Changes in bioactive compounds and antioxidant activity of Gaziantep and Kastamonu garlic during black garlic production

Hatice Kubra SASMAZ¹ • Turkan UZLASIR¹ • Serkan SELLI² • Hasim KELEBEK¹

¹ Department of Food Engineering, Faculty of Engineering, Adana Alparslan Turkes Science and Technology University, 01250 Adana, Türkiye

² Department of Food Engineering, Faculty of Agriculture, Cukurova University, 01330 Adana, Türkiye

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Correspondence: Haşim Kelebek E-mail: hkelebek@atu.edu.tr

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Abstract

Garlic (Allium sativum L.), a member of the Alliaceae family, has been widely used in cuisine and traditional medicine since ancient times. Black garlic is produced by fermentation of fresh garlic under controlled conditions for a certain period at high temperature (60-90°C) and high humidity (70-90%). According to the Turkish Statistical Institute (TURKSTAT) data, Kastamonu and Gaziantep garlic varieties are the most cultivated garlic varieties in our country. Changes in protein, sugar content, antioxidant capacity (DPPH and ABTS methods), total phenolic content, 5-hydroxymethylfurfural (HMF) content, and organosulfur compound profiles were investigated in samples taken from Kastamonu and Gaziantep fresh garlic at 7, 14, 21, and 28 days of black garlic production under 65°C temperature and 70% humidity conditions. With these analyses, the differences between black garlic and fresh garlic and the changes in black garlic during the production process were revealed in detail. It was determined that the amount of total phenolic content and antioxidant capacities increased in the black garlic production processes of both regions compared to fresh garlic. While sucrose was fresh garlic's dominant sugar, fructose was black garlic's dominant sugar. Among the organosulfur compounds, allicin was dominant in fresh garlic and SAC in black garlic. It was determined that SAC was formed after the enzymatic conversion of y-glutamyl-S-alk(en)yl-L-cysteine and y-glutamyl and the temperature and fermentation time used in black garlic production increased the formation of SAC. The protein content ranging between 5.8%-7.3% in fresh garlic was 13.1-14.2% in black garlic. Fresh and black garlic from the Gaziantep region was determined to have higher total phenolic content, antioxidant capacity, and organosulfur compound contents. Keywords: Black garlic, Antioxidant capacity, HMF, Organosulfur compounds

INTRODUCTION

Garlic (*Allium sativum L.*), a member of the Lilliaceae family, is considered to be the most important vegetable produced in this family after onion (Kim et al., 2004, Hamma, 2013). According to FAO data, garlic constitutes 2.4% of the 1.14 billion tons of vegetables produced in the world in 58.3 million hectares in 2020. Worldwide, 28 million tons of garlic are produced on 1.6 million hectares (FAOSTAT, 2020). Garlic is a vegetable grown in many regions of our country and can adapt well to the environmental conditions of our country. According to TURKSTAT data, more than 136 thousand tons of garlic were produced in our country in 2020. Kastamonu province ranks first with 23 thousand tons of dry garlic production.

Garlic and its products are widely consumed due to their unique taste and rich

nutritional value (Condurso et al., 2019; Tao et al., 2016; Zhang et al., 2016; Huang et al., 2015). This versatile ingredient has been a staple in global cuisine for millennia, valued not only as a food item but also for its medicinal properties (Moutia et al., 2018). Garlic contains organosulfur compounds such as allicin, alliin, and ajoene, as well as antioxidant-rich phenolic compounds such as catechin, epicatechin, resveratrol, coumaric, chlorogenic acid, carbohydrates (sucrose, glucose), minerals, amino acids, and vitamins such as vitamin A, B1, B2, niacin, and C (Raghu et al., 2012).

