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Processing-induced modifications in bioactive compounds of black garlic: a comparative analysis with white garlic

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Abstract

This study aimed to examine the differences in chemical and bioactive components between black garlic and white garlic under various processing conditions. The processing conditions significantly lowered the pH of black garlic while increasing its total acidity and organic acid levels, with citric acid being the dominant organic acid. Total phenolic compounds (TPC) and antioxidant activity in black garlic increased as a result of processing conditions. Notably, aging caused a significant increase in the levels of 5-hydroxymethylfurfural (HMF) and S-allyl-L-cysteine (SAC). Mineral analyses showed that magnesium and selenium levels in black garlic rose with extended aging. Correlation and Principal Component Analysis (PCA) revealed that the health-beneficial components of black garlic were significantly enhanced through processing, differentiating it from white garlic.

Keywords: antioxidant; black garlic; S-allyl-L-cysteine; PCA; selenium

Introduction

Garlic (*Allium sativum* L.), a member of the Alliaceae family, is known for its shallow root system and various health benefits, and it is recognized as Generally Recognized as Safe (GRAS) (Stępień *et al*., 2024; Utama *et al*., 2024). It is widely used in traditional medicine due to its antioxidant, anti-inflammatory, anticancer, antibacterial, and blood lipid-regulating properties (Wu *et al*., 2024; Stępień *et al*., 2024). The bioactive components of garlic can vary depending on the preparation or processing method. Black garlic is produced by fermenting and aging fresh garlic for 10 to 80 days at controlled temperatures of 60–90°C and humidity levels of 60–80% (Stępień *et al*., 2024; Augustyńska-Prejsnar *et al*., 2023; Matsuse *et al*., 2024; Liu *et al*., 2024). During this process, white garlic loses its pungency due to the breakdown of the alliin compound, developing a sweet-sour taste and becoming odorless (Stępień *et al*., 2024). As black garlic forms, the allicin compound transforms into the water-soluble antioxidant S-allyl-L-cysteine, which is known for its antioxidant, anticarcinogenic, neuroprotective, anti-allergic, antidiabetic, and anti-inflammatory effects (Augustyńska-Prejsnar *et al*., 2023; Wu *et al*., 2024; Santos *et al*., 2024; Stępień *et al*., 2024). The fermentation and aging process also leads to the formation

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of 5-hydroxymethylfurfural (5-HMF), an intermediate of the Maillard reaction, which has been linked to various health concerns, including carcinogenicity, tumor formation, and liver and kidney toxicity, with an LD50 value reported as 871.12 mg kg^{-1} (Liu *et al*., 2024). Selenium, a crucial micronutrient obtained externally because the human body cannot synthesize it, plays significant roles in the immune system and body development. Selenium has been shown to have anti-aging properties (Bjørklund *et al*., 2022), enhance immune function (Avery and Hoffmann, 2018), prevent cardiovascular diseases (Jenkins *et al*., 2020), and exhibit anti-tumor effects (Ghose *et al*., 2001). Most research has focused on optimizing black garlic production by studying organosulfur compounds, polysaccharides, polyphenols, and other components. However, a comprehensive study using principal component analysis (PCA) to differentiate black garlic is still lacking.

This study aimed to investigate the chemical and bioactive changes in black garlic under varying processing conditions. Specifically, it analyzed changes in pH, total acidity, organic acids, total phenolic content (TPC), antioxidant activity (DPPH and ABTS), mineral content, hydroxymethylfurfural (HMF), and S-allyl-L-cysteine (SAC) levels in black garlic and compared these changes to those in white garlic. Additionally, the study assessed the chemical composition changes in black garlic during different aging periods using correlation analysis and principal component analysis (PCA). The goal was to explore the relationships between the chemical components of black garlic under various processing conditions and their differences from white garlic, thereby contributing to the evaluation of black garlic's potential as a functional food by revealing how its biological and nutritional values change with processing.

Materials and Methods

Araban garlic, which is cultivated in Turkey and has received geographical indication, was used as the material in this study. Araban garlic was collected from a local seller during the June harvest period.

Production of black garlic

Araban garlic was weighed at 250 g. The weighed fresh garlic was then placed in a climate conditioning device (Nüve, TK600) at Atatürk University Food Engineering Laboratories and subjected to an aging process for 10, 20, and 30 days at 60°C and 80% relative humidity to obtain 10-day (BG10), 20-day (BG20), and 30-day (BG30) samples (Zhang *et al.*, 2015).

