in a_w between Chinese sausages containing MDCM and control. The initial a_w of all sausages were approximately 0.85 and this value was still maintain until 56 days of storage which met the requirement by Thai standard for this type of sausage. The determination of pH value in processed meat products was one of the most important factors because it is related with color, texture and microbial growth. The pH values of Chinese-style sausages made from MDCM were increased as larger MDCM quantities were used because of high pH value of MDCM (6.65). The pH values of those sausages varied from 6.45 to 6.77, whereas control sausage was 6.35. After 14 days of storage, the pH value was slightly decreased in all sausage samples, especially 100% MDCM, as a consequence of the acidification caused by lactic acid bacteria presented in Chinese sausage (TAN *et al.*, 2006).
After 28 days of storage, Chinese sausages formulated with more than 30% MDCM were decreased (P<0.05) in hardness while control sample was not decreased much until end of storage. The 30%MDCM added sausage had similar trend with control and less change was shown in textural properties. DAROS *et al*. (2005) showed that the protein value of mechanically deboned poultry meat was lower than hand deboned chicken meat. Therefore, the possible reason for decreasing textural properties in this study would be affected by lower protein value of MDCM. Myofibrillar protein is the main functional protein responsible for the conformational structure and eating quality of meat and meat products would undergo denaturation mechanical deboning process leading to a reduction in their functionalities.

4. CONCLUSIONS

The addition of MDCM higher than 30% in the Chinese-style sausages significantly decreased lightness (L*) and increased (a*) values after 14 days of storage. The hardness properties of the samples containing MDCM were decreased as increased MDCM content in Chinese-style sausages. However, the textural properties of sample containing 30% MDCM were acceptably quite close to control. Lipid oxidation and sensory evaluation are recommended for further study to evaluate the acceptance of the products.

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REFERENCES

Daros F.G., M.L. Masson and S.C. Amico. 2005. The influence of the addition of mechanically deboned poultry meat on the rheological properties of sausage. J. Food Eng. 68:185-9.

Perlo F., P. Bonato, G. Teira, R. Fabre, and S. Kueider. 2006. Physicochemical and sensory properties of chicken nuggets with washed mechanically deboned chicken meat: Research note. Meat Sci. 72:785-8.

Savadkoohi, S., K. Shamsi, H. Hoogenkamp, A. Javadi, and A. Farahnaky. 2013. Mechanical and gelling properties of comminuted sausages containing chicken MDM. J. Food Eng. 117:255-262.

Tan F., K. Hsu, and H. Ockerman. 2006. Effect of ripening temperature on quality characteristics of Chinese-style sausage. J. Muscle Foods. 17:367-74.

DISTRIBUTION OF MICROORGANISMS AND QUALITY CHANGES OF COMMERCIAL TRIMMED AROMATIC COCONUT

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ABSTRACT

Aromatic young coconut is a well-known Thai fresh produce around the world. It has been a major economic significance with a great demand from both national and international markets. The commercialized ready to serve fresh coconut was prepared by trimming and PVC film wrapping; then distributed to local and worldwide market. In order to prolong its shelf-life against microbial growth and color appearance change, antimicrobial and antibrowning agents are considered using during postharvest treatment on the coconut. Sulfiting agents such as potassium metabisulfite (KMS) are generally used to overcome both problems. Therefore, this postharvest treatment is purposely to inhibit microbial growth and indirectly to prolong its shelf-life. Nonetheless, microbial growth still has been found randomly. This study was performed to study microbial distribution during the storage at 2°C and the RH around 81-83% of 3% KMS-treated and PVC film wrapped young coconut. Through the 4 week-cold storage, microbial distribution of the outer area and coconut water of the trimmed coconut were analyzed. Aerobic plate count (APC) was found to be in the range of 0.83-2.01 log CFU/fruit, whereas yeast and mold (YM) was found less than 0.17 log CFU/fruit. For coconut water, APC and YM were found to be in the range of n.d.-1.21 log CFU/ml and n.d.-0.23 log CFU/ml, respectively. In addition, at the shoulder area of the trimmed coconut, TPC was observed to be mostly found in the range of 0.10-1.36 log CFU/fruit through the cold storage period. Nevertheless, coconut water was analyzed for its pH, total soluble solid and titratable acidity which ranged in pH 6.26-6.97, 7.53-8.53°Brix, and 0.031-0.040%, respectively. Outer color changes were analyzed as browning index which ranging from 19.26-27.16. The data suggested that using low temperature together with KMS was successfully inhibit some microorganisms particularly yeast and mold, while it was slightly affected chemical properties of the coconut juice. Interestingly, the distribution of total microorganisms was found mostly at the top area of the trimmed coconut when it was compared to other areas.

Keywords: aromatic coconut, coconut water, metabisulfite, microorganism, shelf-life

1. INTRODUCTION

A trimmed aromatic coconut is currently popular worldwide, whereas, major problems of the trimmed aromatic coconut during export at low temperature are microbial spoilage and mesocarp color changes. The microbial spoilage usually causes by molds, yeasts (MACIEL *et al.,* 2013) and spoilage bacteria (GABRIEL and JULIUS, 2016). Brown color on mesocarp surface of trimmed coconut occurred due to its exposure to light as of oxidation reaction. The total soluble solids and titratable acidity were in a range of 5-7°Brix and 0.03-0.09%, respectively, during 6 week-storage of Makapuno coconut (LUENGWILAI *et al.,* 2014). The objective of this study was to evaluate microbiological, chemical and physical changes of KMS-dipped trimmed aromatic coconuts that were storage at 2°C under the relative humidity (RH) around 81-83 % for four weeks.

2. MATERIALS AND METHODS

Young coconuts (*Cocos nucifera* L.) were collected from factory located at Samutsakorn province, Thailand. The fruits were trimmed and dipped in 3% KMS (Potassium metabisulfile) (STAR-FRESH 9, Thailand) solution for 10 min, wrapped with polyvinylchloride (PVC) film and stored at 2°C, 81-83% RH. The trimming was done in a similar shape as shown in JARIMOPAS and RUTTANADAT (2007). Qualities of coconuts were determined by randomly selection of three fruit samples in each week for 4 weeks.

2.1. Chemical determinations

Total soluble solids (TSS), titratable acidity (TA) and pH of coconut water were evaluated. TSS was determined using hand refractometer (Atago, model N-1E, Japan). TA was determined by titration samples with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator (AOAC, 2000). The pH of coconut water was measured by pH meter (Denver, model UB-10, Japan).

2.2. Physical determinations

Percent weight loss was calculated by weighing fruit individually before and after storage at room temperature. The difference as a percent of the initial weight was then calculated. Color of KMS-dipped trimmed aromatic coconuts was measured from three parts of fruit (shoulder, body and bottom) by spectrophotometer **(**Hunter Lab, UltraScan PRO, USA.**)** and calculated as browning index.

2.3. Microbiological determinations

Coconut water and three surface areas of trimmed coconut were determined. The whole areas of the shoulder, body and bottom from trimmed coconut are 150, 300 and 80 cm^2, respectively, which were swabbed for microbial determinations. Aerobic plate count and yeast and mold counts were determined according to BAM (1998, 2000).

3. RESULTS AND DISCUSSION

The qualities of trimmed coconut were investigated. The pH of coconut water was approximately 5.14-6.90 during storage for 4 weeks (Fig. 1A.). At the beginning, pH of

coconut water was 5.14, it increased to 6.26, 6.37, 6.97 and 6.50 after storage for 1, 2, 3 and 4 weeks, respectively. The results corresponded to the previous report that the pH of coconut water from a burnt coconut was 6.21-6.46 after storage for 2 weeks (JANGCHUD *et al*., 2007). The trend of TSS was risen up toward the 4th week (Fig. 1B.). The TSS of coconut water was increased from 7.16°Brix to 8.53°Brix within 1 week. Then the TSS was quite stable toward 4 week-storage.

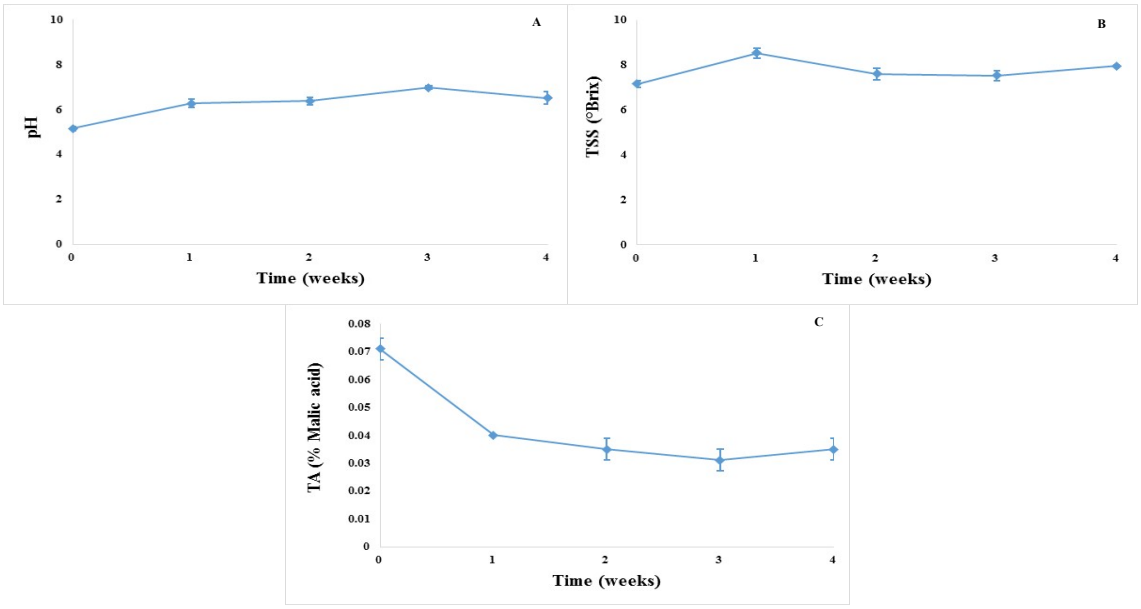

Figure 1. Changes in pH (A) TSS (B) and TA (C) of trimmed coconut during cold storage.

Meanwhile, the trend of TA of coconut water during cold storage for four week was reduced from 0.071% at the initial week to 0.035% at the final week (Fig. 1C.). In agreement with JANGCHUD *et al.* (2007), the TA of burnt coconut water were 0.033-0.044% during 2 week-storage.

The highest browning index was found at the shoulder area of trimmed coconut, whereas the lowest value was detected at the bottom area. Nonetheless, the browning index of all three areas were rather stable through the 4 week-storage (Fig. 2A.). At the beginning, the browning index were 27.75, 24.86 and 17.97 for shoulder, body and bottom areas, respectively. When the coconuts were stored for 4 weeks the browning index of shoulder, body and bottom were 27.16, 21.36 and 18.64, respectively. Weight loss of the coconut after 4 week-storage was ranged from 1.36 to 2.77% (Fig. 2B). Weight loss of coconut in cold storage was normally observed (LUENGWILAI *et al.*, 2014). JANGCHUD *et al.* (2007) also reported weight loss of burnt coconut increased to 1.95% at the 4th week of cold storage. The APC are shown in Fig. 3A and Fig. 3C. The microbial numbers on shoulder body and bottom areas in the initial week were 1.47, 1.90 and 1.32 log CFU/area, respectively. After 1 week-storage, the microbial count of all area were decreased.

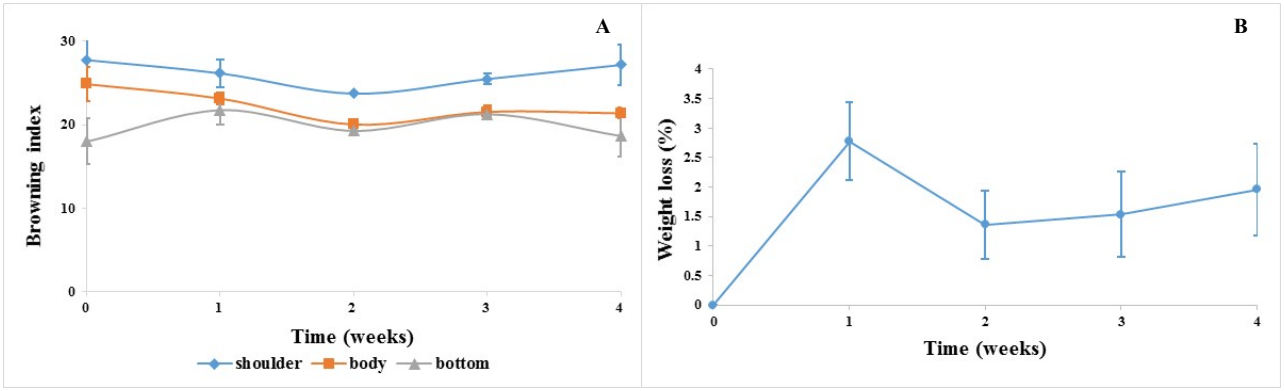

Figure 2. Changes in browning index (A) and weight loss (B) of trimmed coconut during cold storage.

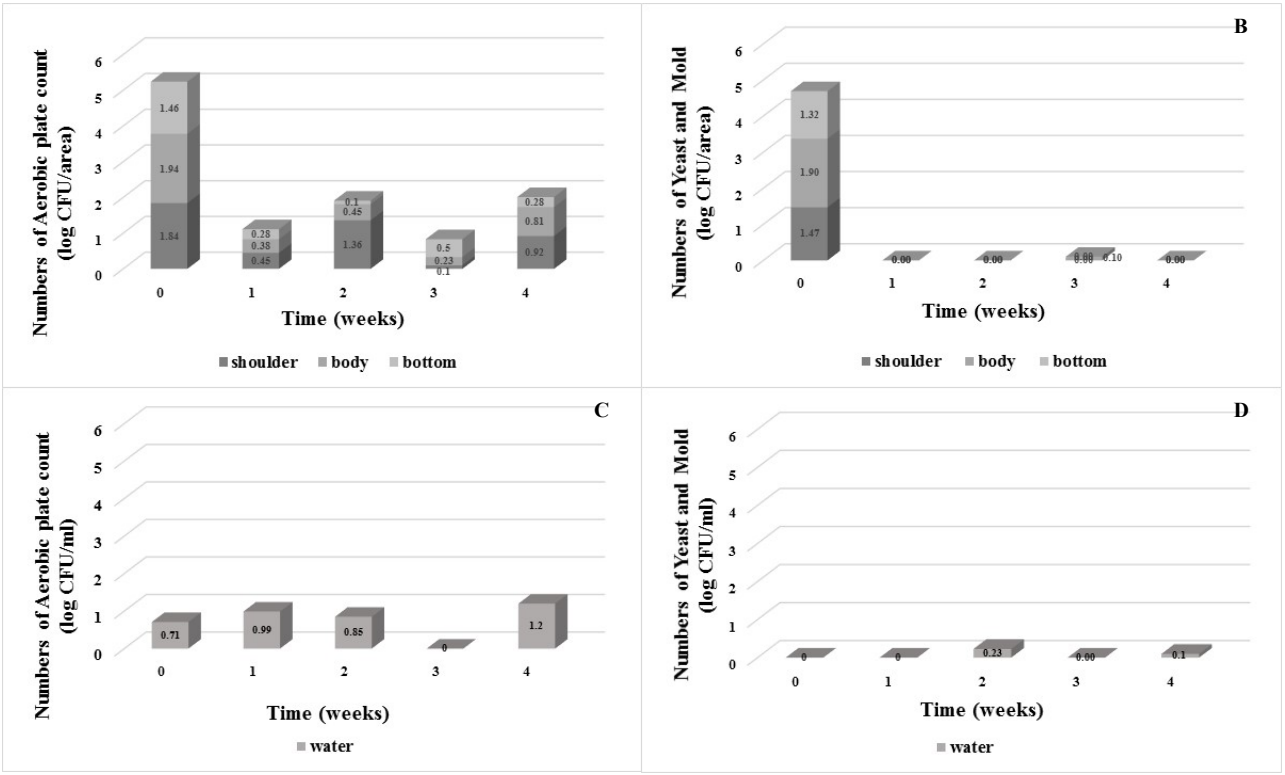

Figure 3. Numbers of aerobic plate count (A), yeasts and molds (B) on surface of trimmed coconut and numbers of aerobic plate count (C), yeasts and molds (D) in coconut water during storage at 2°C for 4 weeks.

In the 3rd week, the APC was not detected in the coconut water; but it was found with the lowest amount on shoulder, body and bottom areas were 0.10, 0.23 and 0.50 log CFU/area, respectively (Fig. 3C). In the 4th week of storage, the APC in the coconut water was 1.20 log CFU/ml; whereas the amount on shoulder, body and bottom areas were 0.92, 0.81 and 0.28 log CFU/area, respectively. The YM on shoulder, body and bottom areas in the initial week were 1.47, 1.90 and 1.32 log CFU/area, respectively (Fig. 3B). There were no YM detected in coconut water in the initial week; while they were detected as 0.23 and 0.10 log CFU/ml in coconut water after cold storage for 2 and 4 weeks, respectively (Fig. 3D). According to report from MOHPRAMAN and SIRIPHANICH (2012), sulfite residues were mostly penetrated into coconut mesocarp at the bottom area. Therefore, high sulfite

residue concentration at that area could resulted in reducing of microbial contamination and oxidation reaction.

4. CONCLUSIONS

Though the microbial count at the bottom area of KMS-dipped trimmed aromatic coconuts were found lower than at the shoulder and body areas. In addition, the highest browning index was shown at the bottom area followed by body and shoulder areas, respectively. The pH and TSS from the coconut increased as storage time increased. The TA of coconut water was reduced when storage for 4 weeks. Browning index on the coconut surface were quite stable, while its weight loss was fluctuated through the storage time. Our results suggested that trimmed coconut stored at 2°C 81-83% RH for 4 weeks were still found microbial spoilage together with some chemical and physical changed; which affect qualities of exported coconuts through their cold storage time.

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REFERENCES

AOAC. 2000. Official methods of analysis of AOAC International, 17th ed. The Association of Official Chemists, Maryland.

Bacteriological Analytical Manual. 1998. Aerobic Plate Count. 8th ed. Available from: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm. Accessed 2017 May 15.

Bacteriological Analytical Manual. 2000. Yeast Mold and Mycotoxins. 8th ed. Available from: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071435.htm. Accessed 2017 May 15.

Gabriel, A. A. and Colambo J.C.R. 2016. Comparative resistances of selected spoilage and pathogenic bacteria in ultraviolet-C-treated, turbulent-flowing young coconut liquid. J. Food Control. 69:134-140.

Jangchud, K., Puchakawimol, P. and Jangchud, A. 2007. Quality changes of burnt aromatic coconut during 28-day storage in different packages. LWT-Food Sci. Technol. 40:1232-1239.

Jarimopas, B. and Ruttanadat, N. 2007. Development of a young coconut fruit trimming machine. J. Food Eng. 79(3):752-7.

Maciel, N.O.P., Pio F.B., Freitas L.F.D., Gomes F.C.O., Jojannn S., Nardi R.M.D., Lachance M.A. and Rosa C.A. 2013. The diversity and antifungal susceptibility of yeasts isolated from coconut water and reconstituted fruit juices in Brazil. Int. J. Food Microbiol. 160:201-205.

Luengwilai, K., Beckles D.M., Pluemjit O. and Siriphanich J. 2014. Postharvest quality and storage life of 'Makapuno' coconut (*Cocos nucifera* L.). Sci. Hortic. 175:105-110.

Mohpraman, K. and Siriphanich J. 2012. Safe use of sodium metabisulfite in young coconuts. Postharvest Biol. Technol. 65:76-78.

THE EFFECT OF CALCIUM LACTATE DIPPING ON QUALITY AND TOTAL ANTHOCYANIN CONTENT OF FROZEN RED SUPERSWEET CORN KERNEL (*ZEA MAYS* L. SACCHARATA)

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ABSTRACT

The color and texture qualities of red supersweet corn (*Zea mays* L. saccharata) kernels are changed during frozen. The objective of this study was to investigate the effect of calcium lactate dipping on quality and total anthocyanin content of frozen red supersweet corn kernel. Red supersweet corn kernels (cv. Siam Ruby Queen) were removed from corn cobs and steam-blanched for 10 min in order to inhibit peroxidase activity. Samples were dipped in calcium lactate solution **(**0.3, 0.5 and 1%**)** at 60°C for 1 min. Color and total anthocyanin content of dipping solution were also evaluated to determine leaching of pigment during dipping process. After dipping, corn kernels were cryogenic frozen and storage at -20°C. Color, texture, drip loss and total anthocyanin content of corn kernel were determined after 3 months of storage. The results showed that significant amount of anthocyanins leached into calcium lactate solution during dipping process when compared to control **(**no dipping**)** and the loss of pigment increased as concentration of calcium lactate increased. After 3 months of storage (-20°C), although there was no significant difference in drip loss, significant color (L*, a* and b*) changes were observed in all samples when compared to control (no dipping). Dipping in calcium solution (0.3%-1%) resulted in significant loss of anthocyanin, approximately 20-27% when compared to control. However, as concentration of calcium lactate increased up to 1%, dipping in calcium lactate solution could significantly help maintain firmness of corn kernels ($p \leq 0.05$), with approximately 16% higher firmness when compared to control. Further study on minimization of pigment loss during calcium lactate dipping should be done in order to successfully maintain quality of frozen red supersweet corn kernels.