The health effects of garlic are attributed to its bioactive compounds, particularly organosulfur compounds that cause bitterness and a pungent odour. Studies have shown that garlic consumption has potential benefits against chronic diseases such as cancer and diabetes (Oyawoye et al., 2022; Queiroz et al., 2009). The characteristic odor of garlic makes it not widely preferred in the food industry despite its potential health benefits. Many people hesitate to consume garlic because of its odor. Black garlic has been developed as an attractive alternative for consumers who want to enjoy the health benefits of garlic. Black garlic has a milder odor and a more pleasant texture. Black garlic is produced by heat treatment of fresh garlic under controlled temperature (60-90 °C) and high humidity (50-95%) without any extra processing or additives (Zhang, et al. 2016). Many reactions occur during the production of black garlic. Depending on these reactions, carbohydrates, organosulfur compounds, amino acids, polyphenols and other antioxidant compounds in fresh garlic undergo significant changes. As a result of this change, studies have determined that the bioactivity of black garlic is higher than fresh garlic (Kim et al., 2011; Qiu et al., 2020). Various changes occur during the fermentation stages in black garlic production. The most important of these changes is the increase in the amount of S-allyl cysteine (SAC) compound as a result of the fermentation process (Haber et al., 1996; Atanasova-Goranova et al., 1997). During the heat treatment of garlic, the Maillard reaction changes the nutrient content, color, texture, and flavor of garlic (Yuan et al., 2016).

This study aims to investigate the biochemical composition, antioxidant capacity, HMF, and sugar content of fresh garlic grown in the Gaziantep and Kastamonu regions of Turkiye during black garlic production. For this purpose, samples were taken on different days of the fermentation process, evaluations were made and changes in protein and sugar content, antioxidant capacity (DPPH and ABTS methods), total phenolic matter content, HMF content, and organosulfur compound profiles were determined.

MATERIALS AND METHODS

Material

Kastamonu fresh garlic samples were obtained from a local market in Taşköprü city of Kastamonu province and Gaziantep fresh garlic samples from the Araban city of Gaziantep province. The specimens were then subjected to a controlled process of black garlic production, which involved exposure to specific temperature and humidity conditions. During the production of black garlic, fresh garlic samples were subjected to temperatures of 65 °C, humidity levels of 70%, and kept in a humidity chamber (HCP105, Memmert GmbH + CO, KG, Germany) for 28 days.

Chemicals

Methanol, sodium hydroxide, ethanol, acetonitrile, Folin–Ciocalteu reagent, and formic acid were purchased from Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, Folin-Ciocalteu phenol reagent, sodium carbonate, Gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, ABD). Sucrose, glucose, and fructose were procured from Merck Company (Darmstadt, Germany). Deionized water was utilized in HPLC to prepare the mobile phases. Standard solutions and other sensitive solutions were prepared daily.

Protein determination

Crude protein content was determined by the Kjeldahl method AOAC (1997).

Sugar determination

For sugar analyses, 2 g of sample was taken, and 20 ml of water was added and extracted with a magnetic stirrer for 30 minutes. At the end of this time, the extract obtained was centrifuged (Hettich Universal 320R) at 5500 rpm at 4°C and the upper clear part was removed and filtered through 0.45 µm filters (Lee and Coates, 2000). The filtrate obtained was injected into the Shimadzu Prominence-I HPLC device and the sugar amounts in the samples were determined. Aminex HPX-87H (300 x 7.8 mm) (Bio-Rad, California, USA) column and 5 mM sulphuric acid solution were carrier phases. The sugar concentrations were determined in the samples by an external standard method using RID (Refractive Index Detector). For this purpose, calibration solutions were prepared from sucrose, glucose, and fructose standards at 5 different concentrations, and sugar amounts were calculated according to the calibration curve.

Determination of antioxidant capacity

Two different methods (DPPH and ABTS) were applied to assess the antioxidant capacity of garlic samples. The ability to suppress free radicals was assessed in a UV-Vis (Agilent Cary 60) spectrophotometer at 515 nm using DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995; Kelebek et al., 2009). The ABTS assay was carried out by the method given by Saafi et al. (2009). By combining it with 2.45 mM potassium bisulfate and leaving it in the dark for 12-16 hours, a 7 mM solution of ABTS (2,2'-azino-bis [3-ethylbenzthiazoline-6-sulfonic acid]) was created. The solution was diluted with sodium acetate (pH 4.5) buffer to an absorbance of 0.70 0.01 at 734 nm using a UV-Vis spectrophotometer. The extract was then combined with 2.98 mL of the prepared buffer and incubated in the dark for 20 minutes at room temperature. The absorbance at 734 nm was then measured with a UV-Vis spectrophotometer (Agilent-Cary 60). The Trolox calibration curve was utilized to calculate antioxidant activity, and the results were expressed as mol Trolox per 100 g.