Method

pH analysis

The pH was determined according to Kang (2016).

Total acidity analysis

Total acidity was measured using acid-base titration as described by Leng *et al*. (2020). In summary, five grams of garlic samples were weighed, crushed, and then diluted to 100 mL with distilled water. A few drops of phenolphthalein indicator were added, and the mixture was titrated with 0.1 mol L^{-1} NaOH solution until a pink color appeared, at which point the volume of NaOH used was recorded.

Extraction method

Phenolic contents are extracted using either organic or inorganic solvents, with the efficiency of the extraction being influenced by the choice of solvent (Takım, 2020). Ağbaş *et al*. (2013) found that ethanol extraction demonstrated superior activity in terms of both phenolic and antioxidant components. The extraction procedure was adapted from the methods described by Choi *et al*. (2014) and Takım (2020). To prepare the garlic samples, they were peeled, chopped into small pieces, and then crushed using a mortar and pestle. The crushed garlic was subsequently lyophilized. The lyophilized garlic was mixed with 50% methanol-water at a garlic-to-solvent ratio of 1:10, subjected to ultrasonic treatment in a water bath for 15 min, and then centrifuged at 6000 rpm for 10 min. The resulting extract was filtered through Whatman No. 1 filter paper and used for the analysis of total phenolic content, DPPH radical scavenging activity, and ABTS assays.

For the extraction process used in organic acid and HMF analyses, 5 g of the sample was soaked in 15 mL of distilled water at +4°C for 24 h. The sample was then homogenized using an Ultraturrax for 3 min. From this homogenized mixture, 5 g was transferred to a 50 mL volumetric flask, treated in an ultrasonic water bath for 30 min, and then centrifuged at 2000 g for 15 min. Subsequently, 10 mL of the supernatant was collected, to which 0.5 mL of Carez 1 (potassium hexacyanoferrate (II)) and Carez 2 (zinc sulfate heptahydrate) solutions were added sequentially. The mixture was then filtered through a 0.45 µm filter paper and analyzed using an HPLC device. Results are presented on a dry weight basis.

Organic acid analysis

The analysis of organic acids was performed with modifications based on the method described by Castellari *et al*. (2000). Organic acid analyses were conducted using an HPLC RID system (Agilent 1260 Infinity Series, Agilent Technologies, Waldbronn, Germany) equipped with a MetaCarb H Plus column. The mobile phase consisted

of 0.045 N sulfuric acid and water in a 99:1 ratio, under isocratic conditions. The flow rate was set at 0.6 mL min^{-1}, with a column temperature of 30°C, a detection wavelength of 210 nm, an injection volume of 10 µL, and a total run time of 40 min. The analysis allowed for the quantification of citric acid, lactic acid, and propionic acid.

Total phenolic content

The TPC of the samples was determined according to Binici *et al*. (2021). Briefly, 0.100 mL of the white garlic and black garlic extracts were taken and sequentially mixed with 0.800 mL of 0.2 N Folin-Ciocalteu reagent (FCR), 0.800 mL of 10% sodium carbonate (Na_2CO_3), and 0.800 mL of distilled water. The final volume was adjusted to 2.5 mL. Total phenolic content was determined using a spectrophotometer (PG Instrument TV-60) at 760 nm, with gallic acid (GA) as the standard. The samples were kept in the dark for 30 min before measurement at 760 nm.

DPPH assay

The DPPH assay was carried out with modifications based on the method described by Binici *et al*. (2021). In this procedure, 0.02 mM $DPPH^-$ was weighed and dissolved in 100 mL of ethanol. The prepared extracts were adjusted to various concentrations, with the final volume set to 2.5 mL. The mixture was then incubated in the dark for 30 min and measured at a wavelength of 517 nm using a spectrophotometer.

ABTS assay

The $ABTS^+$ assay for garlic samples was conducted with modifications based on the methods outlined by Tahir (2022). To prepare the $ABTS^+$ solution, 7 mM $ABTS^+$ was combined with 5 mL of 2.45 mM potassium persulfate, resulting in a total volume of 10 mL. This mixture was placed in a foil-wrapped bottle and left in a dark environment for 16 h to allow stabilization. The stabilized solution was then diluted with ethanol to achieve an absorbance of 0.700 at 734 nm. For the assay, 10 µL of garlic extract was added to 1.990 mL of the $ABTS^+$ solution, mixed thoroughly, and after a 6-min incubation period, the absorbance was measured at 734 nm using a spectrophotometer. The concentration of the extracts that achieved 50% $ABTS^+$ radical scavenging activity (IC_{50}) was determined from the graph plotting extract concentration against radical scavenging activity (%) and was expressed in mg mL^{-1}.