Keywords: anthocyanin content, calcium lactate, frozen, quality, red supersweet corn

1. INTRODUCTION

Recently, red supersweet corn (*Zea mays* L. saccharata) has been new conventionally bred in Thailand by Sweet Seeds Co., Ltd. consuming fresh and beverage. Frozen corn kernels are new value-added products, which could be used as food ingredients. One of the most general processes for quality storage is freezing process that low temperature can reduce deteriorative reactions and restrict growth of microorganism (SIMANDJUNTAK *et al.*, 1996). Calcium lactate has been normally used as an alternative calcium source for firming agent without bitterness and flavor difference, which be found in calcium chloride (Monsalve-Gonzalez *et al.*, 1993; LUNA-GUZMÁN and BARRET, 2000). Many studies have to focus on firmness tissue but there is little study on maintaining anthocyanin stability. The objective of this research was to determine the effect of calcium lactate dipping on quality and total anthocyanin content of frozen red supersweet corn kernel.

2. MATERIALS AND METHODS

Red supersweet corn, cv. Siam Queen Ruby (SR1006), was purchased from Sukhothai province, Thailand. The corn samples were harvested, ice packed and transported to Kasetsart University within 2 h. They were kept at 4°C for 24 hr before husking and kernel removal by hand. Corn kernels were steam-blanched for 10 min and rapidly cooled in ice water for 3 min. Then the samples were dipped 0.3%, 0.5% or 1% calcium lactate solution at 60°C for 1 min. Dipping solutions were immediately collected to measure color and total anthocyanin content. The samples without dipping in calcium lactate were used as control. Corn kernels were then frozen using cryogenic freezer (Bangkok Industrial Gas CO., Ltd., BKK, Thailand) until core temperature of kernel reached -20°C. All Samples was stored at -20°C for 3 months. After 3 months of storage, samples were evaluated for color, total anthocyanin content, texture and drip loss.

2.1. Color analysis and total anthocyanin content

Corn kernels were extracted with 0.1% HCl-80% methanol using the method modified from YANG *et al.* (2009). Color value of corn kernel extract was measured using color analyzer (CIELAB: L*, a* and b*) (HunterLab, VA, USA). Total anthocyanin content was determined according to pH differential method as described by GIUSTI and WROLSTAD (2005) and was expressed as mg of cyaniding-3-glucoside equivalents (C3G) per 100 g dry weight (DW).

2.2. Texture

The method of texture analysis was modified from MARQUES *et al*. (2014). Briefly, frozen corn kernels were thawed at 4°C and then texture was analyzed (SHANG *et al*., 2016). A TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK) was used to perform maximum force of hardness (N). The kernel was compressed by 25 mm diameter of flat-cylinder probe.

2.3. Drip loss

Drip loss (DL) of frozen corn kernel was determined with weight of the frozen corn kernel and paper after thawing (GONÇALVES *et al.*, 2011). The drip loss was expressed as percentage of water lost during thawing process.

2.4. Statistical analysis

The experiments were set up according to complete randomized design. All data were reported as means ± SD from three replicates. One-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) were used for comparing means. The statistical significance level was measured by p-value ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

The result showed that after dipping corn kernels in calcium lactate solution, redness (a*) values and total anthocyanin contents of calcium lactate solutions increased as calcium lactate concentration increased (Fig. 1A and 1B), which indicated that color pigment from corn kernels significantly leached into calcium lactate solution as concentration of calcium lactate increased.
This leaching effect is significantly observed when dipping in 1% calcium solution. As the calcium concentration increased, Lightness (L*) of dipping solution decreased, while yellowness (b*) increased (data not shown).

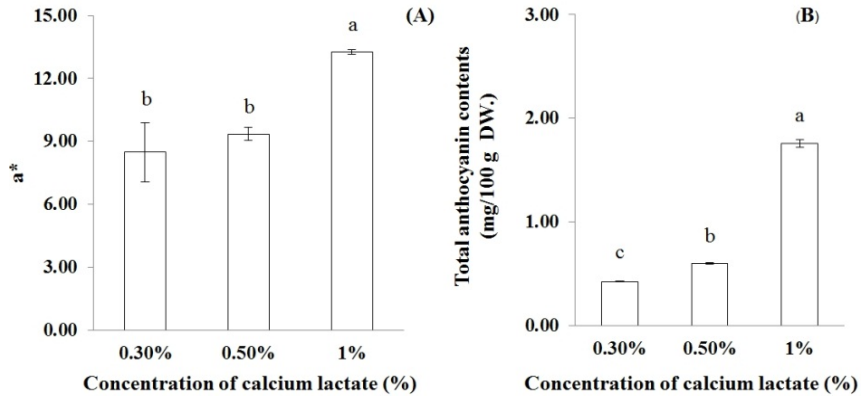

Figure 1. Redness (a*) value (A) and total anthocyanin content (B) of dipped calcium lactate solutions at 0.30%, 0.50% and 1%. Values are expressed as mean ± SD.

After 3 months of storage (-20°C), there was no significant difference in drip loss among treatments ($p>0.05$, data not shown). Drip loss of red corn kernel varied between 18.07% and 18.52%. Frozen sample during storage may cause recrystallization of ice crystal that effected on loss of water holding capacity resulting in thawing (VAN BUGGENHOUT *et al.*, 2006). Significant redness value (a*) changes and total anthocyanin loss were observed in all dipped samples when compared to control (no dipping) (Fig. 2A and 2B). Dipping in calcium solution (0.3%-1%) resulted in significant loss of anthocyanin, approximately 20-27% when compared to control. When compared to control, corn kernel dipping in calcium solution showed significant loss of yellowness (b*) and increase of lightness (L*)

(data not shown). The results corresponded to previous results of dipping solution color. Significant loss of pigment in 1% calcium treatment may be due to the high osmotic pressure of 1% calcium lactate solution, when compared to other (SILVA *et al*., 2014). However, as concentration of calcium lactate increased up to 1%, dipping in calcium lactate solution could significantly help maintain firmness of corn kernels ($p \leq 0.05$), with approximately 16% higher firmness when compared to control (Fig. 3). According to previous research, firmness could be enhanced by addition of calcium owing to membrane stabilization and calcium pactate formation with increasing of stiffness of middle lamella, cell walls and reducing speed of polygalacturonase activity (WHITE and BROADLEY, 2003). Further study on minimization of pigment loss during calcium lactate dipping should be done in order to successfully maintain quality of frozen red supersweet corn kernels.

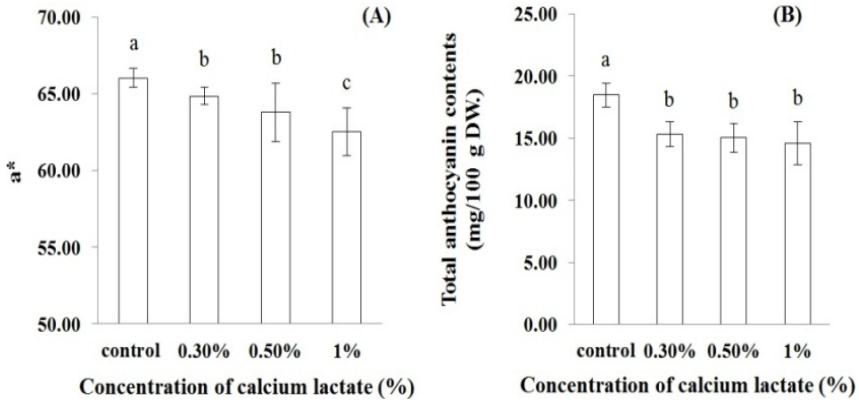

Figure 2. Redness color (A) and total anthocyanin content (B) of corn kernel during 3 months of storage. Values are expressed as mean ± SD.

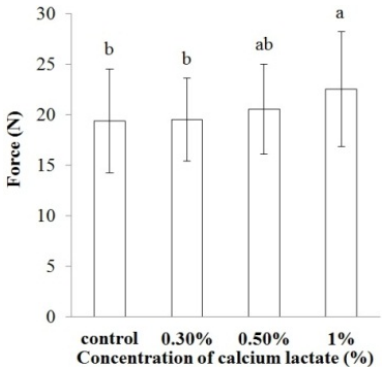

Figure 3. Texture of corn kernel expressed by hardness (N) during 3 months of storage. Values are expressed as mean ± SD.

4. CONCLUSIONS

Dipping in calcium lactate solution up to 1% could help maintain texture of frozen red supersweet corn kernel. However, significant loss of color and total anthocyanins were observed. Therefore, further study should be done to identified treatment that could maintain both color and texture of frozen red supersweet corn kernel.

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REFERENCES

Giusti M.M. and Wrolstad R.E. 2005. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy: Handbook of Food Anlytical Chemistry, p 19-31. Wiley-Interscience, Hoboken, NJ.

Gonçalves E.M., Abreu M., Brandão T.R.S. and Silva C.L.M. 2011. Degradation kinetics of colour, vitamin C and drip loss in frozen broccoli (*Brassica oleracea* L. ssp. Italica) during storage at isothermal and non-isothermal conditions. Int. J. Refrig. 34:2136-2144.

Luna-Guzman I., and Barrett D.M. 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupe. Postharvest Biol. Technol. 19:61-72.

Monsalve-Gonzalez A., Barbosa-Canovas G.V., and Cavalieri R.P. 1993. Mass transfer and textural changes during processing of apples by combined methods. J. Food Sci. 58:1118-1124.

Marques B.C., Jorge L.M.d.M., and Jorge R.M.M. 2014. Hydration kinetics, physicochemical composition, and textural changes of transgenic corn kernels of flint, semi-flint, and dent varieties. Food Sci. Technol. (Campinas) 34:88-93.

Simandjuntak V., Barret D.M., and Wrolstad R.E. 1996. Cultivar and frozen storage effects on muskmelon (Cucumis melo) colour, texture and cell wall polysaccharide composition. J. Sci. Food Agric. 71:291-296.

Shang H., Li L., Xiaojie H., Jianxin S., and Xianjun M. 2016. Effects of different thawing methods on microstructure and texture of raspberries (cv. Heritage). Agro Food Ind. Hi. Tech. 27:59-64.

Silva K.S., Fernandes M.A. and Mauro M.A. 2014. Effect of calcium on the osmotic dehydration kinetics and quality of pineapple. J. Food Eng. 134:37-44.

Van Buggenhout S., Messagie I., Maes V., Duvetter T., Van Loey A., and Hendrickx M. 2006. Minimizing texture loss of frozen strawberries: effect of infusion with pectinmethylesterase and calcium combined with different freezing conditions and effect of subsequent storage/thawing conditions. Eur. Food Res. Technol. 223:395-404.

White P.J., and Broadley M.R. 2003. Calcium in plants. Ann. Bot. 92:487-511.

Yang Z.D., Chen Z.J., Yuan S.L., Zhai W.W., Piao X.S., and Piao X.L. 2009. Extraction and identification of anthocyanin from purple corn (Zea mays L.). Int. J. Food Sci. Technol. 44:2485-2492.

SHELF LIFE ASSESSMENT OF GREEN ASPARAGUS PACKAGED IN POLYPROPYLENE MACRO-PERFORATED AND MICRO-PERFORATED

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ABSTRACT

The very short shelf-life of asparagus (*Asparagus officinalis*, L.) is mainly related to its high respiratory activity which continues after harvesting. The perishable nature of the product poses the challenge to develop effective storage methods to extend its post-harvest life. The loss of quality is mainly perceived by consumers in the wrinkling of stems, hardening, loss of the green colour and brightness. The aim of this study was to evaluate the quality of green asparagus ('Vegalim') packaged in polypropylene macro-perforated with a piercing density of 7 holes/cm^2 and micro-perforated coextruded PP with a row of central holes spaced each 3 cm having an oxygen transmission rate of 38800 cc/m^2. The quality evaluation of asparagus was assessed by measuring changes in the main physical-chemical parameters and by visually monitoring the product, during a period of 32 days of cold storage. Weight loss, instrumental texture and colour degradation were measured. Data were analysed with a specific testing software. Image analysis was performed. The weight loss of the samples, which is mainly due to moisture loss, was 16% in macro-perforated film, while it was negligible in micro-perforated film (mean value 0.4%). Maximum shear force and cutting energy were measured cutting asparagus spears into three parts: tip (0-6 cm, measured from the apex of the spear), middle portion (6-12 cm) and base (12-18 cm). For samples packaged in the macro-perforated film, hardness increased in all three parts throughout the experimental period. Shear force of the middle portion of samples packaged in micro-perforated film did not change during the 32 days of the experiment. Hue and Chroma were similar in both samples. The shelf life of refrigerated green asparagus could be extended to 32 days with packaging in micro-perforated film. In literature temperatures of 5°C were considered adequate for keeping the shelf life of asparagus about 11-14 days, it is relevant how the utilization of the correct plastic film to wrap asparagus is the goal for any companies producing this vegetable to significantly extend their marketability.

Keywords: extension, green asparagus, micro-perforated polypropylene, shelf life, storage

1. INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a delicate vegetable, which is known to be a rich source of some functional compounds beneficial for human health (WAMBRAUW *et al.*, 2016) and it is consumed in many parts of the world.
Asparagus of any cultivar may be consumed in three color forms resulting from the cultivation method adopted, i.e. white, pale-colored or green (FUENTES-ALVENTOSA *et al.*, 2013).
The number of varieties of asparagus is constantly changing. Many of them have also been modified to meet the requirements and expectations of both producers and customers. It is believed that there is no perfect variety of asparagus in all conditions and the differences between them are often not clear (KOHMURA *et al.*, 2008). Among the green asparagus 'Vegalim' is a male hybrid, largely cultivated in warm climates, such as the Mediterranean one, also because it combines medium early production with high yields, with an excellent tip closure, high weight and uniform diameter of spears (LIMGROUP, 2017).
Green asparagus looses freshness easily and has very short shelf life due to the loss of crispiness, high postharvest respiration rate (Irving and Hurst, 1993), wilting, rotting, wooden and fibrosis (DUAN *et al.*, 2016). The aim of this study was to evaluate the possibility to extend the shelf life of green asparagus variety 'Vegalim' through a change of the packaging system.

2. MATERIALS AND METHODS

Green asparagus (cultivar *Vegalim*) were kindly provided by Asparago Sovrano Consortium. Samples were transported to the Di3A laboratories, divided into 2 homogeneous lots and packaged in polypropylene macro-perforated (PP macro) with a piercing density of 7 holes/cm^2 (Bemis Le Trait sas, France) and the second lot in Coralife Swaf C (Corapack s.r.l., Italy), micro-perforated coextruded PP with a row of central holes spaced each 3cm having an oxygen transmission rate of 38800 cc/m^2 (Coralife C). All samples were stored under refrigerated conditions at 4±1°C and 90-95% RH until analyses. The determination of quality parameters was performed on 3 replicates for each batch after 0 (packaging day), 7, 14, 23 and 32 days of storage.
Weight loss was determined by weighing all samples with a Chyo Balance MK-500C (±0.01 g) at the beginning and end of the each sampling during storage period. The difference between the two values was considered and expressed as weight loss rate (%) as reported by KITAZAWA *et al.* (2011).
The texture properties were evaluated measuring the maximum shear force using a ZwickRoell z 0.5 Texture Analyzer (Zwick GmbH & Co., Ulm, Germany) provided with a 500 N nominal force load cell and a stainless steel probe (length 5 mm). Cutting tests were carried out at the following conditions: preload 0.2 N, test speed 20 mm/min, cutting asparagus spears into three parts: tip (0-6 cm, measured from the apex of the spear), middle portion (6-12 cm) and base (12-18 cm) as reported by TZOUMAKI *et al.* (2009). Firmness was expressed as the maximum force (Fmax, N): data were determined on triplicate samples and were stored and elaborated by the Testxpert II v. 2.2 software (Zwick GmbH & Co., Ulm, Germany).
In order to quantify the color changes in green asparagus, their images were acquired through CanoScan LiDE 220 (Canon, Japan) and then analyzed with software Image-Pro® Plus 7.0 (Media Cybernetics Inc., Rockville, USA). The image acquisition presupposed a careful standardization of conditions, both in terms of light and in spatial and geometrical ones. The model utilized for the analysis of the images during this shelf life study is HSI

(hue, saturation, intensity) that describes colors as like as they are perceived by human eyes. Hue and Chroma were determined as described by RIZZO and MURATORE (2009).

3. RESULTS AND DISCUSSION

Weight loss is a physiological event in fresh vegetables that can be limited by controlling storage temperature and humidity, but also by using appropriate packaging. The weight loss rate was particularly different between samples. As expected this value ranged from 3.9% to 15.8% in asparagus packed in the macro-perforated PP (Fig.1, A), while it was less than 1% during storage in Coralife C (Fig.1, B). Results confirmed, the differences in their technical characteristics in terms of OTR; macro-perforated PP reduced weight loss and was able to maintain hygienic conditions as well as mechanical damage (RIZZO and MURATORE, 2009). Of course, both positive results were highly increased by C, guaranteeing the quality of fresh asparagus and its respiration rate, keeping the initial fresh weight until 32 days just using the appropriate storage temperatures.

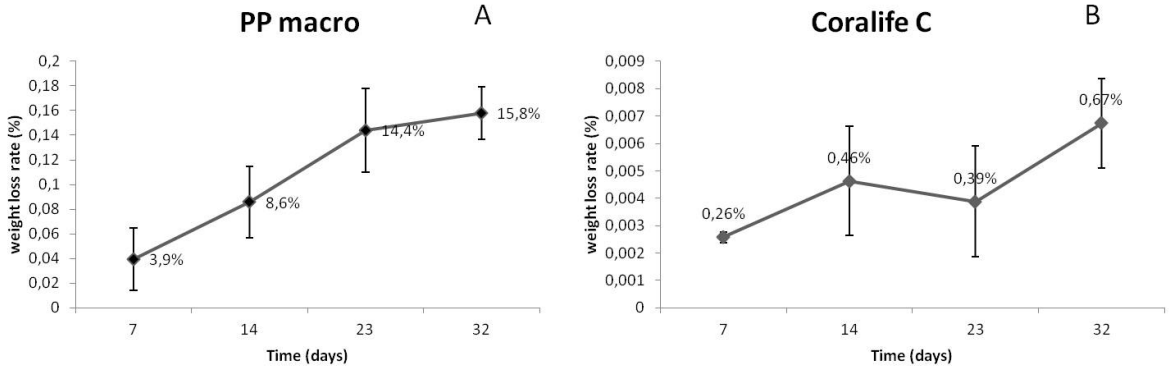

Figure 1. Weight loss rate (%) of green asparagus cv. Vegalim packed in the macro-perforated PP (A) and in the micro-perforated Coralife C film (B) PP during 32 days of storage.

Changes in texture of asparagus have been related to the increase of fibrousness occurring after harvest. Fig. 2 represents the texture of the three distinct asparagus parts for the different packaging solutions. Regarding the apical part of asparagus spears, both samples (Coralife C, PP macro) retained their texture values during storage at levels similar to the fresh asparagus, while the firmness values in the middle part and in the basal parts showed a small increase compared to values at the day of harvest (day 0); in particular, values for the macro-perforated PP were always higher than those of Coralife C.
The mean and standard deviation values of area, reported as grey values measured during shelf life, are reported in Fig. 3. The different features showed a similar trend during the storage period, no significant differences were monitored during the experiment. Hue, saturation and intensity in grey level were calculated as reported in GRILLO *et al.* (2014) and results were reported for macro-perforated PP (A) and Coralife C (B) during storage. Also, Hue and Chroma were similar in both samples, suggesting that image analysis and colorimetric values were not indicatives in this experiment.

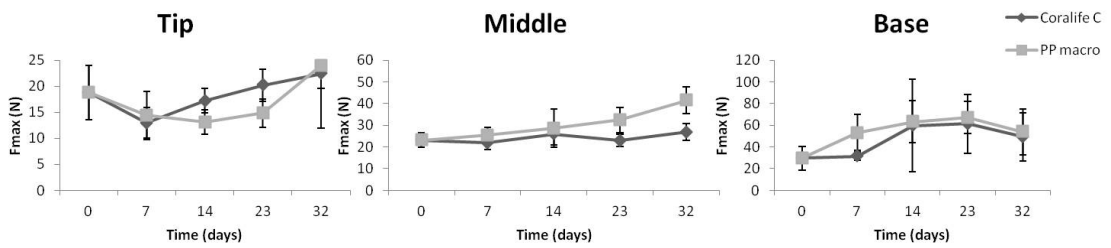

Figure 2. Texture, expressed as maximum shear force, determined in three parts (tip, middle, base) of asparagus samples packed in the macro-perforated PP and in the micro-perforated Coralife C film during 32 days of storage.

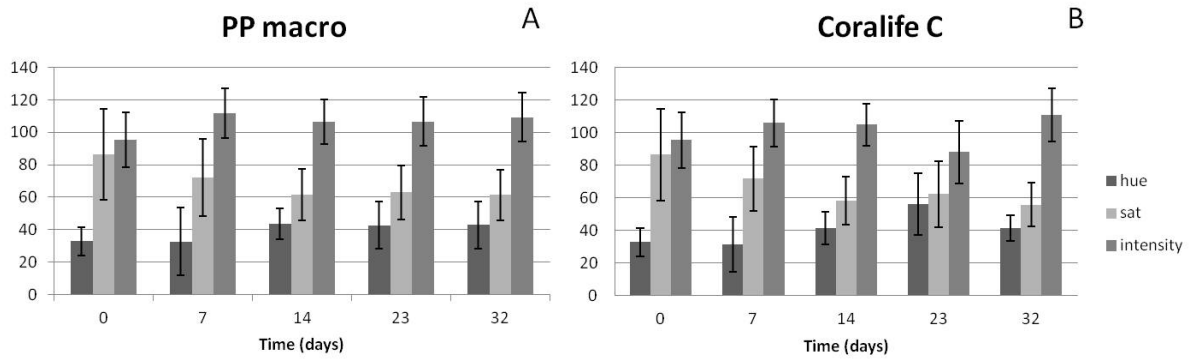

Figure 3. Hue, saturation and grey intensity level for samples packed in the macro-perforated PP and in the micro-perforated Coralife C film during 32 days of storage.