Total Phenolic Compounds (TPC) Analysis

TPC analysis was performed using the Folin-Ciocalteu reagent method, as described by Singleton and Rossi (1965). A spectrophotometer cuvette was filled with 200 L of the extract/standard solution and 1.5 mL of Folin-Ciocalteu reagent (1:10). After five minutes, 1.5 mL of 6% sodium carbonate solution was added to the tubes, which were then stored in the dark for 90 minutes at room temperature. A UV-Vis spectrophotometer was used to detect absorbance at 765 nm. We generated a 500 ppm gallic acid solution for the calibration curve and reported the results in mg/100 g.

Determination of HMF content

Hydroxymethylfurfural (HMF) levels in fresh and black garlic samples were determined using the method published by Kelebek et al. 2016. In brief, 0.4 g of the substance was mixed with 5 ml of distilled water. The mixture was then concentrated to 10 mL using 100 L of Carrez 1 and 100 L of Carrez 2. The resulting blend was ultrasonically treated for 30 minutes in a water bath before centrifuging at 5500 rpm at 4 °C. The filtrate was directly fed into an Agilent 6430 Triple Quadruple mass spectrometer equipped with high-performance liquid chromatography (LC-DAD-ESI-MS/ MS) on a Phenomenex Luna C18 column (250 4.6 mm, 5 m) (Torrance, California, USA). A positive ion electrospray ionization (G1948 B) source is used in the spectrometer (Agilent 6430 LC-MS/MS). The detected ions for the HMF complex were m/z 109 and m/z 127. For quantification, the signal response of an ion with a m/z of 109 was observed. Chromatograms at 285 nm detection wavelength were used to collect data (Kelebek et al. 2016).

Analysis of organosulfur compounds

The extraction of the samples was prepared according to the method of Zhu et al. (2016) with modifications. Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) connected to an Agilent 1260 LC with a Diode Array Detector was used to identify organosulfur compounds. Organosulfur compounds were determined by LC-DAD-ESI-MS/MS using a Phenomenex Luna C18 column (250 x 4.6 mm, 5 µm) (Torrance, California, USA) in positive mode.

Statistical analysis

Statistical analysis was performed by One-Way ANOVA using SPSS 22.0 (version 22, SPSS Inc., Chicago, IL, USA). Duncan's test measured differences in the content levels of the results and means with *p*-values less than 0.05 were indicated to be statistically significant.

RESULTS AND DISCUSSION

Protein content

The protein contents of fresh garlic grown in the Gaziantep and Kastamonu regions and black garlic produced from this garlic on the 7th, 14th, 21st, and 28th days are given in Table 1. While the amount of protein in Gaziantep and Kastamonu fresh garlic was 5.76% and 7.33%, respectively, it was determined that there was a 2-fold increase in the amount of protein until the 21st day in the black garlic production process, and a decrease was observed after the 21st day. Nassur et al. (2017) reported the protein content of fresh garlic as 10.62% and the protein content of black garlic as 11.75% in their study. Liu et al. (2018) found that the protein content of fresh garlic was 5.32% and the protein content of black garlic was 10.26%.