Mineral content

Mineral content was determined according to Zor *et al*. (2024). Briefly, approximately 0.5 g of the sample was subjected to digestion using a mixture of 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2, making a total of 8 mL of acid. Following the digestion process, the sample volume was adjusted to 14 mL. From the diluted sample, 1 mL was taken and further diluted to 16 mL, resulting in a 16-fold dilution. These dilutions were incorporated into the final calculations. The results are expressed in ppm for Mg, Ca, and Fe, and in ppb for Cu and Se.

5-HMF analysis

A novel method for HMF analysis was established using HPLC, featuring an Ace C18 column (5 µm, 250 × 4.6 mm). The analysis was performed with a mobile phase composed of methanol and water (80:20, v/v), a flow rate of 1 mL min^{-1}, an injection volume of 5 µL, and detection at a wavelength of 284 nm. The identification of HMF was accomplished using an HMF standard. A calibration curve was generated by plotting the peak areas against the concentrations of HMF solutions prepared at various concentrations.

SAC analysis

The extraction process was conducted by combining and modifying the methods described by Chen *et al*. (2020) and Bae *et al*. (2012). In this procedure, 1 g of garlic samples was dissolved in 10 mL of distilled water (1:10 w/v) and subjected to ultrasonic treatment in a water bath for 30 min. The mixture was then filtered through Whatman No. 2 filter paper, and the resulting filtrate was further filtered using a 0.45 µm syringe filter. Subsequently, 20 µL of the filtrate was injected into an HPLC-UV-DAD system. For the analysis of S-allyl-L-cysteine, standard solutions were derivatized with dansyl chloride in a basic borate buffer. A 100 µg mL^{-1} S-allyl-L-cysteine stock solution was initially prepared in methanol, and standard solutions ranging from 0.1 to 50 µg mL^{-1} were prepared from this stock. To each standard solution, 0.1 mL was taken, followed by the addition of 0.25 mL dansyl chloride and 0.65 mL borate buffer (pH 9.2). The mixture was vortexed for 30 s and left to react at room temperature for 15 min. The resulting turbid solutions were filtered through a 0.22 µm filter, producing final standard concentrations ranging from 0.01 to 5 µg mL^{-1} after dilution. A new HPLC method was developed for the analysis of S-allyl-L-cysteine, utilizing an Ace C18 column (5 µm, 150 x 4.6 mm) with a mobile phase consisting of 50 mM sodium acetate buffer (pH 5) and methanol (35:65, v/v). The analysis was performed at a flow rate of 1 mL min^{-1}, with an injection volume of 10 µL and detection at 250 nm.

Statistical analysis

The data collected in the study were analyzed using correlation and variance analyses with IBM SPSS Statistics 25.0 software (IBM, Armonk, New York). Duncan's multiple comparison tests were performed to identify significant differences between the groups at the p = 0.01 and 0.05 levels. To identify groupings among the black garlic

samples, principal component analysis (PCA) was conducted using SIMCA 14.1 software (MKS UMETRICS, Umea, Sweden).

Results

The pH, total acidity, and organic acid content of black garlic (BG) processed under various conditions are presented in Table 1. Compared to white garlic (WG), the total acidity of BG increased significantly, while its pH decreased markedly from 4.64 to 3.86, compared to 6.53 in WG ($p < 0.01$). Analysis of the organic acids in BG revealed an increase in their levels due to different processing conditions, compared to WG (Table 1, $p < 0.01$). Citric acid emerged as the most abundant organic acid, with levels ranging from 246.83 to 425.13 mg kg^{-1} in BG, compared to 283.02 mg kg^{-1} in WG.