4. CONCLUSIONS

The shelf life of refrigerated green asparagus could be extended to 32 days with packaging in a micro-perforated film. In literature temperatures of 5°C were considered adequate for keeping the shelf life of asparagus about 11-14 days, however, the choice of a suitable packaging film should be addressed by any company aiming at extending the quality and the marketability of this valuable product.

ACKNOWLEDGEMENTS

We thank Asparago Sovrano, asparagus producer consortium (C.da Imbaccari Sovrano, Piazza Armerina, Enna, Italy) for the spear supply.

REFERENCES

Duan X., Liu S., Jia L., Zhang X., Sun L., Li Y., Fan Z. and Zhang Q. 2016. Effects of pre-cooling, low temperature transportation and different retailing methods on the quality of green asparagus. J. Food Eng. Technol. 5(2):15-19.

Fuentes-Alventosa J.M., Jaramillo-Carmona S., Rodriguez-Gutierrez G., Rodriguez-Arcos R., Fernandez-Bolanos J., Guillen-Bejarano R., Espejo-Calvo J.A. and Jimenez-Araujo A. 2009. Effect of the extraction method on phytochemical composition and antioxidant activity of high dietary fibre powders obtained from asparagus by-products. Food Chem. 116(2):484-490.

Grillo O., Rizzo V., Saccone R., Fallico B., Mazzaglia A., Venora G. and Muratore G. 2014. Use of image analysis to evaluate the shelf life of bakery product. Food Res. Int. 62:514-522.

Irving D.E. and Hurst P.L. 1993. Respiration, soluble carbohydrates and enzymes of carbohydrate metabolism in tips of harvested asparagus spears. Plant Sci. 94:89-97.

Limgroup 2017. Agricultural research. https://www.limgroup.eu/es/esp%c3%a1rragos/ Accessed 15/06/2017.

Kitazawa H., Motoki S., Maeda T., Ishikawa Y., Hamauzu Y., Matsushima K., Sakai H., Shiina T. and Kyutoku Y. 2011. Effects of Storage Temperature on the Postharvest Quality of Three Asparagus Cultivars Harvested in Spring. J. JPN. Soc. Hortic. Sci. 80(1):76-81.

Kohmura H., Watanabe Y. and Muto N. 2008. Polyphenol content, antioxidant activity and surface colour of asparagus spears cultivated under different conditions of sunlight. Acta Hortic. 776:255-260.

Rizzo V. and Muratore G. 2009. Effects of packaging on shelf life of fresh celery. J. Food Eng. 90:124-28.

Tzoumaki M.V., Biliaderis C.G., Vasilakakis M. 2009. Impact of edible coatings and packaging on quality of white asparagus (Asparagus officinalis, L.) during cold storage. Food Chem. 117: 55–63.

Wambrauw D.Z.K., Kashiwatani T., Komura A., Hasegawa H., Narita K., Oku S., Yamaguchi T., Honda K. and Maeda O. 2016. Effect of supplemental light on the quality of green asparagus spears in winter 'Fusekomi' forcing culture. Environ. Control Biol. 54 (3):147-152.

OXIDATIVE STABILITY OF HUMAN BREAST MILK DURING FREEZE-STORAGE

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ABSTRACT

Human breast milk is considered as the best nutrition for newborn infants. However, due to the diverse factors of mothers' modern lifestyles, breast milk is mostly pumped and stored longer periods up to one year in the freezer. With high amounts of polyunsaturated fatty acids, breast milk could undergo dramatic fat oxidation potentially resulting in the formation of rancid flavor which could led to the rejecting of many infants. In this study, we aimed to monitor the oxidative stability of breast milk during frozen storage (-18 °C) as affected by storage time, thawing temperature and types of container. Results from this study are beneficial in order to make a recommendation for mothers how to avoid the rancid flavor of breast milk as much possible. In our study, the hexanal content was measured by using a headspace solid-phase microextraction gas chromatographic method as an indicator of fat oxidation. Result showed that the hexanal contents were gradually increased from 340 ppb (3-month) to 400 ppb (4-month), 520 ppb (5-month) and 635 ppb (6-month) throughout the storage period. With three thawing temperatures (4, 30 and 50°C) of 3-month frozen milk, 50°C thawed milk samples gave the highest hexanal content (430 ppb). With three types of container of 4-month frozen milk, low density polyethylene bag (LDPE), clear glass bottle and amber glass bottle; the hexanal contents were 400, 385 and 300 ppb, respectively. Therefore, several recommendations could be made from our study to avoid rancidity of breast milk during storage: (1) breast milk should kept frozen as short a time period as possible, first-in first-out is highly recommended, (2) frozen breast milk should be thawed at 4°C, and (3) breast milk should be stored out of direct sunlight.

Keywords: container, frozen storage, human breast milk, oxidative stability, rancidity, storage time, thawing temperature

1. INTRODUCTION

Human breast milk is considered as the best nutrition for newborn infants. Breast milk contains many complex proteins, lipids and carbohydrates as well as many bioactive compounds, which could promote the growth, development and immune status of the infants. However, new mother today need to deal with modern lifestyle and the increasing working requirements, which greatly interfere with routine breastfeeding. Therefore, breastfeeding has not done directly, but rather breast milk is pumped and stored longer periods up to one year in the freezer. The long period of freeze-storage does not result in the formation of any potentially microbial or biochemical harming compounds. However, the flavor changes after 2 months storage in freezer has been reported (SPITZER *et al.*, 2010).

Fat is a crucial component of breast milk which provide energy and the optimum development of central nervous system. Unsaturated fatty acids dietary essentials are rich in breast milk (SPITZER and BUETTNER, 2010). During long period freeze storage, with high amounts of PUFA, breast milk could undergo dramatic fat oxidation caused rancid flavor along with the formation of potent odorants which could led to the rejecting of many infants. Numerous methods, such as the measurement of conjugated dienes, peroxide value and malondialdehyde formation, have been studied to measure lipid oxidation of human milk. However, these methods could lack specificity due to the interference with non-lipid components in the food matrix, or have variable reactivity that depends on method conditions. Quantification of hexanal thus has been recommended to access the lipid oxidation of human milk (ELISIA and KITTS, 2011). Hexanal is a secondary product of lipid oxidation, which was identified as the major volatile aldehyde generated from lipid peroxidation in human milk. Since hexanal is a sensitive and useful chemical indicator for assessing peroxidation reactions in human milk. Hexanal in human milk could be easily quantified using solid phase microextraction-gas chromatography/flame ionization detector (ELISIA and KITTS, 2011). In this study, we aimed to monitor the oxidative stability of breast milk during frozen storage as affected by storage time, thawing temperature and types of container.

2. MATERIALS AND METHODS

2.1. Human breast milk samples

Human breast milk was collected from five healthy mothers (age 25-40) who were in lactation period from 3 to 12 months postpartum by means of an electric breast pump. The milk samples from five mothers, which were a combination of foremilk and hind milk were combined into one pool samples. This sample was then divided into three aliquots to monitor the oxidative stability of breast milk during frozen storage as affected by storage time, thawing temperature and types of container. Milk samples were either immediately evaluated and analyzed, or subjected to the respective storage treatments detailed below.

(1) Storage time: fresh pumped breast milk was sub-divided into 5 aliquots (200 mL each). Each aliquot was kept in milk storage bag made from low-density polyethylene (LDPE). One being analyzed immediately (0-month or fresh milk control sample); the other four were stored in freezer at -18°C for 3, 4, 5 and 6 month. After that the sample was thawed at 25°C. Thawed milk of each sample was used for color (whiteness), pH and hexanal analyses.

(2) Thawing temperature: three samples of 3-month frozen breast milk kept in LDPE bags were thawed (in bag) by soaking in water at 3 different temperature conditions; 4°C, 25°C and 50°C. Thawed milk samples were used for hexanal analysis.
(3) Container: fresh pumped breast milk was sub-divided into 3 aliquots. Each aliquot was kept in LDPE bag, clear glass bottle and amber glass bottle in freezer (-18°C) for 4 months. After that the sample was thawed at 25°C. Thawed milk of each sample was used for hexanal analysis.

2.2. pH and whiteness analyses

The pH of the sample was measured by using pH meter. Whiteness of the sample was carried out by measuring lightness (L*), redness (a*) and yellowness (b*) using an UltraScan Pro spectrophotometer (HunterLab, VA, USA) with a D65/8° illumination source. The color measurements were performed at room temperature (25±3°C). Whiteness of each sample was calculated using the equation:

$$\text{whiteness} = 100 - ((100-L^*)^2 + a^{*2} + b^{*2})^{1/2}$$

2.3. Hexanal contents

HS-SPME (Head space-Solid Phase Microextraction) was used for hexanal extraction by using Carboxen™/polydimethylsiloxane (CAR/PDMS) StableFlex™ fiber (Supelco; Bellefonte, PA) 85 μm. The fiber was exposed to the headspace of the sample for 15 min at 45°C. Following adsorption, the fiber was immediately thermally desorbed in the Gas Chromatography (Agilent 1909 J-413) for 15 min at injector temperature of 250°C and was held for 2 min. Column HP-5 (5% Phenyl Methyl Siloxane, 30.0m x 320 μm x 0.25 μm) was used. The hexanal was quantitatively determined using standard curve of compound and the results were expressed as ppb of hexanal of sample.

2.4. Statistical analysis

A randomized block design (RCBD) was used in this study. All experiments were conducted in duplicate and the mean and SD were reported. Statistical analysis was performed using Duncan's multiple range test with significance decided at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Oxidative stability as affected by storage time

The hexanal contents gradually increased from 100 ppb to 340, 400, 520 and 635 ppb when storage period was increased from 0 month to 3, 4, 5 and 6 months, respectively (Fig. 1). According to NORTON (2003), the odor detection threshold of hexanal in milk was 340 ppb. Therefore, from our results, only fresh pumped breast milk and 3-month stored milk had hexanal contents lower than that of the odor threshold for hexanal. The increase of hexanal could be explained due to the lipolytic oxidation processes occurred by auto-oxidation processes and enzymatically catalyzed such as lipases or oxidation promoting enzymes. These reactions caused the formation of free fatty acids and other several oxidation products from polyunsaturated fatty acids which were related with fishy and

metallic odors resulting in the induce flavor changes with high sensory impact (SPITZER *et al.*, 2010). Therefore, long time storage of human breast milk more than 3 months could possibly lead to the rejection from babies.

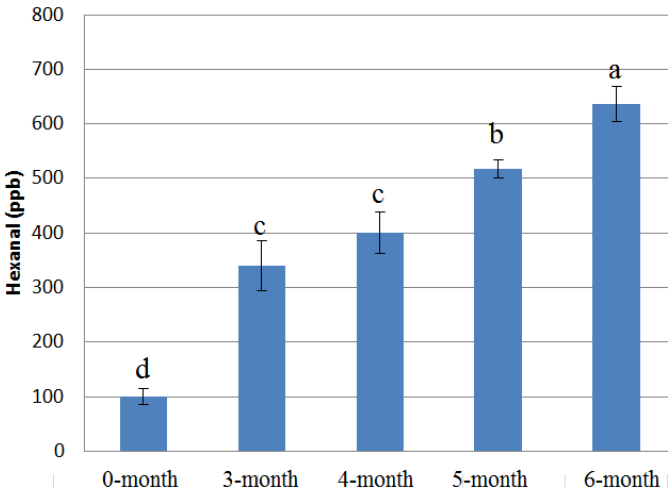

Figure 1. Hexanal contents of human breast milk that had been stored in freezer (-18°C) for 0-6 months.

During 0-6 months of storage, the pH and whiteness of milk samples were in the range between 6.66-6.70 and 78.0-78.5, respectively (Table 1). There was no significant different between the samples indicated that although the oxidation was occurred during storage, the storage period up to 6 month did not have affected on pH and whiteness of human breast milk samples.

Table 1. The pH and whiteness of human breast milk that had been stored in freezer (-18°C) for 0-6 months.

Storage (month)	pH	Whiteness
3	6.66 ± 0.01^{ns}	78.0 ± 0.06^{ns}
4	6.70 ± 0.01	78.1 ± 0.20
5	6.68 ± 0.02	78.2 ± 0.83
6	6.67 ± 0.06	78.5 ± 0.12

Each value is expressed as mean ± standard deviation (n=3).
^{ns}No significant differences ($P \leq 0.05$) between the samples within the column.

3.2. Oxidative stability as affected by thawing temperature

Hexanal contents in thawed milk samples at 4 and 25°C were 210 and 270 ppb, respectively (Fig. 2) which were lower than that of the odor threshold for hexanal. Increase thawing temperature to 50°C caused the dramatically increased of hexanal content (430 ppb). This could be due to 50°C is an optimum temperature of lipase resulting in the

increase in the rate of oxidation processes (RAY et al., 2013). Therefore, thawing at high temperature (50°C) is not recommended.

3.3. Oxidative stability as affected by storage containers

No significant difference of hexanal contents in breast milk stored in LDPE bag (400 ppb) and clear glass bottle (385 ppb). Storing milk in amber glass bottle provided, as expected, caused the lower of hexanal content (300 ppb) due to the protection of light-exposure (Fig. 3).

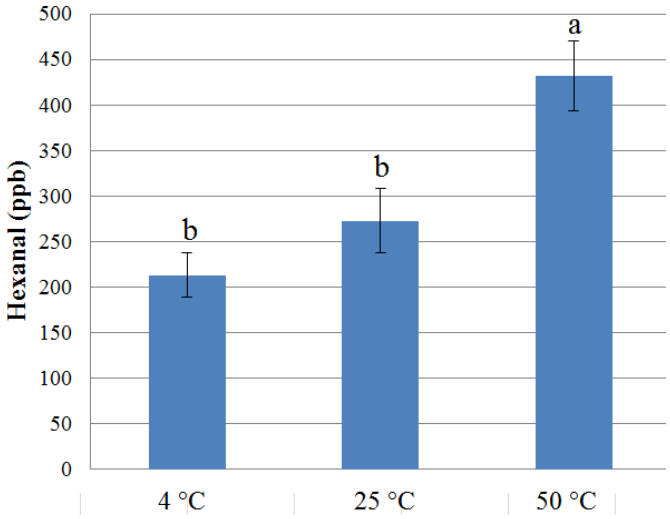

Figure 2. Hexanal contents of 3-month frozen human breast milk that was thawed at 4, 25 and 50 °C.

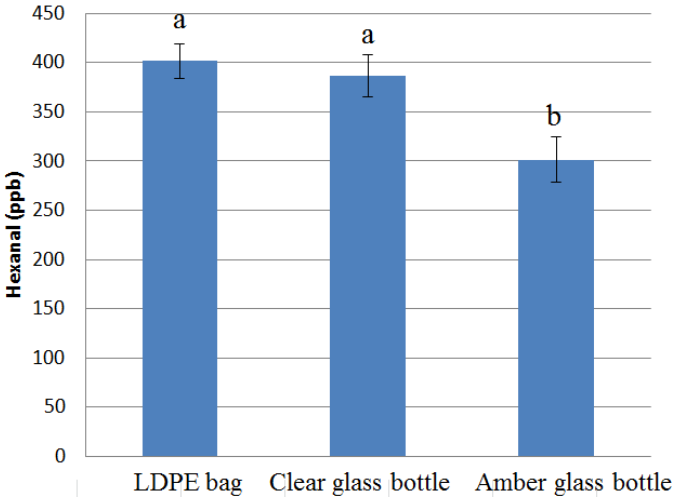

Figure 3. Hexanal contents of 4-month frozen human breast milk that was stored in LDPE bag, clear glass bottle and amber glass bottle.

4. CONCLUSIONS

According to our results presented, the several recommendations could be made for all mothers to reduce the formation of rancid flavor of stored milk. First, breast milk should be stored in freezer as short as possible, less than 3 months would be the best. Using "first in first out" system is highly recommended. Second, thawing frozen milk at refrigerated temperature (4°C) is the best. Thawing in warm water (>50°C) is not recommended. Third, store milk in amber container or avoid stored milk to expose light during freeze-storage is also highly recommended.

ACKNOWLEDGEMENTS

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REFERENCES

Elisia I., and Kitts D. 2011. Quantification of hexanal as an index of lipid oxidation in human milk and association with antioxidant components. J. Clin Biochem Nutr. 49:147-52.

Norton J. 2003. Efficacy of odor scavengers in reducing odor compounds in water, milk and soymilk. (Master 's Thesis). Virginia Polytechnic Institute and State University. Virginia, USA.

Ray P.R., Chatterjee K., Chakraborty C., and Chatak P.K. 2003. Lipolysis of milk: Review. Int. J. Agr. Sci Vet Med. 1: 8-74.

Spitzer K., and Buettner A. 2010. Characterization of aroma changes in human milk during storage at -19 °C. Food Chem. 120:240-6.

Spitzer J., Doucet S., and Buettner A. 2010. The influence of storage conditions on flavor changes in human milk. Food Qual. Prefer. 21:998-1007.

PREDICTION OF SECONDARY SHELF-LIFE OF CROISSANTS: SURVIVAL ANALYSIS AND CATA QUESTIONS METHODOLOGIES

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ABSTRACT

The secondary shelf life of croissants made by frozen dough was estimated by using the survival analysis methodology. Check-all-that-apply (CATA) questions were also performed in order to evaluate sensory perception of the product by consumers. Two formulations of frozen butter croissant (A, B) were stored at -18°C until experimental analysis. The samples were removed from the freezer and baked for 20 minute at 180°C. Samples were stored for 3, 7, 16, 20, 23 and 27 hours at 25°C and 65% of relative humidity (RH). Ninety-six subjects participated in the consumer test. They were asked to eat each sample, and answer the question:" Would you normally consume/buy this croissant? Yes or No?" and provide a sensory product characterization using check-all-that-apply (CATA) questions composed of 16 terms. Results showed that the survival analysis can be used to successfully determine the secondary shelf life of croissants. Weibull distribution was used to describe the rejection function. The shelf life was estimated as the storage time that corresponded to 50% consumers rejection in 22±2 h and 11±2 h, respectively for formulation A and B. Significant differences among samples were found in terms of both negative (*stale, hard, dry)* and positive *(soft, fresh)* attributes, and, as expected, frequency of use of the positive attributes decreased with storage time, whereas the contrary occurred for the negative ones.

Keywords: check-all-that-apply, croissant, secondary shelf life, survival analysis

1. INTRODUCTION

Secondary shelf life can be defined as the period of time after the opening of the packaging in which, in specific environmental condition, the food preserve a required level of acceptability for consumption (NICOLI, 2012). For croissants made from frozen dough, secondary shelf life can be defined as the period of time after the opening of the packaging and after baking in which it preserves a desired level of acceptability for consumption. Croissant is a popular bakery product usually eaten for breakfast in many parts of the world. As function of composition, the main mechanisms of alteration are related to moisture migration from product to environment and to fat oxidation. For not packed croissant, microbiological alterations did not represent a limiting factor for product shelf life. Thus, croissant shelf life depends mainly from sensory properties. Nevertheless, as reported by HOUGH *et al.* (2006), food products do not have sensory shelf lives of their own; rather they will depend on the interaction of the food with the consumer. For this reason consumers are the most appropriate tool for determining food product sensory shelf life. In order to estimate sensory shelf life based on consumer rejection of a food product, survival analysis could be applied. This methodology focuses the shelf life risk on the consumer's rejection of the product, estimating the product shelf life as the time necessary to reach a fixed percentage of consumer rejection.
The check-all-that-apply (CATA) method is a descriptive sensory method applying multiple choice questions in which assessors select from a list all the terms that they consider applicable to describe a set of products (GIACALONE *et a*l., 2013), without any explicit method constraint on the number of terms that can be selected (ARES *et a*l., 2013; FARAH *et a*l., 2017). Therefore, the objective of this study were: (i) to predict the secondary shelf life of croissants made by frozen dough by using the survival analysis methodology (ii) to evaluate sensory perception of the products by using the check-all-that-apply (CATA) questions (iii) to evaluate how different formulations affect product shelf life and sensory perception.

2. MATERIALS AND METHODS

2.1. Materials

Two formulations of frozen butter croissant (A, B) were obtained from the producer industry (Fresystem S.p.A). Samples B has the double quantity of fat respect of samples A.

2.2. Storage conditions

Samples were stored at -18°C until experimental analysis. The samples were removed from the freezer and baked for 20 minute at 180°C. Samples were stored for 3, 7, 16, 20, 23 and 27 hours at 25°C and 65% of relative humidity (RH). A reverse storage design has been applied: samples stored for 3, 7 and 27 hours or 16, 20 and 23 hours were analyzed at the same time.

2.3. Consumers acceptability test

A total of 96 consumers (croissant consumers, recruited from faculty, staff and students of University of Naples Federico II), 31% male and 69% female, ranging in age from 18 to 60 participated in this study. Informed consent was obtained from all participants. To assess the acceptability of the products, they were asked to eat each sample, answer the

question:" Would you normally consume/buy this croissant? Yes or No?". Chek-all-that-applay (CATA) questions were used to characterise the samples (ARES *et al.*, 2013). CATA questions of a list of terms were defined by a focus group with ten non-trained assessors (consumers). The final CATA list had 16 terms in total (fragrant, friable, sweet, sugar icing, soft, flavour of croissant, butter flavour, rancid flavour, stale, hard, dry, thick, browning, sticky, frothy, presence of sugar grain).