	Protein (%)	Sucrose (g/kg)	Glucose (g/kg)	Fructose (g/kg)	Total sugar (g/kg)	HMF (g/ kg)
Gaziantep fresh garlic	5.86 ± 0.67^{t}	3.43±0.19 [†]	2.81±0.27 [′]	1.35±0.01 [′]	7.60±0.07 ^j	Nd
G7	8.34±0.11 ^{de}	35.37±0.23 [°]	7.72±0.04 ⁹	32.38±0.04 ⁹	75.48±0.22 ⁹	0.09±0.01 ^f
G14	8.27±0.21 ^d	34.92±0.02 [°]	9.74±0.03 ^f	68.98±0.07 ^f	113.66±0.07 ^f	0.14±0.01 [°]
G21	12.59±0.66 [°]	22.85±0.10 [°]	16.78±0.02 ^d	168.45±0.12 ^d	208.09±1.25 ^d	0.63±0.03 [°]
G28	9.85±0.54 ^{bc}	17.49±0.01 ^d	21.92±0.01 ^b	$219.34\pm0.10^{\circ}$	258.76 ± 0.01^{b}	0.86 ± 0.01^{b}
Kastamonu fresh garlic	7.33±0.32 [°]	6.18±0.01 [°]	3.29±0.03 [′]	2.29±0.56 [′]	11.76±0.58 [′]	Nd
K7	9.28±0.48 [°]	$24.56 \pm 0.08^{\circ}$	4.16±0.06 ^h	19.65±0.07 ^h	48.38±0.09 ^h	0.10±0.01 ^f
K14	10.03±0.30 ^{bc}	35.03±0.02 [°]	12.79±0.05 [°]	95.31±0.02 [°]	143.14±0.05 [°]	0.19±0.01 ^d
K21	13.10±0.06 [°]	24.07±0.01 ^b	19.43±0.01 [°]	189.50±0.01 [°]	233.02±0.01 [°]	0.65±0.02 ^c
K28	9.87±0.13 ^{bc}	3.58±0.01 ^f	27.44±0.10 [°]	272.86±0.30 [°]	303.89±0.20 [°]	0.95±0.01 [°]

Table 1. General composition of garlic

^{a-h}Different exponential letters in the same column indicate a significant difference between the samples (p<0.05). Nd: Not detected. G7; Black garlic produced from Gaziantep fresh garlic in 7 days, G14; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 7 days, K14; Black garlic produced from Kastamonu fresh garlic in 14 days, K14; Black garlic produced from Kastamonu fresh garlic in 14 days, K21; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 28 days.

Sugar content

Carbohydrates constitute 26-30% of fresh garlic and contain 23% fructans. Fructans are a group of oligopolysaccharides consisting of fructose units linked by β -2,1 bond and constitute 70-80% of the dry matter of garlic (Huang et al., 2011; Li et al., 2017). Fructans are hydrolyzed to fructose and glucose by heat treatment applied during black garlic production. Thus, black garlic becomes sweeter and the texture softens (Hofmann et al., 2000; Yuan et al., 2016). The sucrose, glucose, and fructose compounds in fresh garlic from the Gaziantep and Kastamonu regions and black garlic produced from these regions were determined and presented in Table 1. Analysis revealed that sucrose was the predominant sugar in fresh garlic, followed by glucose and fructose. Additionally, it was observed that the levels of sucrose, glucose, and fructose increased with the fermentation period in the black garlic production process, with fructose becoming the dominant sugar after 14 days. Casas et al. (2017) determined the highest increase in fructose (from 0.38 to 44.73 g/100 g/100 g DW) and glucose (from 0.21 to 2.51 g/100 g DW) in black garlic using high-performance liquid chromatography (HPLC). Choi et al. (2008) examined the physicochemical properties of black garlic and determined the fructose, arabinose, glucose, and sucrose compounds of fresh garlic as 63.9 mg/100 g, 51.1 mg/100 g, 91.6 mg/100 g, and 76.3 mg/100 g, respectively. In black garlic, fructose (63.9 mg/100 g), arabinose (114.5 mg/100 g), glucose (181.7 mg/100 g), and sucrose compounds were identified.