The total phenolic content (TPC) and antioxidant levels of BG under different processing conditions are provided in Table 2. The TPC in WG was 16.88 mg GAE g^{-1}, while in BG, it ranged from 25.44 to 46.27 mg g^{-1}. The DPPH IC_{50} values varied between 4.67 and 23.88 mg mL^{-1}. In WG, the DPPH IC_{50} was 23.88 mg mL^{-1}, which decreased in BG due to different processing conditions, indicating enhanced antioxidant activity (Table 2). There is an inverse relationship between IC_{50} values and antioxidant activity, with higher IC_{50} values indicating lower antioxidant activity (Binici *et al*., 2024). In our samples, BG aged for thirty days exhibited higher antioxidant activity than WG, with a DPPH IC_{50} value of 4.67 mg mL^{-1}. The ABTS IC_{50} value was 5.02 mg mL^{-1} in WG, while in BG, it ranged from 2.64 to 3.57 mg mL^{-1} (Table 2). As the aging time increased, the ABTS IC_{50} value decreased, indicating a rise in antioxidant activity.

The mineral content of BG under different processing conditions is shown in Table 3. Magnesium was the most abundant mineral, while selenium was the least, as presented in Table 3. Generally, the mineral content of BG increases with aging. The magnesium content in WG was 27.10 ppm, while in BG, it ranged from 27.56 to 31.16 ppm, increasing with longer maturation times.

The HMF and SAC content of BG under different processing conditions are presented in Table 4. While HMF was not detected in WG, it was found in BG in amounts ranging from 57.63 to 70.86 mg kg^{-1}, with levels increasing as aging time extended.

Separation of garlic samples using correlation and principal component analysis (PCA)

The correlation matrix for black garlic under different processing conditions is presented in Table 5. A strong negative correlation exists between pH and total acidity (–0.992) as well as pH and TPC (–0.902). This suggests that as pH values increase, total acidity and TPC decrease. Additionally, pH values are significantly correlated with antioxidant activity, mineral content, HMF, and SAC levels. A strong positive correlation is observed between selenium content and HMF (0.747), SAC (0.770), and propionic acid (0.838), indicating that an increase in selenium levels is associated with an increase in HMF, SAC, and propionic acid concentrations. Figure 1–1C display the PCA plots for black garlic processed under different conditions. The combined contribution of PC1 and PC2 accounted for 94.7% of the total variance, indicating that the results obtained in this study are well-suited for PCA, making the method effective and useful for analysis. White garlic samples were more distinctly separated compared to black garlic (Figure 1A). TPC, Fe, Cu, Mg, and citric acid clustered on the left side of the plot (Figure 1B). Furthermore, it was observed that black garlic aged for thirty days had higher levels of HMF, Se, SAC, TPC, Fe, Mg, Cu, total acidity, propionic acid, and citric acid compared to white garlic (Figure 1C).

Table 1. pH, total acidity and organic acid content of garlic according to different processing conditions.

Samples/analyses	pH	Total acidity (g 100^{-1})	Organic acids		
			Citric acid (mg kg^{-1})	Lactic acid (mg kg^{-1})	Propionic acid (mg kg^{-1})
WG	6.53±0.01^{a}	0.43±0.04^{d}	283.02±3.09^{c}	11.02±0.27^{b}	16.42±0.40^{d}
BG10	4.64±0.01^{b}	1.24±0.01^{c}	246.83±0.15^{d}	7.23±0.03^{d}	61.27±0.25^{c}
BG20	4.25±0.01^{c}	1.33±0.01^{b}	314.88±0.04^{b}	8.44±0.23^{c}	124.64±0.64^{b}
BG30	3.86±0.02^{d}	1.42±0.01^{a}	425.13±3.91^{a}	17.20±0.33^{a}	138.09±0.15^{a}
Sig.	**	**	**	**	**

n:3, Data shown as mean ± standart deviation. Values on the same column with different superscript letters are statistically different. **: p<0.01. Results are given on dry weight basis. WG: white garlic, BG10: aged black garlic 10 days, BG20: aged black garlic 10 days, BG30: aged black garlic 30 days.

Table 2. TPC and antioxidant content of garlic according to different processing conditions.

Samples/analyses	TPC (mg GAE g^{-1})	DPPH IC50 (mg mL^{-1})	ABTS IC50 (mg mL^{-1})
WG	16.88±0.37^{d}	23.88±0.15^{a}	5.02±0.03^{a}
BG10	25.44±0.73^{c}	20.92±0.17^{b}	3.57±0.01^{b}
BG20	39.86±0.29^{b}	9.44±0.20^{c}	2.99±0.05^{c}
BG30	46.27±0.19^{a}	4.67±0.36^{d}	2.64±0.02^{d}
Sig.	**	**	**

n:3, Data shown as mean ± standart deviation. Values on the same column with different superscript letters are statistically different. **: p<0.01. Results are given on dry weight basis. WG: White Garlic, BG10: aged black garlic 10 days, BG20: aged black garlic 10 days, BG30: aged black garlic 30 days.