2.4. Data analysis

A full factorial design was used with a total of 1152 samples. Acceptability data were analyzed by using the survival analysis methodology (HOUGH *et al.*, 2006). The key concept of this methodology is to focus the shelf life hazard on the consumer rejecting the product. Defining a random variable T as the storage time at which the consumer rejects the sample, the survival function S(t) can be defined as the probability of a consumer accepting a product beyond time t, that is $S(t) = P(T > t)$. Alternatively, the cumulative distribution function F(t) can be defined as the probability of a consumer rejecting a product before time t, that is $F(t) = P(T \leq t)$. Because of the discrete nature of the storage times, T will never be observed exactly, hence the censored nature of the data (HOUGH *et al.*, 2006). Suppose that consumers are presented with samples stored at times a, b and c. If a consumer rejects the sample at the first storage time observed, then $T \leq a$, and the data is left-censored. If a consumer accepts the sample stored at time a, but rejects the sample stored at time b, then $a < T \leq b$, and the data is interval-censored. Finally, if a consumer accepts all samples, then $T > c$, and the data is right-censored. Data has been described by Weibull model at two parameters:

$$F(t) = 1 - exp\left(-\left(\frac{t}{\eta}\right)^{\beta}\right) \quad (1)$$

The model parameters estimation of the rejection function, F(t), is obtained maximizing the likelihood function of the censored data by using the software Weibull++ (Reliasoft). Shelf life was defined as the time to reach a reject function of 50% (NICOLI, 2012).
The CATA results were evaluated by the frequency that each attribute was selected for each sample and statistical differences ($p < 0.05$) were assessed by Cochran's Q test. Multiple factor analysis was applied to relate the two data sets (acceptance and CATA) (ARES *et al.*, 2013).

3. RESULTS AND DISCUSSION

Figure 1 shows the rejection function versus time of storage of the samples A and B. For both samples, Weibull distribution describe very well the experimental data (confidence level: 0.9). For sample A, β was 2.7 ($2.1 < \beta < 3.3$) and η was 25 h^{-1} ($24 < \eta < 28$), where as for samples B, b was 1.69 ($1.4 < \beta < 2$) and η was 14 h^{-1} ($10 < \eta < 17$).

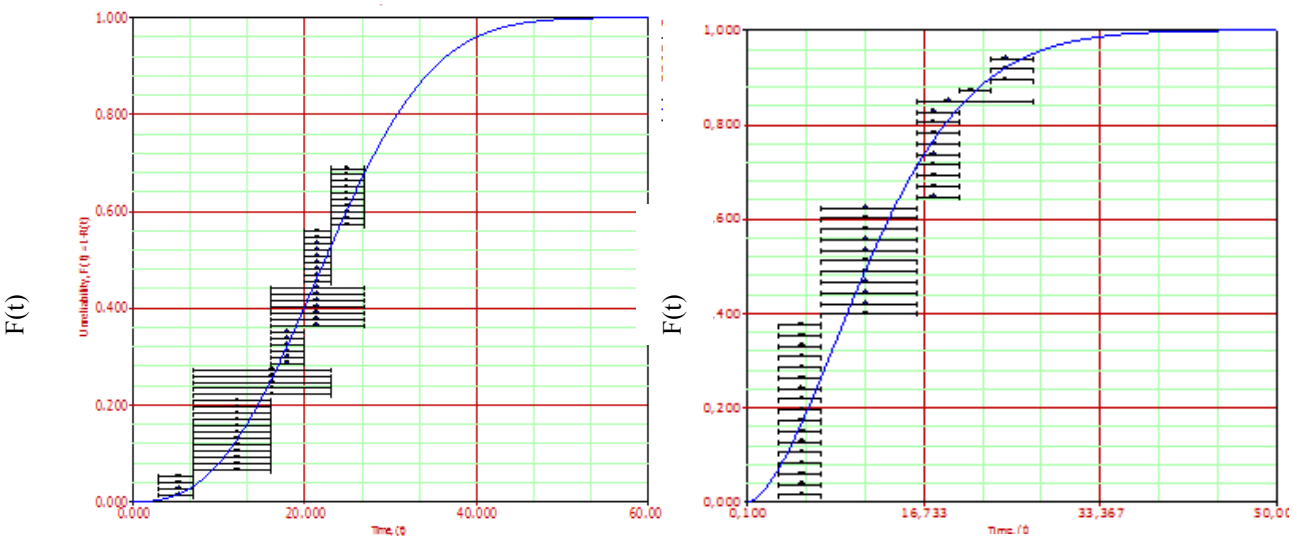

Figure 1. Estimation of consumers rejection function (F(t) versus storage time for sample A (left) and B (right).

For 50% of consumers rejection, the shelf life of samples A was 22±2 hours, whereas for samples B, the shelf life was 11±2 hours. Thus, results showed that the increase in fat content has a negative effect of product shelf life. Tables 1 and 2 present the frequencies that each attribute was indicated by consumers for sample A and B, respectively, at different storage times. Only significant attributes were reported. That was no statistical difference among samples ($p > 0.05$) for icing, typical croissant flavour, batter flavour, rancid flavour, browning for sample A and for friable, sticky, frothy and browning for sample B. Therefore, those attributes cannot be pointed out as responsible for acceptance or rejection of a particular sample.

Table 1. Cochran's Q test results for each attribute for sample A at different storage times.

Attributes/times	p-values	3 h	7h	16h	20h	23h	27h
Fragrant	0,0001	0,256 (b)	0,222 (b)	0,022 (a)	0,067 (ab)	0,044 (a)	0,033 (a)
Friable	0,0001	0,300 (b)	0,244 (b)	0,067 (a)	0,078 (a)	0,056 (a)	0,033 (a)
Sweet	0,034	0,400 (b)	0,322 (ab)	0,272 (ab)	0,315 (ab)	0,174 (a)	0,239 (a)
Soft	0,0001	0,456 (bc)	0,489 (c)	0,315 (ab)	0,315 (ab)	0,228 (a)	0,217 (a)
Rancid flavour	0,004	0,022 (a)	0,011 (a)	0,098 (a)	0,054 (a)	0,109 (a)	0,076 (a)
Stale	0,0001	0,011 (a)	0,044 (ab)	0,196 (b)	0,152 (ab)	0,239 (b)	0,185 (b)
Hard	0,0001	0,022 (a)	0,011 (a)	0,076 (ab)	0,130 (ab)	0,141 (b)	0,196 (b)
Dry	0,0001	0,056 (a)	0,044 (a)	0,239 (b)	0,250 (b)	0,272 (b)	0,348 (b)
Thick	0,0001	0,122 (a)	0,144 (a)	0,120 (a)	0,174 (ab)	0,152 (ab)	0,283 (b)

*Same letters in a row indicate there is no statistical difference among samples at 5% significance level according to Cochran's Q test.

Table 2. Cochran's Q test results for each attribute for sample B at different storage times.

Attributes	p-values	3h	7h	16h	20h	23h	27h
Fragrant	0.001	0,137 (b)	0,053 (ab)	0,084 (ab)	0,011 (a)	0,011 (a)	0,063 (ab)
Sweet	0.000	0,126 (b)	0,158 (b)	0,011 (a)	0,021 (a)	0 (a)	0,011 (a)
Sugar icing	0.003	0,021 (ab)	0,053 (b)	0 (a)	0 (a)	0 (a)	0 (a)
Soft	0.000	0,305 (b)	0,284 (b)	0,074 (a)	0,053 (a)	0,053 (a)	0,021 (a)
Typical croissant flavour	0.002	0,042 (ab)	0,084 (b)	0,042 (ab)	0 (a)	0,011 (a)	0 (a)
Butter flavour	0.001	0,558 (b)	0,537 (b)	0,453 (ab)	0,347 (a)	0,379 (ab)	0,347 (a)
Rancid flavour	0.000	0,263 (a)	0,305 (ab)	0,347 (abc)	0,526 (c)	0,432 (abc)	0,453 (bc)
Stale	0.000	0,074 (a)	0,221 (ab)	0,200 (ab)	0,432 (c)	0,305 (bc)	0,421 (c)
Hard	0.000	0,032 (a)	0,053 (a)	0,116 (ab)	0,232 (bc)	0,221 (bc)	0,316 (c)
Dry	0.000	0,137 (a)	0,179 (a)	0,389 (b)	0,495 (b)	0,516 (b)	0,484 (b)

*Same letters in a row indicate there is no statistical difference among samples at 5% significance level according to Cochran's Q test.

Acceptability of croissant were high related with the typical attributes of fresh croissant (soft, friable, fragrant, typical croissant flavor), whereas the attributes responsible of rejection were mainly rancid flavor, stale, hard, for both samples (Fig. 2).

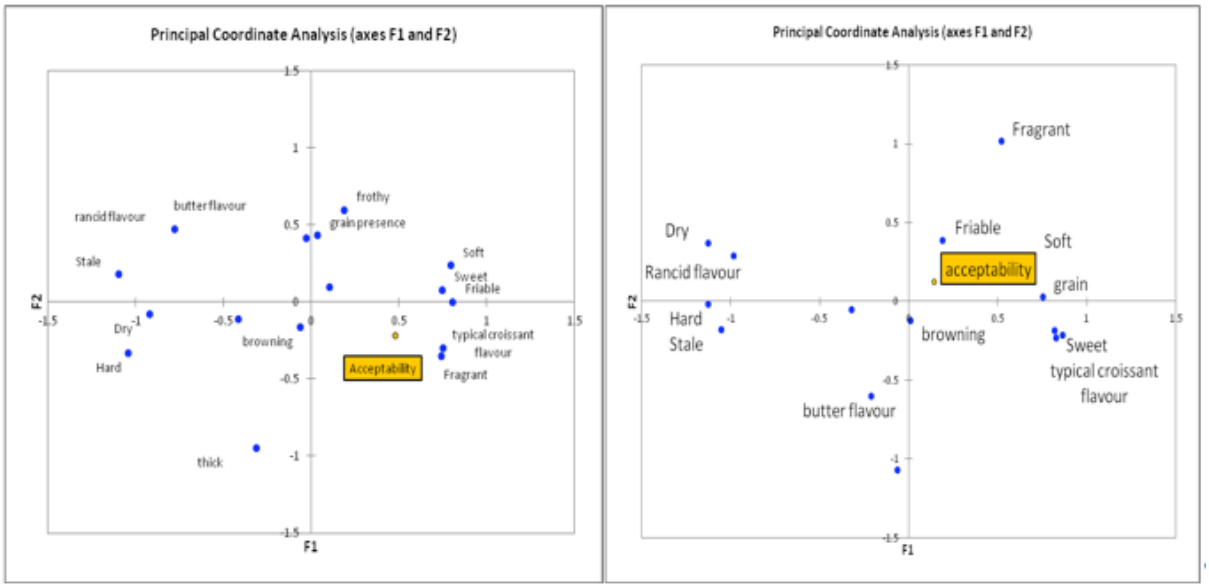

Figure 2. Multiple factor analysis associating data from CATA test and acceptance test of sample A (left) and samples B (right).

4. CONCLUSIONS

The shelf life was estimated as the storage time that corresponded to 50% consumers rejection in 22±2 h and 11±2 h, respectively for formulation A and B. The increment of butter content has a negative impact on product acceptability. Regarding CATA questions results, significant differences among samples were found in terms of both negative (*stale, hard, dry)* and positive *(soft, fresh)* attributes, and, as expected, frequency of use of the positive attributes decreased with storage time, whereas the contrary occurred for the negative ones.

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REFERENCES

Ares G., Jaeger S. R., Bava C. M., Chheang S. L., Jin D., Gimènez, A. *et al*. (2013). CATA questions for sensory product characterization-raising awareness of biases. Food Qual. Prefer., 30:114-127.

Farah J.S., Araujo C.B., Melo L. (2017). Analysis of yoghurts', whey-based beverages' and fermented milks' labels and differences on their sensory profiles and acceptance. Int. Dairy J., 68:17-22.

Giacalone D., Bredie W.L.P. and Frost M.B. (2013). All-in-one test (AI1): A rapid and easily applicable approach to consumer product testing. Food Qual. Prefer., 27, 108e119.

Hough G., Garitta L. and Gomez G. (2006). Sensory shelf-life predictions by survival analysis accelerated storage models. Food Qual. Prefer., 17:468-473.

Nicoli M.C. 2012. An introduction to food shelf life: definition, basic concept, and regulatory aspects. In: "Shelf Life Assessment of Food". M.C. Nicoli (Ed.), p 1-15. CRC Press, Boca Raton, FL.

EFFECT OF OHMIC HEATING ON TYROSOL AND ANTIOXIDANT ACTIVITY IN AROMATIC COCONUT WATER

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ABSTRACT

Tyrosol (4-hydroxyphenylethanol) is one of the most important simple phenols which have been used for medical purpose with antioxidant activity, immune stimulant and antibiotic properties. The aim of this study was to investigate the effects of ohmic heating on tyrosol content, total phenolic content (TPC), antioxidant activity and physicochemical quality of aromatic coconut water that was pasteurized using ohmic heating (100-300 V; 50 Hz, 74 °C for 15 s) and conventional heating (74°C for 15 s). Tyrosol content was determined using high performance liquid chromatography (HPLC) coupled with diode array detector. TPC and antioxidant activity were evaluated using Folin-Ciocalteu assay and using 2, 2-diphenly-1-picrylhydrazyl (DPPH) radical scavenging assay, respectively. When compared to the fresh sample, aromatic coconut water pasteurized using ohmic heating could retain 97% of tyrosol, while those pasteurized using conventional heating could retain only 90%. The results also showed that TPC of aromatic coconut water pasteurized using ohmic heating was not significantly different from that of fresh aromatic coconut water ($p>0.05$). There were no significant differences in antioxidant activity, total soluble solid and pH values among all samples ($p>0.05$), while higher turbidity values were detected in both pasteurized samples. In conclusion, aromatic coconut water pasteurized by ohmic heating had higher tyrosol retention than conventional heating. Therefore, ohmic heating could possibly used as an alternative technology for preserving quality and nutrients of aromatic coconut water.

Keywords: antioxidant activity, coconut water, ohmic heating, phenolic content, tyrosol

1. INTRODUCTION

Tyrosol is phenyl ethanol derivative which is characterized as simple phenol. It has been used for medical purpose due to its antioxidant activities and acts as protective agent in countering sensory motor dysfunction in the brain (BU *et al.*, 2007). Tyrosol has been mostly founded in olive oil (KLEN *et al.*, 2014). Aromatic coconut water is natural beverage that has high demand in a global market due to its high nutritional value. It contains several phytochemicals, especially phytohormones (YONG *et al.*, 2009) and phenolic compounds (CHANG and WU, 2011). Commercial coconut water products are usually processed using conventional thermal processing. However, the external heat transfer in conventional heating lead to over processed on food product (KAMONPATANA, 2012) which may cause destruction of nutritive compounds (VALENCIA-FLORES *et al.*, 2013). To overcome heat transfer problem in conventional heating, ohmic heating present as an innovative food processing method in which food can be heated by the passage of an electrical current through the food material. With ohmic heating process, aromatic coconut water is rapidly heated therefore phytochemicals could be possibly retained better. The objectives of this study were to investigate the effects of ohmic heating on tyrosol content, total phenolic content, antioxidant activity and physicochemical quality of aromatic coconut water.

2. MATERIALS AND METHODS

Thermal pasteurization treatment was applied to the aromatic coconut water (maturity at 5-6 months) that was packed in polyvinylidene chloride (PVDC) plastic pouch. In ohmic heating, devices of experiment included of ohmic chamber, data logger, multimeter, thermocouple, and stainless-steel electrodes (The distance between electrodes was 30 cm). 12 L of sodium sulphate solution, as carrier medium of the current, was filled to ohmic chamber. The sample pouches were placed in the rack inside ohmic chamber. The ohmic heating set-up for the experiment was set following the schematic diagram as shown in Fig. 1.

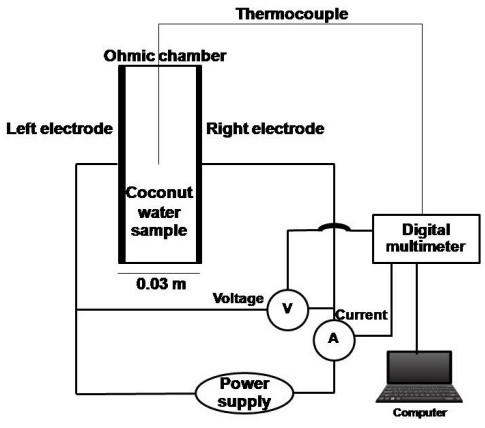

Figure 1. Schematic diagram of the ohmic heating circuit.

The sample pouches were heated in thermal pasteurization temperature (74°C for 15 s) according to DAMAR *et al.* (2009). The voltage used was in the range of 100 to 300 volt and the data was recorded by data longer (Yokogawa Electric Corporation, Japan). While in

the conventional heating, the sample pouches were heated to 74°C for 15 s in 12 L of boiling water. After thermal treatment, sample was kept at -20°C prior to analysis. Tyrosol was analyzed using HPLC-DAD (POTHINUCH and TONGCHITPAKDEE, 2011). Total phenolic content (TPC) was measured using Folin-Ciocalteu assay and expressed on gallic acid equivalent (KIM *et al.*, 2002). Retention of tyrosol and TPC of sample were calculated as percentage when compared to control sample (100%). Antioxidant activity was measured using DPPH radical scavenging activity assay and expressed in trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent (AOAC, 2000). The pH, total soluble solid (TSS), and turbidity were determined according to AOAC (2000). The data were subjected to analysis of variance (ANOVA) using SPSS. The comparison of means was carried out by Fisher's Least Significant Difference (LSD) (p-value<0.05). The measurements were performed in two replications.

3. RESULTS AND DISCUSSIONS

3.1. Tyrosol identification in aromatic coconut water

Tyrosol in ohmic heating and conventional heating sample was marked with an arrow at 15.4 min and was detected in the maximum wavelength of 275.8 nm (Fig. 2).

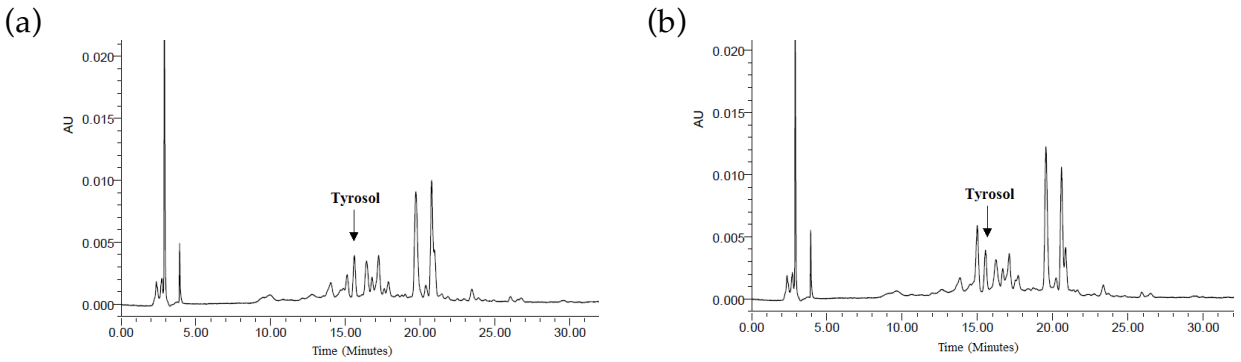

Figure 2. HPLC chromatogram of aromatic coconut water under ohmic heating (a) and conventional heating (b) at 275.8 nm of maximum wavelength.

Tyrosol identification was confirmed by spiking with internal standard and comparison of retention time and UV-spectrum with the authentic external standard. That identification method was in agreement with those reported by previous literatures (KLEN, 2014; OBIED *et al.*, 2007).

3.2. Effect of ohmic heating on tyrosol, total phenolic content (TPC), and antioxidant activity

Conventional heating resulted in 10% and 7% losses of tyrosol and TPC, respectively, while ohmic heating only resulted in 3% losses for both tyrosol and TPC (Table 1). However, statistical analysis showed there were no significant differences in the tyrosol and TPC retentions between ohmic heating samples when compared to the control and conventional heating samples (p>0.05). Meanwhile, conventional heating resulted in significantly higher losses of tyrosol and TPC content when compared to control (p<0.05).

The rapid heating process during ohmic heating significantly reduced exposure time of sample to heat and may allow better retention of heat sensitive phytochemicals (MERCALI *et al.*, 2013).

Table 1. Retention of tyrosol and total phenolic content of aromatic coconut water under ohmic and conventional heating pasteurization.

Sample	Tyrosol and TPC retention (%)	
	Tyrosol	Total phenolic content
Control	100±0.0[a]	100±0.0[a]
Ohmic heating	97±1.4[ab]	97±0.8[ab]
Conventional heating	90±0.5[b]	93±2.5[b]

Value are expressed as means ± standard deviation (SD), n=2. The different letters in column indicate statistically significant difference at $p < 0.05$

Although there were some losses of tyrosol and TPC in sample after ohmic heating and conventional heating process, there were no significant differences on antioxidant activity in the sample ($p>0.05$) (Table 2). This may be because heating process could improve aroma or volatile compound in aromatic coconut water which composed of aromatic ring that contribute antioxidant activity (PRADES *et al.*, 2012 and JIRAPONG *et al.*, 2012).

3.4. Effect of ohmic heating on pH, total soluble solid (TSS), and turbidity

The result showed there were no significant differences on pH and TSS values among the samples ($p>0.05$) (Table 2). The result also showed that pasteurization using both methods slightly increased turbidity of coconut water ($p<0.05$) (Table 2). Many factors have been reported to have effects on turbidity of coconut water, including oil content, protein, mineral content, and insoluble material (TANQUECO *et al.*, 2007; VAILLANT *et al.*, 2008).

Table 2. Scavenging activity, pH, TSS, and turbidity values of aromatic coconut water under ohmic and conventional heating pasteurization.