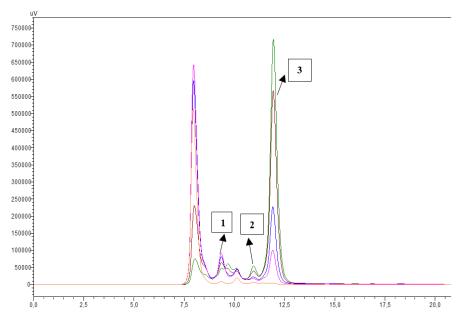
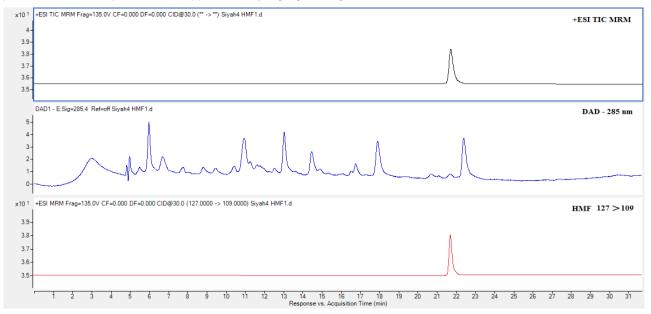


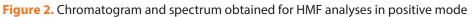
Figure 1. Chromatogram of the sugar determined in garlic (1; Sucrose, 2; Glucose, 3; Fructose)

HMF amount

The amount of HMF was determined during the production process of black garlic, and the data obtained are presented in Table 1. LC-MS/MS was employed for identification, with the study conducted in positive ion mode based on 127>109 multiple reaction monitoring (MRM) transitions. Notably, HMF was not detected in fresh garlic.

During the production process of black garlic derived from garlic cultivated in the Gaziantep and Kastamonu regions, the HMF content was found to range between 0.09 and 0.86 g/kg in Gaziantep black garlic and 0.10 and 0.95 g/kg in Kastamonu garlic. Notably, in some studies, the maximum HMF content in black garlic at the end of the production process has been reported to be approximately 5 g/kg (Zhang et al., 2016).





Antioxidant capacity and total phenolic content

The antioxidant capacity of fresh garlic grown in Gaziantep and Kastamonu regions and black garlic produced from this garlic were determined by two different methods, DPPH and ABTS, on the 7th, 14th, 21st, and 28th days (Table 2) and a statistically significant difference was observed (p<0.05). The antioxidant capacity potential of fresh garlic grown in Gaziantep and Kastamonu regions was determined as 53.69 µmol Trolox /100 g and 56.85 µmol Trolox/100 g by the DPPH method and 188.14 µmol Trolox /100 g and 109.55 µmol Trolox /100 g by ABTS method, respectively. It was determined that antioxidant capacity increased in the black garlic production process depending on the increase in production time. It was determined that the antioxidant capacity of black garlic produced from fresh garlic grown in the Kastamonu region.

The prominent feature of black garlic is its potent antioxidant activity. Studies show that black garlic can effectively scavenge DPPH, ABTS, hydroxyl, nitrite, and superoxide anion radicals (Jeong et al., 2016; Kim, Nam, et al., 2012; Lee et al., 2009; Zhang et al., 2016). Black garlic plays a similar regulatory role in lipid peroxidation and superoxide dismutase activity, with its effect on reducing free radical production (Bae et al., 2014; Kim, Nam, et al., 2012; Sato, Kohno, Hamano et al., 2006). In particular, antioxidant activity increases during the processing of black garlic. This increase is due to the formation of new antioxidant compounds such as S-allyl cysteine (SAC), polyphenols, carboline derivatives, melanoidins, Amadori and Heyns compounds along with increases in the amounts of compounds such as HMF and pyruvate (Qiu et al., 2020). This reinforces the unique antioxidant properties of black garlic.