Table 3. The mineral content of garlic according to different processing conditions.

Samples/analyses	Mg (ppm)	Cu (ppb)	Se (ppb)	Ca (ppm)	Fe (ppm)
WG	27.10±0.62^{b}	632.10±70.17^{b}	100.32±25.23^{b}	2.10±0.03^{a}	0.64±0.07^{a}
BG10	27.56±2.51^{b}	646.20±61.56^{b}	142.65±51.02^{ab}	1.73±0.03^{b}	0.66±0.05^{a}
BG20	27.58±0.36^{b}	859.23±20.39^{a}	203.13±30.94^{a}	1.71±0.02^{b}	0.78±0.03^{a}
BG30	31.16±0.42^{a}	889.23±22.05^{a}	207.63±28.95^{a}	1.71±0.02^{b}	0.74±0.07^{a}
Sig.	*	**	*	**	ns

n:3, Data shown as mean ± standart deviation. Values on the same column with different superscript letters are statistically different. **: p<0.01, ppm: mg kg^{-1}, ppb: µg kg^{-1}. Results are given on dry weight basis. WG: White Garlic, BG10: aged black garlic 10 days, BG20: aged black garlic 10 days, BG30: aged black garlic 30 days.

Table 4. 5-HMF and SAC amounts of garlic according to different processing conditions.

Samples/analyses	5-HMF (mg kg^{-1})	SAC (ug g^{-1})
WG	nd	11.98±0.24^{d}
BG10	57.63±0.02^{c}	31.90±0.15^{c}
BG20	63.86±0.16^{b}	35.13±0.03^{b}
BG30	70.86±0.15^{a}	43.96±0.09^{a}
Sig.	**	**

n:3, Data shown as mean ± standart deviation. Values on the same column with different superscript letters are statistically different. **: p<0.01, nd: not determined. Results are given on dry weight basis. WG: White Garlic, BG10: aged black garlic 10 days, BG20: aged black garlic 10 days, BG30: aged black garlic 30 days.

Discussion

This finding aligns with the study by Choi *et al*. (2014), which reported a pH reduction in BG from 6.33 to 3.74 after 35 days of aging. Similarly, Shin *et al*. (2008) found that the pH of BG decreased from 6.40 to 5.29. Sasmaz *et al*. (2022) identified various organic acids in both WG and BG, including lactic, citric, pyruvic, malic, succinic, oxalic, fumaric, pyroglutamic, acetic, and ascorbic acids. Previous studies have shown that citric, formic, malic, lactic, and fumaric acids are more prevalent in WG (Bonasia *et al*., 2020; Liang *et al*., 2015; Petropoulos *et al*., 2018). The increase in organic acids in BG relative to WG is likely attributed to the formation of short-chain carboxylic acids through alkali group breakdown and the Maillard reaction during processing (Ahmed and Wang, 2021). Due to limited studies on organic acids in BG, comprehensive data is scarce. The TPC increased as the aging process progressed, consistent with findings by Choi *et al*. (2014), who observed an increase from 25.81 mg GAE g^{-1} to 58.25 mg GAE g^{-1} after 35 days of aging. Wu *et al*. (2007) also reported that heat treatment increased free phenolic acids and compounds while decreasing ester, glycoside, and ester-bound fractions, leading to a rise in free phenolic forms. Chua *et al*. (2022) reported DPPH IC_{50} values for BG aged 12 days ranging from 3.91 to 16.31 µg mL^{-1}, compared to 24.12 µg mL^{-1} in fresh garlic. Our DPPH IC_{50} values were higher than those found in other studies, possibly due to differences in extraction methods and garlic varieties. Choi *et al*. (2014) reported ABTS values in BG ranging from 92.43 to 245.45 mM TE g^{-1} after 35 days of aging, with an increase observed on days other than the 35th day. Kim *et al*. (2012) found the ABTS IC_{50} value in BG to be 6.5 mg mL^{-1}. Various methods are used to assess the antioxidant activity of naturally occurring bioactive substances in foods, with DPPH and ABTS assays being widely employed for this purpose due to their simplicity and ease of application (Choi *et al*., 2014; Kim *et al*., 2012). Šnirc *et al*. (2023) reported

Table 5. Correlation table of garlic according to different processing conditions.