Parameter	Control	Ohmic heating	Conventional heating
DDPH (mg/100 g trolox equivalents)	16.71±0.78 [a]	16.71±0.08 [a]	16.69±1.02 [a]
DPPH (% inhibition)	20.17±1.00 [a]	20.17±1.00 [a]	20.10±1.00 [a]
pH	5.13±0.01[a]	5.13±0.01[a]	5.15±0.00[a]
TSS (^{0}Brix)	7.00±0.00[a]	7.00±0.00[a]	7.00±0.00[a]
Turbidity (NTU)	60.47±0.01[a]	63.60±0.01[b]	63.30±0.01[b]

Value are expressed as means ± standard deviation (SD), n=2. The different letters in column indicate statistically significant difference at $p < 0.05$.

4. CONCLUSIONS

Ohmic heating show potential benefits in retaining tyrosol and TPC in aromatic coconut water. However, further study of its effects on other phytochemicals and volatile compounds is needed to confirm the benefit of this technology.

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REFERENCES

AOAC. 2000. Official Methods of Analysis of AOAC International, 17th ed.; AOAC International: Rockville, MD.

Bu Y., Rho S., Kim J., Lee D.H., Kim S.Y., Choi H. and Hocheol K. 2007. Neuroprotective effect of tyrosol on transient focal cerebral ischemia in rats. Neuroscience Letters. 414: 218-221.

Chang C.L. and Wu R.-T. 2011. Quantification of (+)-Catechin and (-)-Epicatechin in Coconut Water by LC-MS. Food Chemistry. 126: 710-717.

Damar S. Balaban M.O. and Sims C.A. 2009. Continuous dense-phase CO_2 processing of a coconut water beverage. International Journal of Food Science and Technology.10: 1-8.

Jirapong A. Uthairatanakij S. Noichinda S. Kanlayanarat and C. Wongs-Aree. 2012. Comparison of volatile compounds between fresh and heat-processed aromatic coconut. Asia Pacific Symposium on Postharvest Research, Education and Extension. Thailand.

Kamonpatana P. 2012. Mathematical modeling and microbiological verification of ohmic heating of solid-liquid mixtures in continuous flow ohmic heater system. Ph. D. Dissertation, The Ohio State University, Athens, Ohio.

Kim D., Lee K.W. Lee H. J. and chang Y.L. 2002. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. Journal of Agricultural and Food Chemistry. 50: 3713-3717.

Klen T.J. 2014. Olive fruit phenols in olive oil processing: the fate and antioxidant potential. Ph. D. Dissertation, University of Nova Gorcia, Nova Grocia.

Mercali G.D., Jaeschke D.P., Tessaro I.C. and Ligia D.F.M. 2013. Degradation kinetics of anthocyanins in acerola pulp: Comparison between ohmic and conventional heat treatment. Food Chemistry. 136: 853-857.

Obied H.K., Bedgood D.R. Jr., Prenzler, P.D. and Kevin R. 2007. Chemical screening of olive biophenol extracts by hyphenated liquid chromatography. Analytica Chimica Acta. 603: 176-189.

Pothinuch P. and Tongchitpakdee S. 2011. Melatonin contents in mulberry (Morus spp.) leaves: Effects of sample preparation, cultivar, leaf age and tea processing. Food Chemistry 128: 415-419.

Prades A., Picard-palmade L., Dornier M. and Pain. 2012. Impact of ohmic heating on coconut water volatile compounds. The European Federation of Food Science & Technology Annual Meeting. 20-23 November. Montpellier, France.

Vaillant F., Pérez A.M., Acosta O. and Dornier M. 2008. Turbidity of pulpy fruit juice: A key factor for predicting cross-flow microfiltration performance. Journal of Membrane Science. 325(1): 404-412.

Tanqueco R.E., Rodriguez F.M., Laude R.P. and Cueno M.E. 2007. Total free sugars, oil and total phenolics content of stored coconut (*Cocos nucifera* L.) water. Philippine Journal of Science, 136, 103-108.

Valencia-flores D.C., Hernandez M., Guamis B. and Victoria F. 2013. Comparing the effects of ultra-high-pressure homogenization and conventional thermal treatments on the microbiological, physical, and chemical quality of almond beverages. Journal of Food Science.78 (2): 199-205.

Yong J.W.H., Ge L., Fei Y. and Tan. S.N. 2009. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules. 14: 5145-5164.

SESSION III

Packaging Technology and Shelf-life

SHELF-LIFE OF 2-ACETYLPYRAZINE- AND VANILLIN-RELEASING FILMS PREPARED BY VACUUM IMPREGNATION

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ABSTRACT

Aroma is an important attribute of food. However, food processing and storage generally reduce aroma intensity. Therefore, active packaging has been performed in order to preserve, replace or increase food aroma. Vanillin (vanilla-like) and 2-acetylpyrazine (pandan-like) are among the most popular aroma compounds used in food and beverage, especially ice cream, confectionary and bakery products. The objective of this study was to investigate quality changes during storage of 2-acetylpyrazine- (AF) and vanillin-releasing polypropylene films (VF) prepared by vacuum impregnation. The data collected from gas chromatography-flame ionization detector (GC-FID) showed that aroma content of AF was significantly lower than VF ($p \leq 0.05$). Both films were kept at 35, 45 and 55 ˚C and the film qualities were evaluated after 0, 5, 10, 15 and 20 days of storage. The results revealed that the concentrations of aroma in AF and VF decreased over time, especially at high storage temperature. Reduction of 2-acetylpyrazine followed second order kinetics, whereas reduction of vanillin followed first order kinetics. Aroma detection threshold was used as a critical limit to estimate the shelf-life. Aroma retention of AF was 27, 8 and 2 days, while those of VF were 98, 18 and 16 days at storage temperature of 35, 45 and 55˚C, respectively. The activation energy (E_a) of 2-acetylpyrazine reduction calculated from Arrhenius equation was 132 kJ/mol and E_a of vanillin reduction was 65 kJ/mol. Decrease of aroma concentrations after storage were in agreement with the sensory results. Storage temperature and time also resulted in significant changes in film color ($p \leq 0.05$).

Keywords: 2-acetylpyrazine, active packaging, food aroma, vacuum impregnation, vanillin

1. INTRODUCTION

Food aroma is one of the sensory impressions, which affects consumer acceptance. However, its intensity is generally reduced during food processing and storage. Thus, aroma-releasing film (ARF) has been developed (DIAS *et al.*, 2013) and used as an active packaging in order to preserve, replace or increase food aroma. Vanillin (vanilla-like), the major aroma active compound in vanilla pods (WALTON *et al.*, 2003), is widely used in desserts and beverages. Pandan leaf is also one of the most popular flavoring used in Asian cuisine. 2-Acetylpyrazine, which has pandan note (BUTTERY *et al.*, 1997; WRIGHT, 2010) is generally used by food industry because of commercial availability and high stability. The objective of this study was to evaluate quality changes during storage of 2-acetylpyrazine- (AF) and vanillin-releasing polypropylene films (VF) prepared by vacuum impregnation (VI). Kinetics of aroma release from AF and VF were also investigated. In addition, the application of estimated shelf-life of ARF in food product was demonstrated in this study.

2. MATERIALS AND METHODS

2.1. Preparation of aroma releasing film

VI procedure was adapted from LEE and BOONSUPTHIP (2014) to prepare AF and VF. 2-Acetylpyrazine or vanillin (World Perfume Thailand Co., Ltd., Thailand) was dissolved in ethanol (RCI Lab Scan, Thailand). Ten milligrams of aroma solution (2% w/w) were sprayed on 1 g of polypropylene (PP) film (Highland Industry Co., Ltd., Thailand). The sample was immediately placed in a 3-L hermetically sealed chamber and the vacuum was rapidly established to 74.66 kPa in 30 s and held for 1 min. It was then released to atmospheric pressure within 1 min. The film was washed with ethanol and kept in tightly closed glass jar prior to further analysis within 6 h.

2.2. Shelf-life of aroma releasing film

ARF was stored at 35, 45 and 55 ˚C and the aroma content was determined after 0, 5, 10, 15 and 20 days of storage. One gram of ARF was extracted by 10 mL of diethyl ether in ultrasonic bath (Crest, US) for 30 min and *tert*-butylbenzene was used as internal standard. The extract was dried over 1 g of anhydrous Na_2SO_4 and concentrated to 1 mL by gentle N_2 stream. The analysis was performed by gas chromatography-flame ionization detector (6890plus; Agilent Technologies, US) equipped with an Rxi®-5ms column, according to the method of KABIR and LORJAROENPHON (2014).

Sensory analysis and color of 20-day stored ARF in comparison with initial ARF were evaluated as well. One gram of ARF was prepared in Teflon™ sniffing bottles (Nalgene™, US) marked with 3-digit code. The samples were served in random to 30 untrained panelists (10 males and 20 females, aged between 20 to 30 years old) in individual booth. The panelists were asked to rate overall aroma intensity of the sample using category scale (0 = none to 8 = extreme). Film color was measured by Ultra Scan Pro (Hunter Lab, US).

All experiments were performed in triplicate. Analysis of variance (ANOVA) was carried out in SPSS version 12.0 (IBM, US) and the sample means were separated by Duncan's multiple range test at a confidence level of 95%.

3. RESULTS AND DISCUSSION

Various techniques, such as blending (DIAS *et al.*, 2013) and high-pressure impregnation (GONI *et al.*, 2016), have been used to prepare active packaging. However, there has been no published work on ARF prepared by VI. VI is a novel technique, which is used for rapid penetration of solvated compounds into a supporter (ATARES *et al.*, 2008; NERI *et al.*, 2016). VI is simple, rapid and cost-efficient application. In this study, VI was achieved to penetrate vanillin and 2-acetylpyrazine into PP film. VF had higher aroma content than AF ($p \leq 0.05$) (Fig. 1) due to the compatibility between aroma compound and film. PP is non-polar polymer. Thus, lower polarity of vanillin compared to 2-acetylpyrazine contributed to greater solubility in PP film. The polarity of compound varies inversely with log P and the log P values of vanillin and 2-acetylpyrazine are 1.20 and 0.20, respectively (NATIONAL CENTER FOR BIOTECHNOLOGY, 2004).
The aroma contents in ARF decreased during storage at all storage temperatures (Fig. 1). The aroma reduction rate of VF was lower than those of AF. Vanillin was gradually released from VF at 35˚C of storage temperature. In contrast, 2-acetylpyrazine in AF decreased rapidly in just a short period of storage time and it remained constant after that. The lower volatility of vanillin compared to 2-acetylpyrazine might be the reason. Volatility is affected by several factors, such as vapor pressure and molecular weight. The vapor pressures of vanillin (Mw = 194) and 2-acetylpyrazine (Mw = 122) are < 0.01 and 0.188 mmHg at 25˚C, respectively (NATIONAL CENTER FOR BIOTECHNOLOGY, 2004).

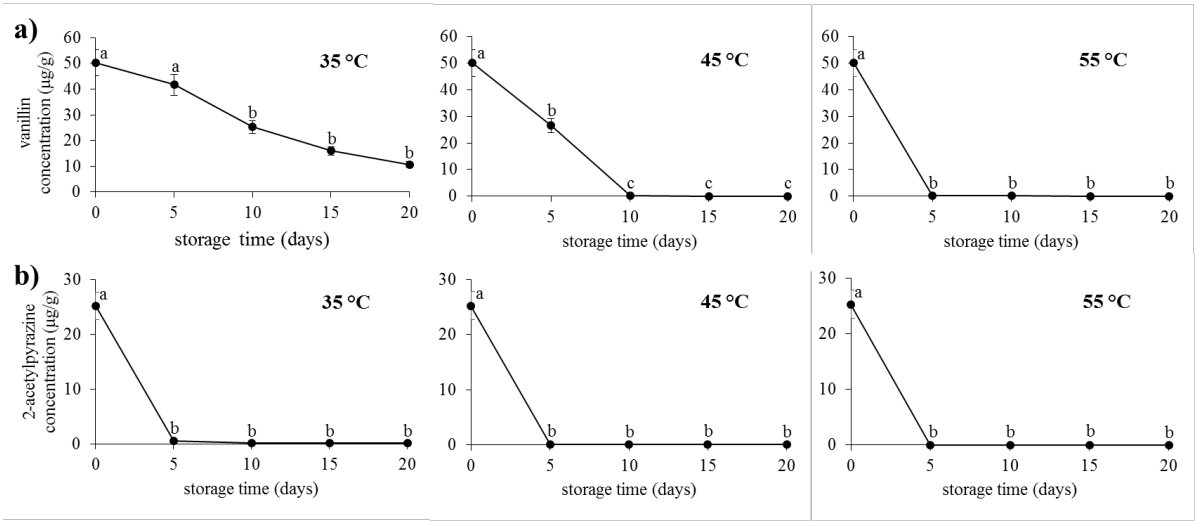

Figure 1. Reduction of aroma concentrations in a) vanillin- (VF) and b) 2-acetylpyrazine-releasing polypropylene films (AF) during storage at different temperatures. Different letters indicate significant differences ($p \leq 0.05$).

The results of chemical analysis were in agreement with sensory analysis. The aroma intensities of 20-day ARFs stored at different temperatures were lower than those of initial ARF sample ($p \leq 0.05$) (Table 1). The color changes of stored ARF were also investigated. Vanillin is a white crystal, while 2-acetlypyrazine is a white-to-pale yellow crystal. These resulted in slightly cloudy appearance of the initial ARF. The L* values of stored ARFs were higher than initial film ($p \leq 0.05$) (Table 1) due to release of aroma compound over storage time. Even though the L* values of ARF stored at studied temperatures were significantly different, they were similar in color appearance.

Table 1. Aroma perception and color of vanillin-(VF) and 2-acetylpyrazine-releasing polypropylene films (AF) after storage at different temperatures.

Sample	Aroma intensity (n=30)		L*	
	VF	AF	VF	AF
0-day film (initial sample)	5.93±1.51a	6.60±1.77a	96.92±0.01d	96.92±0.01d
20-day film stored at 35 ˚C	4.67±1.81a	4.30±1.84b	97.27±0.06a	97.30±0.06a
20-day film stored at 45 ˚C	1.73±1.82b	0.67±1.40c	97.21±0.06b	97.22±0.06b
20-day film stored at 55 ˚C	0.93±1.60b	0.13±0.90c	97.11±0.06c	97.12±0.06c

Means ± standard deviations followed by different letters within a column are significantly different ($p \leq 0.05$).

Among chemical, physical and sensory properties of ARF, reduction of aroma compound was selected for kinetics study. Release of vanillin followed first order kinetics, while reduction rate of 2-acetylpyrazine was categorized as second order kinetics. As the storage temperature was increased, it was observed an increase in rate constant (k) of aroma reduction in both VF and AF (Table 2). These k values as a function of temperature were shown in Fig. 2. The activation energy (E_a), calculated from Arrhenius equation, of aroma reduction from VF and AF were 65 and 132 kJ/mol, respectively. Moreover, Q_{10}, which is useful to determine the reduction rate of aroma from ARF stored at 35˚C can be calculated. The Q_{10} number of VF was 5.44, whereas the Q_{10} number of AF was 3.38.

Table 2. Rate constants (k) and correlation coefficient (r^2) of aroma reduction and estimated shelf-life of vanillin- (VF) and 2-acetylpyrazine-releasing polypropylene films (AF) stored at different temperatures.

Storage temperature	VF (first order kinetics)			AF (second order kinetics)		
	k (min^{-1})	r^2	shelf-life (days)	k (min^{-1})	r^2	shelf-life (days)
35˚C	0.081	0.984	98	0.563	0.795	27
45˚C	0.435	0.897	18	1.640	0.934	8
55˚C	0.368	0.775	16	13.332	0.730	2

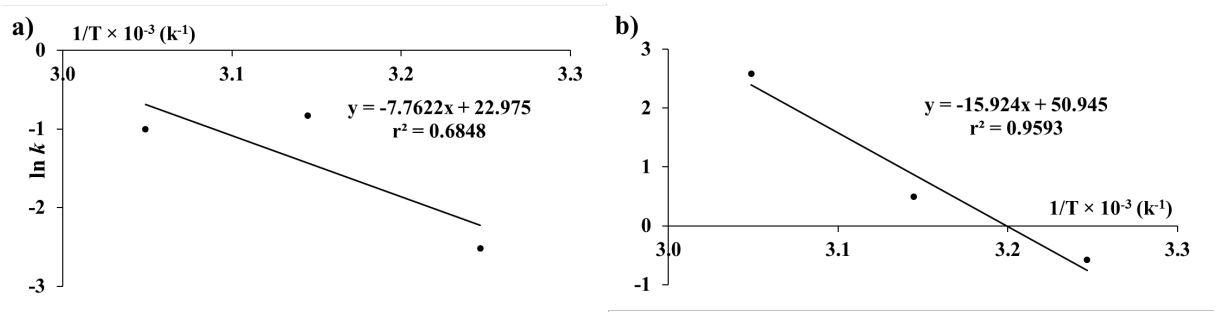

Figure 2. Rate constant (k) of aroma reduction from a) vanillin-(VF) and b) 2-acetylpyrazine-releasing polypropylene films (AF) as a function of temperature.

The odor threshold was used as a critical limit to determine the end of ARF shelf-life. The odor detection thresholds of vanillin and 2-acetylpyrazine in water are 0.025 $\mu g/g$ (SCHUH and SCHIEBERLE, 2006) and 0.062 $\mu g/g$ (BUTTERY, 1999), respectively. The aroma retention of VF was longer than those of AF at all storage temperatures (Table 2). Higher aroma concentration in initial VF and lower volatility of vanillin were the reasons. Among different storage temperatures, 35˚C could be focused since it was reported as the average daily temperature in Thailand (THAI METEOROLOGICAL DEPARTMENT, 2017). Although shelf-life of AF at 35˚C was about three times shorter than VF, AF could be applied as a food packaging material to maintain the aroma quality of some foods over storage life. For example, SMITH *et al.* (2004) reported that the shelf-life of baked goods was less than 30 days.

4. CONCLUSIONS

Aroma-releasing film is successfully prepared by vacuum impregnation technique. Both 2-acetylpyrazine- and vanillin-impregnated films are appropriate to use as food packaging materials. However, vanillin shows higher penetration efficiency into PP film and slower release over storage time compared to 2-acetylpyrazine.

REFERENCES

Atares L., Chiralt A., and Gonzalez-Martínez C. 2008. Effect of solute on osmotic dehydration and rehydration of vacuum impregnated apple cylinders (cv. Granny Smith). J. Food Eng. 89:49-56.

Buttery R.G. 1999. Flavor chemistry and odor thresholds. In: R. Teranishi, L. Wick and I. Hornstein (Eds.), p 353-365. Flavor chemistry: Thirty years of progress, Kluwer Academic/Plenum Publishers, New York.

Buttery R.G., Ling L.C., and Stern D.J. 1997. Studies on popcorn aroma and flavor volatiles. J. Agric. Food Chem. 45:837-843.

Dias M.V., Medeiros H.S., Soares N.F.F., Melo N.R., Borges S.V., Carneiro J.D.S. and Pereire J.M.T.A.K. 2013. Development of low-density polyethylene films with lemon aroma. LWT - Food Sci. Technol. 50(1):167-171.

Goni M.L., Ganán N.A., Strumia M.C. and Martini R.E. 2016. Eugenol-loaded LLDPE films with antioxidant activity by supercritical carbon dioxide impregnation. J. Supercrit. Fluids 111:28-35.

Kabir A. and Lorjaroenphon Y. 2014. Identification of aroma compounds in coconut sugar, proceedings of 52nd Kasetsart University Annual Conference: Agro-Industry, p. 239-246, Kasetsart University, Thailand, February 4-7.

Lee S.J. and Boonsupthip W. 2014. Physical, chemical, and sensory properties of antioxidant-enriched raw and cooked rice by vacuum-drying impregnation in a semidry state. Cereal Chem. 91(5):445-452.

National center for biotechnology information. PubChem Compound Database; CID=1183, 30914, https://pubchem.ncbi.nlm.nih.gov/compound (accessed July 1, 2017).

Neri L., Biase L.D., Sacchetti G., Mattia C.D., Santarelli V., Mastrocola D. and Pittia P. 2016. Use of vacuum impregnation for the production of high quality fresh-like apple products. J. Food Eng. 179:98-108.

Schuh C. and Schieberle P. 2006. Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: quantitative differences between tea leaves and infusion. J. Agric. Food Chem. 54:916-924.

Smith J.P., Daifas D.P., El-Khoury W., Koukoutsis J. and El-khoury A. 2004. Shelf life and safety concerns of bakery products-A review. Crit. Rev. Food Sci. Nutr. 44:19-55.

Thai meteorological department. https://www.tmd.go.th/climate/climate.php?FileID=6 (accessed July 1, 2017).

Walton N.J., Mayer M.J. and Narbad A. 2003. Vanillin. Phytochemistry 63(5):505-515.

Wright J. 2010. Flavor bites: 2-acetylpyrazine. Perfumer & Flavorist 35:32-33.

MEASURES TO PREVENT STRESS CORROSION CRACKING OF ALUMINUM CANS FOR CARBONATED BEVERAGES

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ABSTRACT

Common containers for carbonated beverages are PET bottles and aluminum cans. These different materials are chosen based on marketing and convenience reasons. While the shelf life of carbonated beverages packed in PET bottles is affected by the CO_2 permeation through the polymer matrix, aluminum cans allow the gas retention and guarantee a higher quality during storage. Indeed, the shelf life of canned carbonated beverages is commonly ≥1 year. While the longer commercial life is an advantage from a commercial point of view, it increases the probability of occurrence of failure due to corrosion. Stress Corrosion Cracking (SCC) occurring from the outside of the can would prejudice the integrity of the container, provoke the product loss and, what is probably worst, could determine a chain-effect on other adjacent cans if the product is spilled from the damaged can. Normally, aluminum cans are internally lacquered for protecting the metal from corrosion by the food components. Chlorine from the conditioning water bath and storage environmental moisture determines the onset of SSC, while temperature and internal gas pressure speed up the failure of cans. This study aimed at evaluating the potential of a supplementary outer enamel for preventing stress corrosion cracking, and proved that this measure, which does not imply significant process changes or supplementary costs, could effectively reduce the risk of product loss, with consequent need for damaged products withdraw and disposal, and prejudice for the image of the producer.