Phenolic compounds are aromatic or aliphatic compounds characterized by at least one aromatic ring to which one or more hydroxyl (OH) groups are attached. The anti-inflammatory, anti-aging, antiproliferative, and antioxidant properties of phenolic compounds have been established in various studies (Rahman et al., 2021). The total phenolic content of fresh garlic grown in the Gaziantep and Kastamonu regions was 65.12 mg GAE/100 g and 19.59 mg GAE/100 g, respectively. It was determined that the amount of total phenolic matter increased with the increase in the production period of black garlic produced from fresh garlic grown in both regions. In previous studies, it was reported that black garlic had higher total polyphenol content than fresh garlic. Total polyphenol content was reported to be 7-11 times higher than fresh garlic, and total phenolic acid content increased 4 to 8 times (Casas et al., 2017).

	DPPH	ABTS	TPC
	(µmol Trolox /100 g)	(µmol Trolox /100 g)	(mg GAE /100 g)
Gaziantep fresh garlic	53.66±4.06 ^h	188.14±9.98 ^h	65.12±1.71 ⁹
G7	620±4.49 ^f	1081.85±39.47 ^f	165.43±1.03 ^e
G14	1292.38±41.31 ^d	2177.77±6.66 ^d	331.66±4.19°
G21	2598.57±28.53°	3770.74±93.07°	586.81±1.52 ^b
G28	2929.52±42.24 ^a	4350±70.61ª	618.03±13.64 ^b
Kastamonu fresh garlic	56.85±2.66 ^h	109.55±0.86'	19.59±0.93 ^h
K7	548.09±10.72 ^g	841.48±11.18 ⁹	92.69±1.01 ^f
K14	1013.33±14.30 ^e	1655.92±16.32 ^e	211.51±2.02 ^d
K21	2631.42±29.51°	3838.88±4.80 ^c	590.75±2.14 ^b
K28	2782.85±10.78 ^b	3970.74±10.01 ^b	624.24±9.58ª

Table 2. Antioxidant activity and total phenolic content results

^{a-g}Different exponential letters in the same column indicate a significant difference between the samples (p<0.05). G7; Black garlic produced from Gaziantep fresh garlic in 7 days, G14; Black garlic produced from Gaziantep fresh garlic in 14 days, G21; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 28 days, K7; Black garlic produced from Kastamonu fresh garlic in 7 days, K14; Black garlic produced from Kastamonu fresh garlic in 14 days, K21; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 28 days.

Table 3. Retention time and mass spectral properties of organosulfur compounds in garlic

Organosulfur compound	Retention time (min)	Abbreviation	Main ion	Fragment ion
(3R,5S)-5-methyl-1,4-thiazane-3-carboxylic acid	5,72	Cycloalliin	178.23	88.08, 91.04
(+)-S-allyl-l-cysteine sulfoxide	6,32	Alliin	178.15	88-74
(+)-S-(trans-1-propenyl)-l-cysteine sulfoxide	6,57	Isoalliin	178.2	88.00, 160.10
(+)-S-allil-I-sistein	10,64	SAC	162.1	145.1, 73.1
γ-l-glutamil-S-allil-l-sistein	17,38	GSAC	291.2	162,2, 144,8
γ-l-glutamil-S-(trans-1-propenil)- l-sistein	20,5	GSPC	291.2	201,1
γ-l-glutamil-phenylalanine	24,83	γGPA	295.3	178.0, 88.0
Allicin	55,3	Allicin	163.2	73.2, 41.1

Table 4. Organosulfur compound contents of garlic (mg/g)