	pH	Total acidity (%)	TPC (mg g^{-1})	DPPH IC_{50} (mg mL^{-1})	ABTS IC_{50} (mg mL^{-1})	Mg (ppm)	Cu (ppb)	Se (ppb)	Ca (ppm)	Fe (ppm)	HMF (mg kg^{-1})	SAC (µg g^{-1})	Citric acid (mg kg^{-1})	Lactic acid (mg kg^{-1})
Total acidity (%)	−0.992**													
TPC (mg g^{-1})	−0.902**	0.848**												
DPPH IC50 (mg mL^{-1})	0.838**	−0.771**	−0.990**											
ABTS IC50 (mg mL^{-1})	0.993**	−0.974**	−0.943**	0.892**										
Mg (ppm)	−0.531	0.456	0.647*	−0.648*	−0.559									
Cu (ppb)	−0.743**	0.671*	0.921**	−0.934**	−0.801**	0.648*								
Se (ppb)	−0.775**	0.750**	0.822**	−0.808**	−0.809**	0.271	0.708**							
Ca (ppm)	0.965**	−0.982**	−0.782**	0.696*	0.939**	−0.419	−0.626*	−0.714**						
Fe (ppm)	−0.589*	0.544	0.674*	−0.695*	−0.631*	0.201	0.693*	0.480	−0.522					
HMF (mg kg^{-1})	−0.994**	0.999**	0.853**	−0.777**	−0.977**	0.473	0.682*	0.747**	−0.984**	0.556				
SAC (µg g^{-1})	−0.990**	0.970**	0.920**	−0.864**	−0.989**	0.616*	0.757**	0.770**	−0.930**	0.562	0.974**			
Citric acid (mg kg^{-1})	−0.543	0.441	0.809**	−0.862**	−0.614*	0.771**	0.790**	0.583*	−0.332	0.464	0.452	0.633*		
Lactic acid (mg kg^{-1})	0.255	−0.150	−0.644*	0.737**	0.360	−0.443	−0.752**	−0.486	0.055	−0.519	−0.157	−0.301	−0.815**	
Propionic Acid (mg kg^{-1})	−0.927*	0.882**	0.993**	−0.974**	−0.962**	0.580*	0.908**	0.838**	−0.830**	0.708**	0.887**	0.929**	0.736**	−0.595*