Keywords: aluminum can failure, chlorine, coating, gas pressure, prevention of product loss

1. INTRODUCTION

Almost all metal containers for food application are coated with synthetic layers on the side aimed to be in contact with food, in order to minimize migration of the metal to the food and to protect the metal from corrosion by the food components (LEE *et al.*, 2008). Indeed, foods such as vegetables, fruit juices, beer and soft drinks are widely packed in metal cans provided with an internal enamel. Two-piece aluminum cans, which are widely employed for soft drinks, consist of a wall-ironed body integrated with the bottom, and a lid, including the opening, which is double-seamed to the body. Due to the changes to the aluminum foil occurring during processing, cans are internally coated after reaching their final shape.
The shelf life of carbonated beverages mainly depends on the CO_2 retention performances, however the commercial life of such products can be compromised by ruptures and crackings of the material, which can occur in certain conditions. Similarly to "environmental stress cracking" of PET bottles (CORIOLANI *et al.*, 2012), Stress Corrosion Cracking (SCC) of aluminum cans is an infrequent, though extremely undesirable event, which would prejudice the integrity of the container, provoke the product loss and, what is probably worst, could cause a chain-effect on other adjacent cans if the product is spilled from the damaged can.
SCC is based on a variety of mechanisms, and some factors contribute to its occurrence, such as the metal thickness, the scoring procedure applied on the lid for the easy-opening, trace chlorine, moisture, temperature, the pressure inside the can which, in turn, is determined by the carbonation level chosen by the beverage producer and by temperature (DEWEESE et al., 2008): indeed, the increase in the internal pressure with increasing temperatures results from higher headspace gas pressure and decreased CO_2 dissolution in the beverage.
Aluminum cans allow the gas retention and guarantee a higher quality during storage. Indeed, the shelf life of canned carbonated beverages is commonly ≥1 year, which is about double compared with the same products packed in PET (CORIOLANI *et al.*, 2007; LICCIARDELLO *et al.*, 2011). While the long commercial life is an advantage from a commercial point of view, it increases the probability of occurrence of failure due to corrosion. It is widely accepted that efforts aimed at the reduction of product wastes and losses go towards the improvement of sustainability in the food chains (LICCIARDELLO, 2017). With the aim to reduce product losses through commercial life, this study aimed at evaluating the potential of an outer enamel for preventing SCC in canned carbonated beverages.

2. MATERIALS AND METHODS

2.1. Experimental plan

Mini-cans (15 ml content) of Coca-Cola were used for the challenge test: 216 mini-cans were supplied by Sibeg s.r.l. (Catania, Italy) and divided in two batches: one was used as received (A), and one was externally spray-lacquered with a transparent, acrylic based, quick-drying varnish (Arexons, Cernusco S/N, MI) (B). Samples were treated as follows:
A1-B1: not rinsed in warmer unit
A2-B2: rinsed in warmer unit at 30°C with demineralised water
A3-B3: rinsed in warmer unit at 30°C with tap water

A4-B4: rinsed in warmer unit at 30°C with demineralised water, then immersed in the product as to simulate severe product spillage from adjacent cans (occurring failure in stock).
Samples not rinsed in the warmer unit developed dew on the surface, as a consequence of environmental moisture condensation on the cold metal (Fig. 1).
Samples were stored at 40°C and 82% RU and inspected at regular intervals to detect failures.

Figure 1. Condensation occurring on the can surface. After filling with the cold beverage, cans must be warmed above the dew point to prevent "can sweating".

3. RESULTS AND DISCUSSION

All batches except A4 proved to maintain integrity in the severe storage conditions applied. Irrespective of the rinsing in demineralised or tap water, cans from batch A as well as B did not show damages leading to product loss. Cans belonging to batch A4, on the other hand, showed an overall failure level of 71%: indeed, 17 minicans out of 24 showed failures by the end of the challenge test (113 days). The first failure was observed just after 13 days of incubation (Fig. 2).

Figure 2. Failure occurring to a mini-can incubated in contact with the beverage, as to simulate the product spilled from a damaged adjacent can.

It is interesting to consider that, in real warehouse storage conditions, the occurrence of even one failure could represent a severe problem since the product coming out from the damaged can would spill onto the adjacent cans contained in secondary (6-unit shrink wraps) or tertiary (pallet) packaging, thus representing a potential trigger for further failures.

4. CONCLUSIONS

Only the most severe storage condition (contact with beverage) allowed to confirm the effectiveness of the external coating, while the presence of minerals in the rinsing water did not speed up failure occurrence in the tested conditions. The study proved that the application of a coating on the outer surface of aluminum cans could effectively reduce the risk of product failure, which might imply the damaged products withdrawal and disposal, and prejudice for the image and reputation of the producer. This measure should be applied to prevent aluminum can failures which could occur during storage and transportation in conditions which often exacerbate the stress suffered by the thin metal layer.

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REFERENCES

Coriolani C., Licciardello F. and Muratore G. 2012. Stress-cracking of PET bottle as an unpredictable factor limiting the commercial life of carbonated soft drinks. Italian Journal of Food Science, Special Issue of Vol 24(4):170-173.

Coriolani C., Rizzo V., Licciardello F. and Muratore G. 2007. Dependance of the shelf-life of carbonated soft drinks from PET performances as required by "The Coca Cola Company". Italian Journal of Food Science, Special Issue: 453-455.

DeWeese S.K., Ningileri S.T., Das S.K., and Green J.A.S. 2008. Stress-corrosion cracking in aluminum beverage can ends – issues, observations, and potential solutions. Journal of the Minerals, Metals & Materials Society 60(5):50-57.

Lee D.S., Yam K.L., and Piergiovanni, L. 2008. Food Packaging Science and Technology, CRC Press, Taylor & Francis Group.

Licciardello F. 2017. Packaging, blessing in disguise. Review on its diverse contribution to food sustainability. Trends in Food Science and Technology 65:32-39.

Licciardello F., Coriolani C. and Muratore G. 2011. Improvement of CO_2 retention of PET bottles for carbonated soft drinks. Italian Journal of Food Science, Special Issue of Vol. 23:115-117.

CONTROLLED RELEASE OF NATURAL ANTIOXIDANT AND ANTIMICROBIAL SUBSTANCES ON FRESH-CUT FRUITS FROM A LAYER BY LAYER DEVICE

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ABSTRACT

The high demand for convenience products and the potential of increasing the consumption of fresh fruits among the younger generations create a strong interest on fresh-cut fruits. These products require mild interventions for extending the shelf life which can be adequately met by active packaging systems. In this work a Layer by Layer assembly (LbL) coated onto PET sheets proved to be an effective controlled release system of natural polyphenols as antioxidants and chitosan as antimicrobial on the surface of fresh-cut fruits. The alternate layers of the polycation (chitosan) and the polyanion (alginate containing a green tea extract) has been confirmed by spectrophotometric, contact angles and AFM determinations. The modulated release of polyphenols and chitosan was monitored on a simulant of fresh-cut fruits in contact with the LbL assembly at 5°C. Finally, the antimicrobial and antioxidant effects of the LbL assembly device was tested on peach during the 7 day storage at 5°C, with the uncoated PET sheet as control.

Keywords: chitosan, fresh-cut fruits, green tea extract, layer by layer assembly, shelf life extension

1. INTRODUCTION

The growing demand for ready-to-eat food, together with the need for food waste reduction, led to the development of active packaging solutions to extend the shelf-life. The storage life of fresh-cut fruit can be improved by delaying quality decline and reducing microbiological spoilage. Fresh-cut peaches are highly appreciated fruits but they have a short shelf-life due to fast quality decline attributed to browning. Layer by layer assembly (LbL) is a basic technique in the development of multicomponent films on solid supports through controlled adsorption from solutions or dispersions (DECHER, 1997). With the LbL technique, polyelectrolyte multi-nanolayer films can be fabricated on various substrates through repeat deposition, mainly due to the electrostatic attraction between oppositely charged polyelectrolytes (DONATH *et al,* 1998). One of the main uses of LbL assembly is in medical field, particularly in drug delivery of antibiotics or others substances (WOHL and ENGBERSEN, 2012; TANG *et al.,* 2006). However, only few researches have been done on the potential application of LbL as active packaging system for food products. The aims of this work were the development and characterization of a new Controlled Release System (CRS) by the conjugation of chitosan with alginate, including polyphenols, and the application of this novel active packaging device on fresh-cut peaches to reduce their quality decline and onset of decay.

2. MATERIALS AND METHODS

2.1. Materials

Chitosan from shellfish (CS, GiustoFaravelli S.p.A., Milano, Italy) with a degree of deacetylation of 85% and a molecular weight ranging from 50,000 to 60,000 was used. Green tea extract was obtained from DAL CIN GILDO S.p.A. (Concorezzo, MB, Italy). The PET sheets (~250 μm thick) were provided by ILPA S.R.L. (Bazzano, Italy). All the other chemicals were purchased from Sigma-Aldrich (Milano, Italy).

2.2. Layer-by-layer assembly

Two solutions, 0.2 % (w/v), were separately prepared by dissolving chitosan in acetic acid 2 % (v/v), and alginate in water at 25 °C for 3 h with continuous stirring. Into the alginate solution 0.35% of green tea extract was added; the pH was then adjusted to 3.8 and 6.5 for chitosan and alginate-green tea solutions, respectively. The LbL assembly was carried out by the dipping method described by LI et al. (2013) on PET sheets, cut in 7 cm x 3 cm strips until 40 bilayers were achieved through repeated dipping and drying steps. The same procedures were used on coated glass slides for AFM analysis and thickness measurement.

2.3. Optical contact angle

On the coated PET surface, contact angle (OCA) measurement was performed using the sessile drop method (NEWMAN and KWOK, 1999) with optical contact angle apparatus (OCA 15 Plus – Data Physics Instruments GmbH, Filderstadt, Germany). Data acquisition was achieved with a video system equipped with a high-resolution CCD camera and a high performance digitizing adapter. SCA20 software from Data Physics Instruments.

2.4. Atomic Force Microscopy

The LbL coating on glass slides was gently scratched to expose part of the glass substrate for thickness measurement. An atomic force microscope (AFM, AlphaSNOM, WITec GmbH, Germany) was used both to measure the thickness of the deposited multilayers and to characterize their morphology.

2.5. FITC-CHI synthesis and assembly in LbL device

Fluorescein 5(6)-isothiocyanate was conjugated to chitosan (FITC-CHI) using the procedure of Del Hoyo-Gallego et al. (2016) to monitor the LbL construction on PET dedicated sheets. The absorbance at 490 nm of the coated PET sheets was measured after the deposition of 10, 20, 30 and 40 layers of FIT-CHI using a spectrophotometer (mod. L650, Perkin-Elmer, Milano, Italy).

2.6. Release monitoring

To understand the kinetics and effectiveness of release, portions of the coated strips (3 cm x 3 cm), were stirred for 72 h at 5 $\pm$ 0.5 °C into glass flasks with 5 mL of citric acid solution at pH 3.8 to serve as food simulant. The flasks were shaken using Flask Dancer (270292 Boekel Scientific, Feasterville, PA, USA) and the solutions were used for subsequent analyses.

2.7. In vitro antioxidant and microbiological assays

The antioxidant capacity was assessed by the polyphenols release (UV absorbance) and DPPH assay, following the procedure of BRAND-WILLIAMS *et al.* (1995). The absorbance at 278 nm of the simulant in contact with LbL coated PET strips, due to polyphenols release, was measured every 6 h during the first day, then at 48 and 72 h. Antimicrobial activity was tested against strains belonging to official collection, i.e. *Escherichia coli* CECT 434 (Spanish Type Cult. Collect.), *Staphylococcus aureus* ATCC 29213, *Aspergillus niger* NRRL 565 (Agricultural Research Service Cult. Collect.) and *Penicillium chrysogenum* CECT 2802. s), evaluating the growth inhibition halo of the simulant after 6, 24 and 48 h. of contact with LbL coated strips.

2.8. Preliminary in vivo tests

Peaches (*Prunus persica*) at commercial maturity were purchased at the wholesale market and stored at 5 $\pm$ 0.5 °C until use. Peaches of size and stage of ripeness were pre-washed with distilled water, sanitized for 2 min in chlorinated water (1.5 g/L sodium hypochlorite), rinsed with distilled water and gently dried by wiping. Fruits with skin were sliced using a sterile stainless-steel knife. Four slices were placed onto PET trays (19 cm x 12 cm x 4 cm) and coated PET strips were placed in each slice and at the bottom of the tray and stored at 5 $\pm$ 0.5 °C. In control samples uncoated strips were placed (Fig. 3). Samples were collected in triplicate at time zero and after 2, 4 and 7 days. Flesh colour was evaluated with a chromameter using the CIE L*a*b* System (Konica Minolta CR-300 Sensing, Inc., Japan). Total soluble solids (TSS, %) wers determined by a digital refractometer (Atago Co., Ltd, Tokyo, Japan model PR-32). Titratable acidity (TA) was measured by titrating 1:10 diluted juice (obtained from 50 g of sample) with sodium hydroxide 0.1 M by automatic titrator (Compact 44-00, Crison Instruments, SA, Barcelona,

Spain). The antioxidant potential and antimicrobial activity was assessed following the procedure described in Section 2.6.

3. RESULTS AND DISCUSSION

3.1. LbL assembly

All the tests performed on the developed LbL assembly showed a satisfactory regular coating after stacking of 8 layers (Fig. 1). Even the 40 layers stacking of the assembly is progressive and linear (FITC-CHI concentration). The AFM thickness measured after 20, 30 and 40 layers could be fitted linearly ($R^2 = 0.982$) with an average thickness of each layer around 1.6 nm. The roughness of the coated surfaces was also assessed by AFM but didn't reveal differences between the layers of chitosan and alginate.

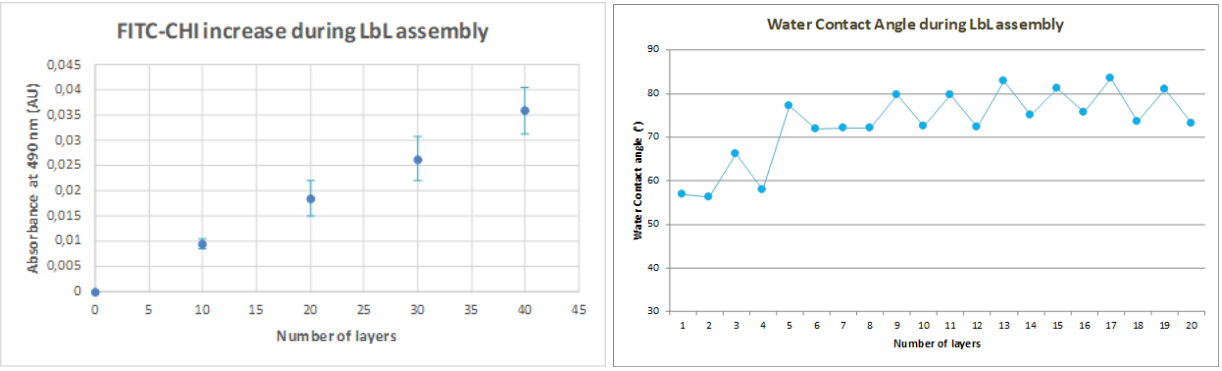

Figure 1. (left) Proof of regular alternate LbL covering, by OCA. (right) Proof of progressive growth of LbL assembly by absorbance at 490 nm of chitosan conjugate.

3.2. Release monitoring

Both chemical and microbiological tests were carried out to prove the release of antioxidant and antimicrobial substances from the LbL device, when in contact with fresh-cut fruits simulant (Fig. 2). The release of antioxidant polyphenols appeared quite fast, thus need to be slowed down for better fruit quality maintenance. However, it must be noted that the simulated trial used a liquid medium, which will definitely accelerate the release of antioxidant ans antimicrobial than when solid medium as in real food is used. The release of effective antimicrobial seems much slower, showing a complete inhibition after 48 hours.

3.3. Shelf life tests

After 7 d of storage, Lightness (L*) and Chroma (C*) were higher in LbL than in control samples. Different studies affirmed that L* can be used as indicator of flesh browning (ROCHA and MORAIS, 2003). GONZALEZ-BUESA *et al.* (2011) demonstrated the correlation between L* and visual consumer perception on peach fruit. Their study reported that L* values higher than 75 did not affect consumer perception of browning. In this work, LbL sample had final L* value around 76 while 74 for the control sample. Also from visual appearance, differences among the different slices can be detected (Fig. 3). The

slices of LbL samples had better attractive appearance than the control samples. The presence of PET strips influenced the weight loss of peach slices, reducing the loss by 50% of initial weight compared with the CTR. With regard TSS and TA, the values obtained were typical of the changes after harvest and there were normal postharvest behavior, with no significant differences among treatments.

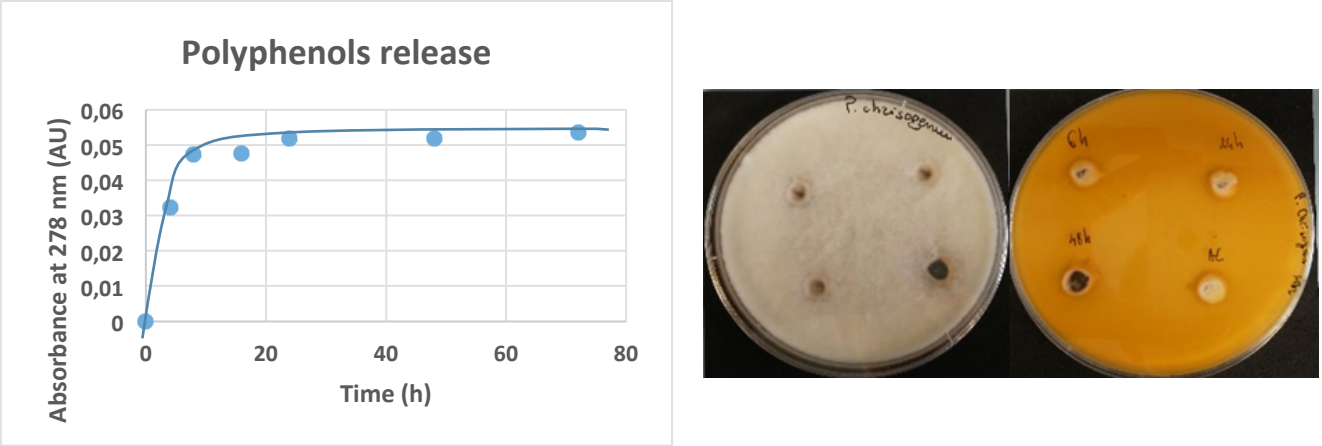

Figure 2. (left) progressive release of antioxidant to the simulant. (right) *Penicillium chrysogenum* inhibition by chitosan release.

Figure 3. (left) Comparison between control and LbL samples after 7 days. (right) Releasing system set-up.

4. CONCLUSIONS

The releasing system developed proved promising in extending shelf life of fresh-cut fruits, considering the wide possibility of modulating the thickness of the active coating as well as the LbL composition in terms of antimicrobial and antioxidant used. The effectiveness of chitosan-alginate multilayers coating deposition, by LbL technique, was demonstrated as possible and useful. The in vitro antimicrobial assays confirmed the effectivity of incorporating green tea antioxidant compounds in the alginate layers and their gradual release from the coated PET. Also gradual release of chitosan was confirmed by chemical and microbiological assays. The application on fresh-cut peach showed interesting results in terms of sensory and chemical parameters. Further investigations are

needed to compare the efficacy of LbL technique against the normal coating procedure or with other active molecules addition.

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REFERENCES

Brand-Williams W., Cuvelier M.E. and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 28:25-30.

Decher G. 1997. Fuzzy nanoassemblies: Toward Layered Polym. Multicomp. Sci. 277:1232-1237.

Del Hoyo-Gallego S., Pérez-Álvarez L., Gómez-Galván F., Lizundia E., Kuritka I., Sedlarik V., Laza J.M. and Vila-Vilela, J.L. 2016. Construction of antibacterial poly(ethylene terephthalate) films via layer by layer assembly of chitosan and hyaluronic acid. Carbohydr. Polym. 143:35-43.

Donath E., Sukhorukov G.B., Caruso F., Davis S.A. and Möhwald H. 1998. Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes. Angew. Chem. Int. Ed. 37:2202-2205.

González-Buesa J., Arias E., Salvador M.L. Oria, R. and Ferrer-Mairal, A. 2011. Suitability for minimal processing of non-melting clingstone peaches. Int. J. Food Sci. Technol. 46: 819–826.

Li F., Biagioni P., Finazzi M., Tavazzi S. and Piergiovanni, L. 2013. Tunable green oxygen barrier through layer-by-layer self-assembly of chitosan and cellulose nanocrystals. Carbohydr. Polym. 92:2128-2134.

Newman A.W. and Kwok D.Y. 1999. Contact angle measurement and contact angle interpretation. Adv. Colloid Interface Sci. 81:167–249.

Rocha A.M.C.N. and Morais A.M.M.B. 2003. Shelf life of minimally processed apple (cv. Jonagored) determined by colour changes. Food Control. 14:13-20.

Tang Z., Wang Y., Podsiadlo P. and Kotov N.A., 2006. Biomedical applications of layer-by-layer assembly: from biomimetics to tissue engineering. Adv. Mater. 18:3203-3224.

Wohl B.M. and Engbersen J.F. 2012. Responsive layer-by-layer materials for drug delivery. J. Control. Release. 158:2-14.