	Gaziantep fresh garlic	G7	G14	G21	G28	Kastamonu fresh garlic	К7	K14	K21	K28
(+)-S-allil-l-sistein	38.31±0.01 ^f	175.35±2.01 ^c	198.33±0.01 ^b	215.98±0.87 ^a	166.81±0.43 ^c	57.28±0.64 ^e	185.95±0.71 [°]	220.67±0.95 ^a	112.80±0.47 ^d	173.30±0.75 [°]
(3R,5S)-5-methyl- 1,4-thiazane-3- carboxylic acid	12.83±0.21 [°]	34.33±0.24 ^b	27.90±0.68 [°]	23.74±0.01 ^d	Nd	16.09±0.01 ^d	42.60±0.01 ^ª	10.43±0.02 ^f	Nd	Nd
(+)-S-allyl-l- cysteine sulfoxide	5.03±0.02 ^e	39.05±0.72 ^b	42.59±0.31 ^ª	26.235±0.40 ^c	Nd	3.43±0.01 ^f	24.22±0.01 ^d	Nd	Nd	Nd
(+)-S-(trans- 1-propenyl)-l- cysteine sulfoxide	6.73±0.01 ^d	23.56±0.26 ^b	32.35±0.01 ^ª	Nd	Nd	4.01±0.01 ^e	7.29±0.01 [°]	Nd	Nd	Nd
γ-l-glutamil-S-allil- l-sistein	4.40±0.01 ^d	7.62±0.01 ^b	Nd	Nd	Nd	9.98±0.01 ^ª	5.47±0.01 [°]	Nd	Nd	Nd
γ-l-glutamil-S- (trans-1-propenil)- l-sistein	194.10±0.10 ^ª	25.95±0.10 ⁹	17.85±0.01 ^h	45.80±0.27 ^d	29.57±0.01 ^f	75.06±0.98 ^b	96.14±0.01 [°]	52.28±0.01 ^d	Nd	32.30±0.01 ^e
γ-l-glutamil- phenylalanine	9.64±0.01 ^ª	6.20±0.10 [°]	Nd	Nd	Nd	9.40±0.01 ^ª	8.28±0.01 ^b	Nd	Nd	Nd
Allicin	300.42 ± 0.01^{a}	2.16±0.01 ^c	1.47±0.01 ^d	0.62±0.01 ^f	0.32±0.01 ^h	116.34±0.21 ^b	2.02±0.01 ^c	1.14±0.01 ^e	0.45±0.01 ⁹	0.24±0.01 [']

^{a-h}Different exponential letters in the same column indicate a significant difference between the samples (p<0.05). Nd: Not detected. G7; Black garlic produced from Gaziantep fresh garlic in 7 days, G14; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 21 days, G28; Black garlic produced from Gaziantep fresh garlic in 7 days, K14; Black garlic produced from Kastamonu fresh garlic in 7 days, K14; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days, K28; Black garlic produced from Kastamonu fresh garlic in 21 days.

CONCLUSION

This study investigated the changes in the black garlic production processes of Kastamonu and Gaziantep garlic, which are Turkey's most produced garlic varieties. Within the scope of the study, samples were taken on the 7th, 14th, 21st, and 28th days of the black garlic production process at 65°C temperature and 70% humidity, and various analyses were performed. According to the results obtained, it was determined that total phenolic substances and antioxidant potentials increased in black garlic production processes of both regions compared to fresh garlic. In addition, it was observed that sucrose was the predominant protein in fresh garlic, while fructose was the predominant protein in black garlic. Regarding protein content, the ratios range from 5.8% to 7.3% in fresh garlic and were found to be around 13.1% to 14.2% in black garlic. As a result, it was determined that fresh and black garlic from the Gaziantep region had higher phenolic matter, antioxidant capacity, and sulfur compound contents. This study provides an important contribution to understanding the changes in the production processes of fresh garlic and the bioactive properties of black garlic obtained from garlic grown in the Kastamonu and Gaziantep regions.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare no competing, actual, potential, or perceived conflict of interest.

Author contribution

Hatice Kubra Sasmaz: conceptualization, data curation, formal analysis, funding acquisition, writing - first draft, writing - review & editing Hasim Kelebek: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing). Serkan Selli: Conceptualization, data curation, formal analysis, funding procurement, original draft writing, review, and editing. Turkan Uzlasir: Conceptualization, data curation, formal analysis, funding acquisition, writing (original draft), writing (review & editing).

Ethics committee approval

Ethics committee approval is not required.

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Data availability Not applicable.

Consent to participate Not applicable.

Consent for publication

Not applicable.