**: p<0.01, *: p<0.05.

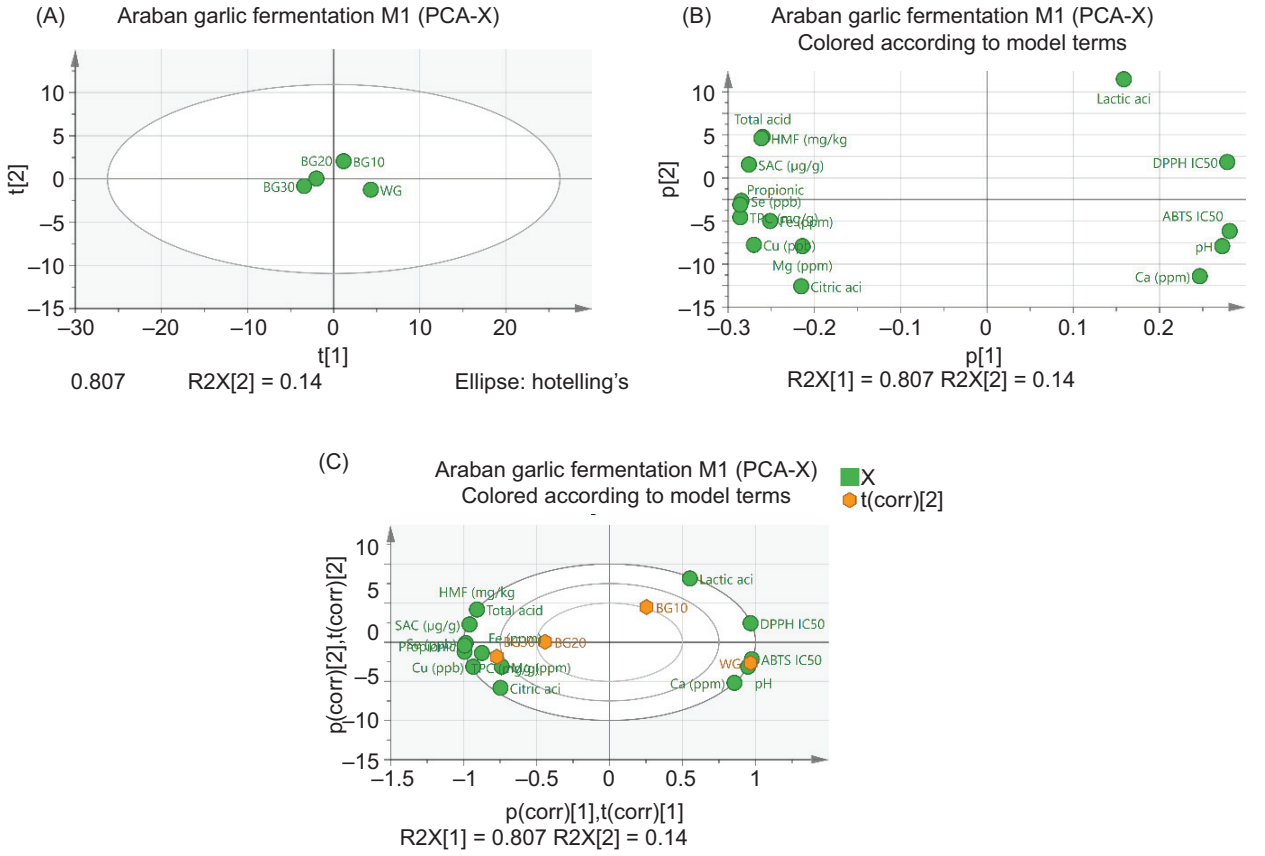

Figure 1. PCA values of garlic according to different processing conditions.

magnesium levels in fresh garlic samples from different cultures between 453.70 and 520.10 mg kg^{-1}. Chua *et al.* (2022) found magnesium content in BG aged 12 days to range from 202 to 385.1 µg mL^{-1}. The increase in mineral content is likely due to weight loss in BG caused by the evaporation of water and volatile compounds during maturation. Kang (2016) suggested that the increase in minerals during BG fermentation was due to variations in temperature and humidity. Selenium, although once considered toxic in the 1950s, is now recognized as vital for preventing diseases like coronary heart disease, Type 2 diabetes, and liver disorders (Genchi *et al.*, 2023). Selenium's presence in BG is particularly noteworthy, as it plays an important role in human health and, given BG's lack of odor, suggests it may be suitable for consumption. HMF is a five-carbon aromatic aldehyde with antioxidant properties formed during Maillard reactions, which are accelerated by high temperatures and acidic conditions. This likely explains the observed increase in HMF levels (Chua *et al.*, 2022). The SAC content in WG was 11.98 µg g^{-1}, while in BG, it ranged from 31.90 to 43.96 µg g^{-1}, increasing with longer aging times. Saputra *et al.* (2024) found SAC, a bioactive compound, to be present at 122 µg g^{-1} in fresh garlic and 335.46 µg g^{-1} in BG. In our study, SAC levels in BG were approximately 3.7 times higher than in WG. Bae *et al.* (2014) reported a 5.78-fold increase in SAC content in BG compared to fresh garlic. The increase in SAC content is likely influenced by various factors, including processing temperature, humidity, duration, variety, and garlic species used in BG production (Manoonphol *et al.*, 2023).

Conclusions

This study demonstrated that black garlic exhibited significant differences in chemical and bioactive components compared to white garlic, which were influenced by processing conditions. The pH of black garlic decreased markedly, while total acidity and organic acid levels, particularly citric acid, increased significantly. Aging enhanced the levels of total phenolic compounds (TPC), antioxidant activity, 5-hydroxymethylfurfural (HMF), and S-allyl-L-cysteine (SAC) in black garlic. Mineral analysis revealed that magnesium and selenium contents increased with aging. Correlation and Principal Component Analysis effectively identified relationships between pH, total acidity, TPC, antioxidant activity, and mineral content, highlighting the differentiation of black garlic from white garlic. In conclusion, black garlic can be

considered a functional food due to its enriched phenolic content and antioxidant activity. These findings support the health benefits of black garlic and suggest the need for further in-depth research in this area.

Author Contributions

Halil İbrahim Binici: Conceptualization, Investigation, Methodology, Writing - original draft, Project administration. Adem Savaş Conceptualization, Writing - original draft, Methodology, Validation, Visualization. Burak Erim Methodology, Conceptualization, Supervision.

Conflict of Interest

The author's declare no conflicts of interest.

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