ADDITION OF GALLIC ACID TO ENHANCE ANTIOXIDATIVE AND PHYSICAL PROPERTIES OF FISH GELATIN FILM FOR EDIBLE OIL POUCH

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ABSTRACT

Fish gelatin film with antioxidant ability could be used as an antioxidant-active packaging. The aims of this research were to enhance antioxidative ability and to improve physical properties of fish gelatin film by incorporation of gallic acid. Film-forming solution composed of 5% protein, 2% (w/w of protein) gallic acid, and 22.5% (w/w of protein) glycerol as a plasticizer, was cast at 25°C, 50% RH for 48 h. The gallic acid-gelatin film was analyzed for total phenolic content (TPC), ABTS and FRAPS assays, tensile strength (TS), elongation at break (EAB), and UV-light transmission. For practical use test, soybean oil (3 ml) was added in the gallic acid-gelatin film pouch (5×5 cm^2) and kept at 25°C, 50% RH for 32 days to evaluate lipid oxidation. The gallic acid-gelatin film significantly displayed high TPC (8.95±1.25 meq of g film), increases in ABTS (29.09±2.38 meq of g film) and FRAP (83.91±5.11 meq of g film) value, as well as an increase in EAB of the film to 86.37 % as compared to that without gallic acid ($p \leq 0.05$). Addition of gallic acid significantly reduced the UV-light passing through the film, i.e., retardation of UV-induced lipid oxidation. Gallic acid resulted in increasing oxygen barrier of the film. Gallic acid might interact with gelatin protein through H-bonding, as shown by FTIR result. Gallic acid-gelatin film pouch could suppress the peroxide value (2.09±0.17 meq of kg oil sample) of the stored soybean oil up to 32 days. In conclusion, addition of 2% gallic acid to fish gelatin film enhanced antioxidative property, increased EAB, and reduced UV transmission of the film. The gallic acid-gelatin film pouch could retard lipid oxidation of the contained-oil during 32 days storage as compared to those without gallic acid and commercial polypropylene (PP) film.

Keywords: active packaging, antioxidant, gallic acid, gelatin film, protein

1. INTRODUCTION

Fish gelatin film is protein-based biodegradable material. Typical properties of gelatin film are high strength, translucent and a good gas barrier, but weakness point is brittle. Antioxidant-active packaging is defined as packaging which providing antioxidant power to retard lipid oxidation during food storage. Fish gelatin was selected to prepare film because the fish gelatin had superior interaction with polyphenol than that commercial bovine hide gelatin as report of GÓMEZ-ESTACA *et al.* (2009). Previous research demonstrated that increasing gallic acid concentration in fish gelatin film resulted in an increase in antioxidant power, TS, EAB, and a reduction of UV absorption (LIMPISOPHON and SCHLEINING, 2017). However, there is no information on quantitative effect of gallic acid on physical properties of fish gelatin film as compared to the one without gallic acid, as well as the retarding effect of gallic acid-gelatin film on lipid oxidation during food storage. Therefore, the objectives of this research were to determine the effect of gallic acid on antioxidant and physical properties of fish gelatin film. Storage test of soybean oil in the gallic acid-gelatin film pouch was also conducted in comparison with those without gallic acid.

2. MATERIALS AND METHODS

Fish gelatin and gallic acid were purchased from Sigma-Aldrich (Vienna, Austria). Gallic acid-gelatin film was prepared according to LIMPISOPHON and SCHLEINING (2017). Final film-forming solution (FFS) was composed of 5% protein (w/w), 2% (w/w of protein) gallic acid, and 22.5% (w/w of protein) glycerol (AppliChem, Darmstadt, Germany) as a plasticizer. The film was cast at 25°C 50% RH for 48 h, and then conditioned at 25°C 50% RH for at least 48 h. Gallic acid-gelatin film was prepared to compare those without gallic acid. The films were analyzed for total phenolic content (TPC), antioxidant activities (radical scavenging by ABTS and electron donating by FRAP) according to LIMPISOPHON and SCHLEINING (2017). Physical and mechanical properties of the films were measured following ASTM D882-97 (1999), UV-light transmittance by following FANG *et al.* (2002), and oxygen permeability (OP) by following ASTM D3985 (1988). Structural interaction of protein in gallic acid-gelatin film in the range of amide I region (1700-1600 cm^{-1}) was determined using FTIR-ATR spectrometer Vertex 70 (Bruker optics, Ettlingen, Germany), since this region was mainly related to changes in peptide chain conformation of gelatin according to PRYSTUPA and DONALD (1996). Two gallic acid-gelatin films (5×5 cm^2) were heat-sealed at heating No. 5 for ~5 sec at three edges to prepare a film pouch by heat sealer (P-200, Fuji Impulse Co., Ltd.). Three ml of soybean oil was added into gallic acid-gelatin film pouch. The containing oil pouch was heat-sealed and kept in darkness at 25°C, 50% RH for 32 days. To monitor lipid oxidation of oil in film pouch during storage, 500 mg of oil was evaluated by peroxide value (PV) following AOCS (1997) for 6 storage-interval times.

3. RESULTS AND DISCUSSION

Table 1 shows antioxidant and physical properties of gallic acid-gelatin film as compared to those without gallic acid and to PP film. Addition of gallic acid into gelatin film significantly increased antioxidant activities ($p \leq 0.05$), including ABTS value of 29.09±2.38 meq/g of film, and FRAP value of 83.91±5.11 meq/g of film. TS value of gallic acid-gelatin film did not change as compared to those without gallic acid ($p > 0.05$). Gallic acid

increased EAB of the film to 86.37% as compared with the film without gallic acid ($p \leq 0.05$). It was suggested that the gallic acid-gelatin film was suitable for a food film wrap. Gallic acid, as a small phenolic molecule, could plasticize protein-based film (ARCAN and YEMENICIOGLU, 2011). OP of gallic acid-gelatin film was slightly higher than those without gallic acid film, but significantly lower than PP film. FTIR spectra peak at 1643 cm^{-1}, representing random coil structure and H-bonds (PRYSTUPA and DONALD, 1996), was higher in gallic acid-gelatin film as shown in Fig. 1B. Gallic acid with hydroxyl groups could interact with side chains of serine in collagen peptide (gelatin) via a hydrogen bond as a report of MADHAN *et al.* (2001); therefore, the peak at 1643 cm^{-1}, representing hydrogen bond, was the majority in gallic acid-gelatin film. Triple helix peak at 1657 cm^{-1} (Fig. 1A), indicating native structural arrangement, was predominant peak in gelatin film. Beta turn peak at 1677 cm^{-1} as shown in Fig. 1A was not observed in the gallic acid-gelatin film. It might be because β-turn peak shifted to lower frequency peat at 1666 cm^{-1} in Fig. 1B. Addition of gallic acid resulted in the presence of structural disorder of gelatin. Consequently, gelatin protein could interact with gallic acid by protein-polyphenol interaction resulting in observed changes in physical properties, i.e., EAB and OP.

Table 1. TPC, antioxidant and physical properties of gallic acid-gelatin film as compared with those without gallic acid and with PP film[1].

Film	TPC[2]	ABTS[3]	FRAP[4]	TS	EAB	OP
	(meq/ g of film)			(MPa)	(%)	($cm^3.\mu m.m^{-2}.d^{-1}.kPa^{-1}$)
Gallic acid-gelatin film	8.95±1.25	29.09±2.38	83.91±5.11	8.89^{b}±0.94	136.98^{b}±18.63	64.55^{b}±0.91
Gelatin film	0.59±0.03	1.03±0.04	5.23±0.01	8.93^{b}±2.29	73.50^{c}±8.96	40.67^{c}±9.52
PP	*	*	*	20.03^{a}±1.57	567.89^{a}±23.84	124.46^{a}±2.30

[1]Data are expressed as mean±standard deviation from three measurements with two treatment replications.

[2]Means in the same column are not significantly different ($p>0.05$) by two independent sample t-test.

[3, 4]Means in the same column are significantly different ($p\leq0.05$) by two independent sample t-test.

Different superscripts in the same column are significantly different means ($p\leq0.05$) by Duncan's multiple range tests.

*Not determined

Results of UV-light transmittance (200-300 nm range) indicated that gallic acid-gelatin film had superior UV-blocking passing through the film at a value of 0.01-3.34% transmittance as compared to gelatin film (0.01-58.75 % transmittance), and PP film (9.36-88.60%). Aromatic structure of phenolic substance like gallic acid and peptide bond in the chains of gelatin could play a role of UV barrier in gallic acid-gelatin film (BAO *et al.*, 2009; WANG *et al.*, 2015).

The gallic acid-gelatin film pouch retarded the PV of the stored soybean oil up to 32 days (Fig. 2) as 2.09±0.17 meq/ kg oil sample. The oil in gallic acid-gelatin film pouch until 32 days can be consumed, since PV of refined oil like soybean oil should be lower than 10 meq/ kg oil as Codex standard for named vegetable oils (COMMISSION, 2001). After 11 days, the PV of the oil kept in the gallic acid-gelatin film pouch was 24% lower as compared to the one kept in commercial PP film pouch as shown in Fig. 2.

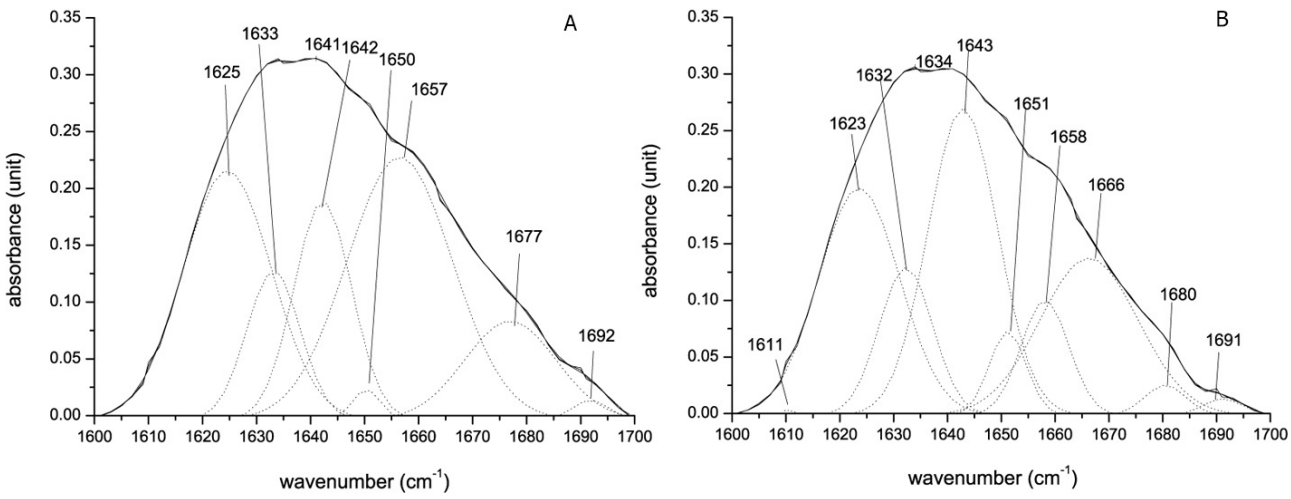

Figure 1. Curve-fitting of FTIR-ATR spectrums corresponding to gelatin film (A) and gallic acid-gelatin film (B).

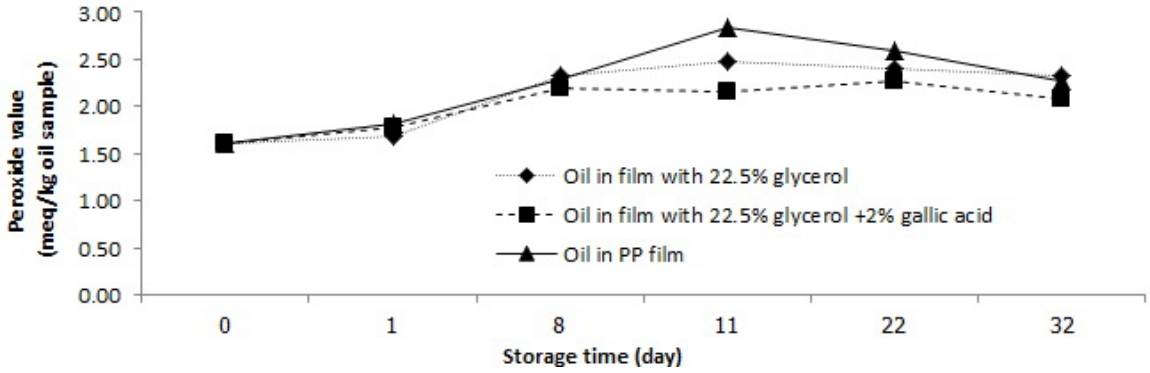

Figure 2. Peroxide value (PV) of soybean oil in different film pouches during storaged at 25 °C, 50 % RH.

4. CONCLUSIONS

Addition of gallic acid into gelatin film increased antioxidant property, extensibility of the film, and reduced UV-light passing through the film. These changes might be due to protein-polyphenol interaction as suggested by FTIR result. Increasing antioxidant property of gallic acid-gelatin film pouch could retard lipid oxidation of the contained soybean oil during 32 day storage.

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REFERENCES

Arcan I., and Yemenicioglu A. 2011. Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. Food Res. Int. 44:550-6.

ASTM. 1988. Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor. In: "Annual book of ASTM standards". p. 607-612. American Society for Testing and Materials, Philadelphia, USA.

ASTM. 1999. Standard test method for tensile properties of thin plastic sheeting. In: "Annual book of ASTM standards". p 163-171. American Society for Testing and Materials, Philadelphia, USA.

Bao S., Xu S., and Wang Z., 2009. Antioxidant activity and properties of gelatin films incorporated with tea polyphenol-loaded chitosan nanoparticles. J. Sci. Food Agric. 89:2692-700.

Commission, CA (2001). "Codex standard for named vegetable oils (CODEX-STAN 210-1999). In: "Report of the 17th Session of the Codex Committee on Fats and Oils, London". p 11-25.

Fang Y., Tung M.A., Britt I.J., Yada S., and Dalgleish D.G.. 2002. Tensile and barrier properties of edible films made from whey proteins. J. Food Sci. 67:188-93.

Gómez-Estaca J, Bravo L, Gómez-Guillén MC, Alemán A, and Montero P. 2009. Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem. 112:18-25.

Limpisophon K. and Schleining G. 2017. Use of gallic acid to enhance the antioxidant and mechanical properties of active fish gelatin film. J. Food Sci. 1:80-9.

Madhan B., Thanikaivelan P., Subramanian V., Raghava Rao J., Unni Nair B. and Ramasami T. 2001. Molecular mechanics and dynamics studies on the interaction of gallic acid with collagen-like peptides. Chem. Phys. Lett. 346:334-40.

Prystupa D.A., and Donald, A.M. 1996. Infrared study of gelatin conformations in the gel and sol states. Polym. Gels Networks. 4:87-110.

Society, A.O.C., and Firestone D. 1997. "Official methods and recommended practices of the American Oil Chemists' Society" 4th ed. AOCS press, IL, USA.

Wang Y., Liu A., Ye R., Li X., Han Y., and Liu C. 2015. The Production of gelatin-calcium carbonate composite films with different antioxidants. Int. J. Food Prop. 18: 2442-56.

MODELING OF ETHANOL VAPOR CONTROLLED RELEASE ACTIVE PACKAGING FOR FRESH PEELED SHALLOTS

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ABSTRACT

Ethanol vapor controlled release active packages can delay microbial proliferations in fresh peeled shallots, a value-added product. This study purposely was conducted to develop mathematical models to predict ethanol vapor concentration accumulated in the active packages. The package physically was a solid plastic tray (9.5× 13×6 cm; width × length × depth) covered with the low density polyethylene (LDPE) film lid. The tray contained 100g peeled shallots and a sachet, kept at 10°C for 10 d. The 3×3 cm sachet was made of either LDPE or Nylon/PE film on one side and aluminum foil laminated on the other. The sachet contained 1g silica gel adsorbent pre-adsorbed with food grade ethanol. The mathematical models were developed through conceptualizing key mass transfer processes in the active packages given assumptions made to describe changes in the sachet, package headspace, plastic film lid, and the shallot with respect to ethanol vapor. The mathematical models were tested against experimental data. The steady-state ethanol vapor concentrations in packages predicted by the models were approximately 1.5 to 2-fold lower than those collected from the experiments. However, the models could reasonably predict trends in changes of ethanol vapor concentrations accumulated in different packages. A lack of fit between models predicted and empirical results was attributed to an accuracy of a model input, namely rate constants of ethanol vapor-shallots interactions. The study highlighted that the mathematical modeling approach and assumptions made was reasonable to simplify key mass transfer of the active packages. Further studies to understand complexities of ethanol vapor-shallots interactions are required for obtaining accurate information on the interaction rates. The mathematical model can be used as a mechanistic tool to facilitate designs of active packages incorporating the ethanol vapor controlled release sachet for fresh peeled shallots or other horticultural produce, given accurate model inputs.

Keywords: active packaging, ethanol vapor, mathematical model, packaging design

1. INTRODUCTION

Fresh-cut fruit and vegetable are susceptible to microbial proliferation leading to significant losses in shelf-life and value. Applications of active packages incorporating with an ethanol vapor controlled release sachet has been proven to delay microbial losses (UTTO, 2014). Designs of active packaging systems by conducting experiments of which a number of factors such as ethanol loads, and sachet or packaging size were taken into accounts would be considered time-consuming and costly tasks. Predictive mathematical models for use in the packaging design have been extensively developed to predict packaging performances such as changes in product weight and temperature, as well as atmospheric compositions. The models appropriately validated could give benefits in screening prior conducting experimental evaluations of prototypes, and in minimizing cost/time requirements (TANNER *et al.*, 2002). At present, developments of the mathematical models for designs of controlled release based active packages for horticultural products of which volatile compounds are either ethanol vapor or others have not been reported. The objective of this work was to develop a global mathematical model that can predict ethanol vapor concentration released and accumulated in the package. The model was developed with a basis of key mass transport processes and their key factors such as film permeability to ethanol vapor (FPE) as well as sachet and package dimensions. Model predictions on ethanol vapor concentration in active packages were experimentally validated. The active package containing fresh peeled shallots as well as an ethanol vapor controlled release sachet were utilized as a demonstrative system because of its effective delaying microbial proliferations UTTO (2016).

2. MATERIALS AND METHODS

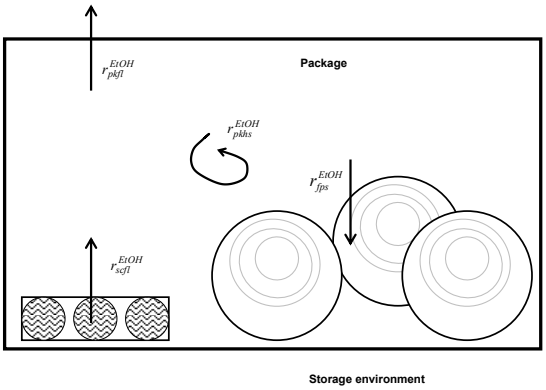
2.1. Physical active package

The active package physically was a solid polypropylene (PP) plastic tray containing 100 g of peeled shallot. Top and roots of shallot cloves were cut and dried skin were taken off. Each clove was peeled by cutting its top layer and peeling away. One sachet was placed on top of the shallots, prior the 80μm LDPE lid was sealed to the tray. The 3×3 cm sachet was made of two materials. One side was Al/PE (120μm) film and the other side was either LDPE (63 μm) or Nylon/PE film (83 μm). The sachet contained one gram of dried silica gel (grade 40, Sigma-Aldrich) pre-equilibrated with ethanol liquid (0.32 ml) for 4 weeks. The trays were kept at 10°C for 10 d. Ethanol vapor concentration (μL L^{-1}) was analyzed using a flame ionization detector gas chromatogram (GC-2014; Shimadsu, Japan) and Porapak Q (a packed column with 2.0 m length, inner diameter 3.00 mm). Other GC operational conditions were reported in UTTO (2016).

2.2. Conceptual model of ethanol vapor mass transfer processes in active packages

Key mass transfer processes governing releases of ethanol vapor in the active package was conceptually modeled and these were shown in Fig. 1-I. Mechanisms of the controlled release package mainly involves ethanol vapor desorbs from the silica gel into the sachet gas phase and permeates to the sachet film into the package headspace (r_{sc}^{EtOH}). Transports from the sachet were simplified as one-dimensional due to a very high barrier to ethanol vapor of Al/PE. Ethanol vapor can both 'interact with the shallots' (r_{fps}^{EtOH}) and 'permeation through the lid to the environment' (r_{pk}^{EtOH}). Sorption of ethanol vapor to the

solid PP tray was assumed to negligible, due to the low ethanol solubility of the tray. The dynamic balance of rates of the processes determines the net rate of ethanol vapor accumulation (r_{pkhs}^{EtOH}) (Fig. 1-I).



Conceptual model (I)

$$V_{pkg}\frac{\partial C_{pkhs}^{EtOHl}}{\partial t}=\frac{P_{scfl,0}^{EtOH}A_{scfl}RT_{pkg}}{L_{scfl}\cdot b_{scfl}^{EtOH}}\left(\exp\left(C_{g,bed}^{EtOH}\cdot b_{scfl}^{EtOH}\right)-\exp\left(C_{pkhs}^{EtOH}\cdot b_{scfl}^{EtOH}\right)\right)$$
$$-\frac{P_{pkfl,0}^{EtOH}A_{pkfl}RT_{pkg}}{L_{pkfl}\cdot b_{pkfl}^{EtOH}}\left(\exp\left(C_{pkhs}^{EtOH}\cdot b_{pkfl}^{EtOH}\right)-\exp\left(C_{env}^{EtOH}\cdot b_{pkfl}^{EtOH}\right)\right)$$
$$-k_{fps,reac}^{EtOH}\cdot C_{pkhs}^{EtOH}\cdot M_{fps}$$

Global mathematical model (Eq. 1) (II)

Figure 1. Conceptualized key ethanol vapor transport processes in active package (I) and a global mathematical model to predict ethanol vapor accumulated in the package (Eq. 1; II).

2.3. Global mathematical model

The global mathematical model (Eq. 1; Fig. 1-II and Table 1) was developed according to (i) Fick's laws for modeling transport rates at sachet film and tray lid of which effective FPE values were exponentially dependent on ethanol vapor concentrations and (ii) first-order reaction for modeling rates of ethanol vapor uptake by the shallots.

Table 1. Nomenclature of variables in Eq. 1.

V_{pkg}	Free volume in package (m^3)	b_{scfl}^{EtOl}	A fitted coefficient for FPE of sachet film ($m^3 \cdot mol^{-1}$)	$C_{g,bed}^{EtOH}$	Equilibrium ethanol vapor concentration above silica gel bed ($mol \cdot m^{-3}$)	A_{pkfl}	Surface area of packaging film (m^2)	$k_{fps,r}^{EtOl}$	Coefficient of ethanol uptake rate by shallot ($m^3 \cdot s^{-1} \cdot kg^{-1}$)
C_{pkhs}^{EtOl}	Ethanol vapor concentration in package headspace ($mol \cdot m^{-3}$)	A_{scfl}	Surface area of sachet film (m^2)	$P_{pkfl,0}^{EtOH}$	A fitted coefficient for FPE of packaging film ($mol \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$)	L_{pkfl}	Thickness of packaging film (m)	M_{fps}	Mass of fresh peeled shallot (kg)
$P_{scfl,0}^{EtOl}$	A fitted coefficient for FPE of sachet film ($mol \cdot m \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$)	L_{scfl}	Thickness of sachet film (m)	b_{pkfl}^{EtOH}	A fitted coefficient of FPE for packaging film ($m^3 \cdot mol^{-1}$)	C_{env}^{EtOH}	Ethanol vapor concentration in environment ($mol \cdot m^{-3}$)	C_{sat}^{EtO}	Saturated ethanol vapor concentration ($mol \cdot m^{-3}$)

Extents of equilibrium ethanol vapor concentration above silica gel bed inside the sachet were varied with ethanol amounts remaining on the bed. The equilibrium sorption data of ethanol vapor was quantified using the gravimetric approach by the Dynamic Vapor Sorption Advantage Instrument (Surface Measurement System, UK) and were modeled

using the Langmuir sorption isotherm equation due to its simplicity and having ability to describe experimental results over wide concentration ranges. The global model utilized simultaneous ordinary differential equations for the sachet, package headspace, and tray lid. These were required to be solved numerically using MATLAB®. Modeling methodology and its assumptions, and model simulating inputs as well as data collection methodologies were reported in UTTO (2016).

3. RESULTS AND DISCUSSION

The initial release peaks of ethanol vapor were observed during the first 6 h (Fig. 2). These were results of great differences between concentrations in sachet and package, immediately after the lid was sealed, causing an initial large driving force for ethanol vapor release from the sachet. The concentrations thereafter slightly decreased to the quasi-steady state levels and were maintained throughout the storage period. Reductions in ethanol vapor concentrations in the package headspace were responses to decreased amounts of ethanol left on the silica gel bed. Higher ethanol vapor concentration accumulated in LDPE-sachet package (Fig. 2) was attributed to FPE value of LDPE, which was relatively higher than that of Nylon/PE. The effective FPE values of LDPE and Nylon/PE were 0.53 and 0.18 $nmol \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$ respectively (estimated at a saturated concentration) (UTTO, 2016).

The model reasonably predicted concentration changing patterns. The concentrations predicted however were 1.5 to 2-fold lower than the empirical ones (Fig. 2). Estimated values of R^2 between empirical and predicted values were 0.48 and 0.07 for LDPE- and Nylon/PE-sachet packages respectively, suggesting significant lack-of-fit of the model predictions.

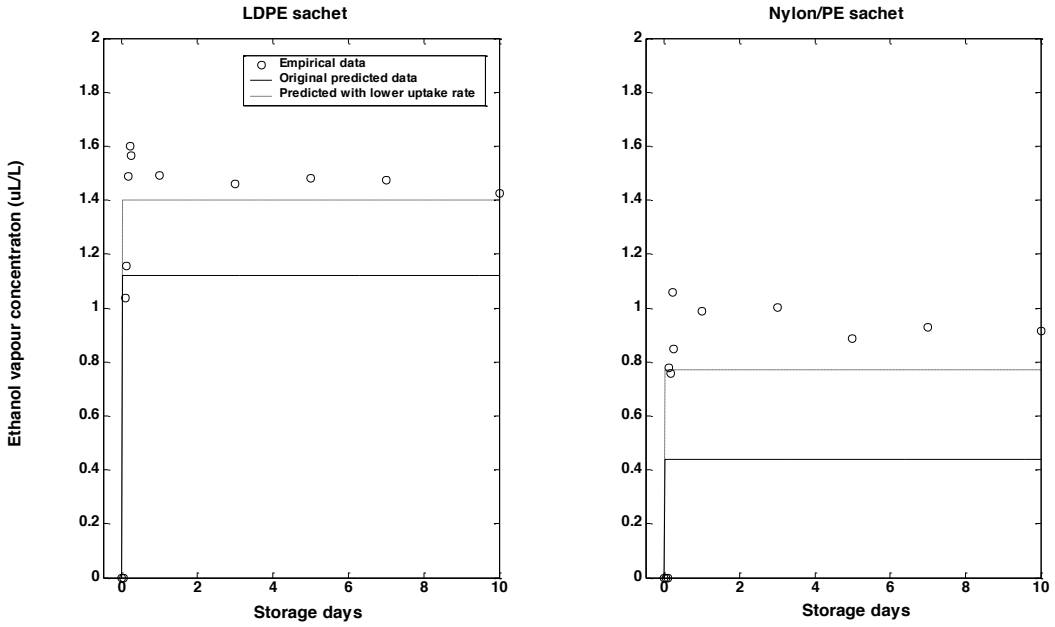


Figure 2. Empirical ethanol vapor concentration (o) and model predictions: solid and dotted lines represent concentrations predicted using original and modified uptake rate constants.

The inaccuracies were presumably attributed to ethanol vapor effects that may delay ethanol uptake rates and other biological changes of the shallot. Whilst there is no report on ethanol effects on the shallots and other *Allium* crops including garlic and onion, the assumption was made regarding evidence reported on that the ethanol vapor delayed changes of chlorophyll in broccoli (SUZUKI *et al.*, 2004) and anthocyanin in cherry (BAI *et al.*, 2011). Key mechanisms of such ethanol effects were reportedly postulated that ethanol slowed down metabolic processes related to ripening and senescence of the produce. Further investigations are required to understand interactions between ethanol vapor and fresh peeled shallots.

In Fig. 2, the model predicted results were much improved when uptake rate constants were arbitrarily lowered by 1.25- and 1.75-fold for LDPE and Nylon/PE sachet-tray, respectively (Fig. 2). The simulation results supported the assumption on that the ethanol vapor could have slowed down the biological activities of the shallots. In future, the validated model can assist designs of active packages by simulating the concentration levels in the package given changes made to packaging components. The simulations are also known as understandings on *what-if* scenarios for example effects of shallot weight changes on the ethanol vapor concentration levels. The designs that cause the concentration levels lower than so-called minimum inhibition levels as those shown in Fig. 2 will be screened out. Because the modeling methodology was developed mechanistically, it could be applied to active package containing a sachet releasing other antimicrobial volatiles and horticultural produce.

4. CONCLUSIONS

The research findings presented that the global mathematical model developed by the modeling methodology including mass transport conceptualizations as well as mathematical equations had abilities to predict changing trends of ethanol vapor concentration in the active package studied. The model's limitation was due to data inaccuracies on shallot's ethanol uptake rates. By understanding these from future studies will improve the global model.

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REFERENCES

Bai J., Plotto A., Spotts R., and Rattanapanone N. 2011. Ethanol vapor and saprophytic yeast treatments reduce decay and maintain quality of intact and fresh-cut sweet cherries. Postharvest Biol. Technol. 62:204-212.

Suzuki Y., Uji T. and Terai H. 2004. Inhibition of senescence in broccoli florets with ethanol vapor from alcohol powder. Postharvest Biol. Technol. 31(2):177-182.

Tanner, D.J. Cleland, A.C., Opara, L.U., and Robertson, T.R. 2002. A generalised mathematical modeling methodology for design of horticultural food packages exposed to refrigerated conditions: part 1, formulation. Int. J. REFRIG. 25(1):33-42.

Utto W. 2014. Factors affecting release of ethanol vapour in active modified atmosphere packaging systems for horticultural products. Maejo Int. J. Sci. Tech. 8:75-85.

Utto W. 2016. Mathematical Model Based Design of Ethanol Controlled Release Active Packaging for Freshly Peeled Shallot. Bangkok, Thailand: Thailand Research Fund, 114 pp.

EFFECT OF LAURIC ACID AND WHEAT GLUTEN ON CHEMO-PHYSICAL AND MECHANICAL PROPERTIES OF ZEIN-BASED EDIBLE FILMS

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ABSTRACT

In this study, the effect of lauric acid and wheat gluten on physico-chemical and mechanical properties of zein-based edible films was studied. Edible films based on thermoplastic zein containing different amount of lauric acid and wheat gluten were obtained through two step procedures: melt mixing and subsequent compression molding. Results highlighted that the addition of lauric acid brought about an increase in tensile modulus and maximum stress and a decrease of water vapor permeability of zein-based edible films. This behavior can be attributed to the increase of the hydrophobic character of the produced films. On the other hand, the addition of wheat gluten induced a reduction of water vapor permeability without affecting their mechanical properties. The best compromise between barrier and mechanical properties was observed for the thermoplastic zein-based films containing 11% and 15% of lauric acid and wheat gluten, respectively.

Keywords: edible film, edible film, gluten, lauric acid, water barrier, zein

1. INTRODUCTION

The development of edible polymers from sustainable sources, mainly polysaccharides and proteins (OLIVIERO *et al.*, 2017), is becoming one of the attempts to reduce the problems caused by the massive production of plastic waste and the decrease in petroleum sources. Conventionally an edible polymer can be defined "*edible*", if all its components are recognized as safe substances such as alcohols, lipids, proteins, polysaccharides and their derivatives (GARCIA *et al.*, 2000). Generally, the functional, organoleptic and nutritional properties of edible films can be modified by adding small amounts of chemical compounds to the formulation (KROCHTA *et al.*, 1997). Mainly the addition of plasticizers, such as glycerol, sorbitol or polyethylene glycol is often used to modify the processability (because the intermolecular forces are reduced and the polymer chains mobility increased), and also the mechanical properties of the produced film (VERDOLOTTI *et al.*, 2016). However, the incorporation of hydrophobic components may cause significant reduction in the functional properties of the film (i.e. gas barrier), since water vapor transfer generally occurs through the hydrophilic portion of the film (PADGET *et al.*, 2000).
This work was undertaken to evaluate the feasibility to produce edible films by using zein, a cereal protein abundant and cheap, as matrix and some sustainable additives, lauric acid and wheat gluten, to enhance physico-chemical and functional performances of zein. Water vapor permeability, thermal and mechanical properties were investigated.

2. MATERIALS AND METHODS

2.1. Materials

Maize zein powder and poly (ethylene glycol), PEG (MW=400) used as plasticizer to prepare thermoplastic zein (TPZ) were purchased from Sigma Aldrich, Italy. In order to improve the properties of TPZ-based edible films, two different chemical modifier additives were used as partial substitutes of zein and PEG: gluten powder (Sigma Aldrich, Italy), hereafter denoted as GLU, and lauric acid (Sigma Aldrich, Italy), hereafter denoted as LA.

2.2. TPZ- based edible films preparation

Powder of edible matrix (GLU and/or zein) and plasticizer (LA and/or PEG) were premixed by hand at room temperature in order to homogenize the mixture and then subjected to temperature (70 °C) and shear stresses in a twin counter-rotating internal mixer (at 50 rpm for 10 min) (Rheomix 600, Haake, Germany) connected to a control unit (Haake PolyLab QC) for thermoplasticization (VERDOLOTTI *et al.*, 2014). Mixing compositions are reported in Table 1. The edible matrix/plasticizer ratio was fixed at 75:25. The materials extracted from the mixer were pressed at 70°C and 50 MPa (P300P, Collin, Germany).

2.3. TPZ- based edible films characterizations

The DSC tests were performed under a N_2 atmosphere by using a differential scanning calorimeter (Q1000, TA Instruments, USA), in a heat-cool-heat mode, with the heating rate and the cooling rate of 10 °C/min in the range from -50 °C to 200 °C for all samples.

The tensile modulus (E), maximum stress ($\sigma_м$) and strain at break ($\varepsilon_в$) were measured at room temperature with a 1 kN load cell using an Instron model 4204 tensile test machine (ASTM D 1708-02). Five samples for each composition were tested and the average values were reported. The water vapor permeability (WVP) was determined using the infrared sensor technique by means of a PermatranW3/31(Mocon, Germany). Permeation tests were performed at 25 °C by setting the relative humidity at the downstream and upstream sides of the film to 0% and to 50% respectively.

Table 1. Classification and weight compositions of TPZ -based edible films.

Edible films	Zein (wt %)	GLU (wt %)	PEG (wt %)	LA (wt %)
TPZPEG25	75	-	25	-
TPZPEG20LA5	75	-	20	5
TPZPEG20GLU10LA5	65	10	20	5
TPZPEG14GLU15LA11	60	15	14	11

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3. RESULTS AND DISCUSSION

DSC analysis was performed in order to determine the compatibility limits between zein and the LA. Usually, above the compatibility limit, the fatty acid in excess does not act as a plasticizer, it does not interact with the film matrix and its melting point can be detected. To quantify the amount of this plasticizer, changes in heat capacity were also measured on LA alone. The DSC curves and the corresponding results for different edible films are shown in Fig. 1 and Table 2. For the TPZ- based materials containing the 5wt% of LA, there was no clear evidence of change in heat capacity or LA melting peaks, indicating that in these samples the lauric acid mobility was hindered by the interactions with zein molecules. On the contrary, lauric acid melting point can clearly be identified in the TPZ-based materials with higher LA content (11wt%) (Fig. 1). This suggested that these composites materials are heterogeneous, containing plasticized zein and a fatty acid phase partially crystallized at ambient temperature. The molar percent of exuded lauric acid (compatibility limit) can be calculated by comparing the melting heat capacities obtained for the fatty acid alone and for the plasticized sample (POMMETA *et al.*, 2003). The plasticizing mole contents corresponded to the compatibility limit between zein and LA. As shown in Table 2, the compatibility limit decreased in presence of gluten.

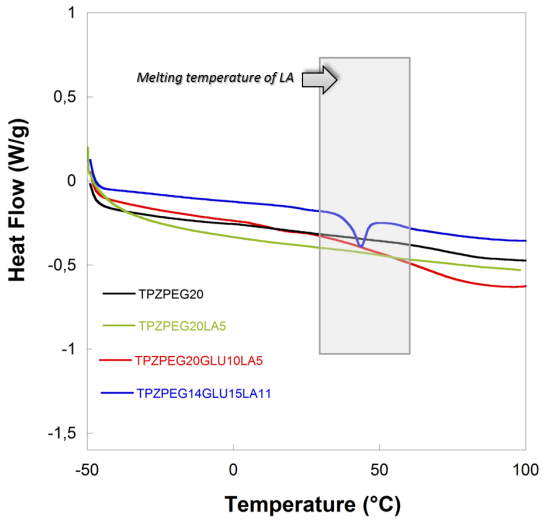

Figure 1. DSC of TPZ-based films.

Table 2. PEL for different edible films.

Edible films	ΔH_f (J/g)	Molar percent of exuded LA (PEL) (%)
TPZPEG25	-	-
TPZPEG20LA5	-	-
TPZPEG20GLU10LA5	-	-
TPZPEG14GLU15LA11	5.3	2.3

The effect of presence and content of LA and GLU on the tensile properties of TPZ and TPZ-based edible films are reported in Table 3. The results highlighted that the use of LA, as partial substitute of PEG plasticizer, combined with the addition of GLU led to higher E (up to 849MPa) and σ_M (up to 12.4) and reduction of ε_B (up to 3%). In particular the values of E and σ_M increased with LA content, while a significant embrittlement (reduction of ε_B) was observed with the increasing of GLU content. This behavior can be attributed to different combined effects: the development of strong interactions (i.e. H bonding) which establishbetween the functional groups of amino acids of the zein with the OH groups of LA (SALERNO *et al.*, 2007) and a reduced plasticization of the bio composite system for the presence of exuded LA and a more content of hydrophobic protein such as GLU.

Table 3. Tensile properties of TPZ- based edible films.

Edible films	E (MPa)	σ_M (MPa)	ε_B (%)
TPZPEG25	268±20	4.2±0.9	105±10
TPZPEG20LA5	290±11	5.3±0.6	89±7
TPZPEG20GLU10LA5	301±19	7.1±0.4	60±6
TPZPEG14GLU15LA11	849±80	12.4±0.5	3±0.8

The water vapor permeability results of TPZ- based edible films containing LA and GLU are shown in Fig. 2 and compared with TPZPEG25. The films containing zein plasticized with PEG have much lower WVP values ($9.5x10^{-11}$ g/m·s·Pa) in comparison with values reported in literature ($8.4x10^{-9}$ g/m·s·Pa) (RYU *et al.,* 2002). Such deviation can be mainly explained by considering the different formulation and procedures for the film preparation. As expected, the partial replacement of PEG by LA in the film formulation affected WVP of TPZ-based films since permeability values decreased by approximately 35%.

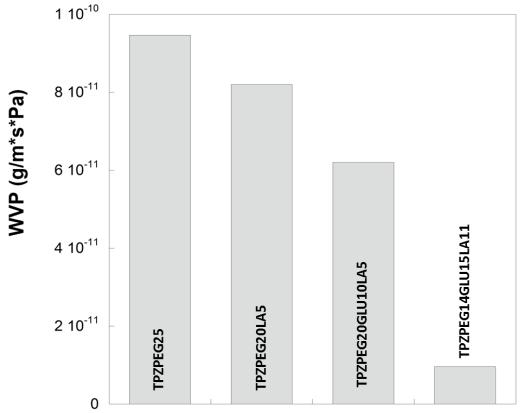

Figure 2. Water vapor permeability (WVP) of TPZ- based edible films.

The existence of specific interactions between LA and zein chains, deduced from the analysis of thermal behavior, was responsible of the enhanced hydrophobicity of the matrix. A significant improvement of the water barrier properties, about 90% decrease, was found for the film named TPZPEG14GLU15LA11 containing the highest content of GLU (15%) and LA (11%) and the lowest content (14%) of PEG, a very hydrophilic plasticizer. This reduction in water permeability is likely to be ascribed to the synergistic effect of three aspects: i) the formation of a network due to the strong interactions between the functional groups of amino acids of the zein with the OH groups of LA (SALERNO *et al.,* 2007) ii) the addition of GLU which exhibits strong hydrophobic features and thus plays a role by repulsing water molecules iii) the reduction of number of polar sites of PEG which brings about a reduction of water sorption and water solubility and thus influences water permeability.

4. CONCLUSIONS

Lauric acid (LA) and gluten (GLU) addition could be used to produce TPZ -based edible films with increased E, σ_M and enhanced water barrier properties. In particular, the addition of GLU decreased the water vapor permeability of TPZ-based edible films, but did not affect mechanical properties. Mechanical and barrier properties were, instead, enhanced with LA addition, due to the existence of specific hydrophobic interactions, as shown from the analysis of thermal behavior. Best compromise between barrier and mechanical properties was observed for the thermoplastic zein-based film with 11% LA and 15% GLU.

REFERENCES

Garcia M.A., Marino M.N. and Zaritzky N.E. 2000. Lipid addition to improve barrier properties of edible starch-based films and coating. J. Food Sci. 65:941-947.

Krochta J.M. and De Mulder-Johnston C. 1997. Edible and biodegradable polymer films: challenges and opportunities. Food Technol. 51:61-77.

Oliviero M., Rizvi R., Verdolotti L., Iannace S., Naguib H.E., Di Maio E., Neitzert H.C. and Landi G. 2017. Dielectric Properties of Sustainable Nanocomposites Based on Zein Protein and Lignin for Biodegradable Insulators, Adv. Funct. Mater. 27(8), DOI 10.1002/adfm.201605142

Padget T., Han I.Y. and Dawson P.D. 2000. Effect of lauric acid addition on the antimicrobial efficacy and water permeability of corn zein films containing nisin. J. Food Process. Preserve. 24:423-432.

Pommeta M., Redl A., Morela M. and Guilberta S. 2003. Study of wheat gluten plasticization with fatty acids. Polymer 44:115-122.

Ryu S.Y., Rhim J.W., Roh H.J. and Kim S.S. 2002. Preparation and physical properties of zein-coated high-amylose corn starch film, LWT-Food Sci. Technol. 35:680-686.

Verdolotti L., Lavorgna M., Oliviero M., Sorrentino A., Iozzino V., Buonocore G. and Iannace S. 2014. Functional zein-siloxane bio-hybrids. ACS Sustain. Chem. Eng. 2:254-263.

Verdolotti L., Oliviero M., Lavorgna M., Iannace S., Camino G., Vollaro P. and Frache A. 2016. On revealing the effect of alkaline lignin and ammonium polyphosphate additives on fire retardant properties of sustainable zein-based composites. Polym. Degrad. Stab. 134:115-125

Were L., Hettiarachchy N.S. and Coleman M. 1999. Properties of cysteine-added soy protein-wheat gluten films. J. Food Sci. 64:514-518.

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