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REFERENCES

Abu-Lafi, S., Dembicki, J.W., Goldshlag, P., Hanuš, L.O., & Dembitsky, V. M. (2004). The use of the 'Cryogenic 'GC/MS and on-column injection for study of organosulfur compounds of the Allium sativum. Journal of Food Composition and Analysis, 17(2), 235-245. https://doi.org/10.1016/j.jfca.2003.09.002

AOAC, 1997. Official methods of analysis of AOAC International 16th ed., Association of Official Analytical Chemists, Washington, DC

- Atanasova-Goranova, V. K., Dimova, P. I., & Pevicharova, G. T. (1997). Effect of food products on endogenous generation of n- nitrosamines in rats. British Journal of Nutrition, 78(2), 335-345. https://doi.org/10.1079/BJN19970151
- Bae, S. E., Cho, S. Y., Won, Y. D., Lee, S. H., & Park, H. J. (2014). Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment. LWT-Food Science and Technology, 55(1), 397-402.
- Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
- Choi, D. J., Lee, S. J., Kang, M. J., Cho, H. S., Sung, N. J., & Shin, J. H. (2008). Physicochemical characteristics of black garlic (Allium sativum L.). Journal of the Korean Society of Food Science and Nutrition, 37(4), 465-471.
- Condurso, C., Cincotta, F., Tripodi, G., Merlino, M., & Verzera, A. (2019). Influence of drying technologies on the aroma of Sicilian red garlic. Lwt, 104, 180-185. https://doi.org/10.1016/j.lwt.2019.01.026
- FAOSTAT (2020) http://www.fao.org/faostat/en/#data/QC. Accessed 15 Apr 2022
- Haber-Mignard, D., Suschetet, M., Bergès, R., Astorg, P., & Siess, M. H. (1996). Inhibition of aflatoxin B1-and N-nitrosodiethylamine-induced liver preneoplastic foci in rats fed naturally occurring allyl sulfides. https://doi.org/10.1080/01635589609514428
- Hamma, I. L., Ibrahim, U., & Mohammed, A. B. (2013). Growth, yield, and economic performance of garlic (Allium sativum L.) as influenced by farm yard manure and spacing in Zaria, Nigeria. Journal of Agricultural Economics and Development, 2(1), 1-5.
- Hofmann, T., & Schieberle, P. (2000). Formation of aroma-active Strecker-aldehydes by a direct oxidative degradation of Amadori compounds. Journal of Agricultural and Food Chemistry, 48(9), 4301-4305. https://doi.org/10.1021/jf000076e
- Huang, C. H., Hsu, F. Y., Wu, Y. H., Zhong, L., Tseng, M. Y., Kuo, C. J., ... & Chiou, S. H. (2015). Analysis of the lifespan-promoting effect of garlic extract by an integrated metabolo-proteomics approach. The Journal of Nutritional Biochemistry, 26(8), 808-817. https://doi.

org/10.1016/j.jnutbio.2015.02.010

- Huang, X. S. Wang, M. & Bai, W. B. (2011). Some characteristics to the related food processing for garlic fructan. Advanced Materials Research, 197, 79–85. https://doi.org/10.4028/www.scientific.net/AMR.197-198.79
- Jangam, G. B., & Badole, S. L. (2014). Garlic (Allium sativum): role in metabolic disorder. In Polyphenols in human health and disease (pp. 611-614). Academic Press. https://doi.org/10.1016/B978-0-12-398456-2.00046-3
- Jeong, Y. Y., Ryu, J. H., Shin, J. H., Kang, M. J., Kang, J. R., Han, J., & Kang, D. (2016). Comparison of Antioxidant and Anti-Inflammatory Effects between Fresh and Aged Black Garlic Extracts. Molecules (Basel, Switzerland), 21(4), 430. https://doi.org/10.3390/molecules21040430
- Kelebek, H. (2016). LC-DAD–ESI-MS/MS characterization of phenolic constituents in Turkish black tea: Effect of infusion time and temperature. Food Chemistry, 204, 227-238. https://doi.org/10.1016/j.foodchem.2016.02.132
- Kelebek, H., Selli, S., Canbas, A., & Cabaroglu, T. (2009). HPLC determination of organic acids, sugars, phenolic compositions, and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. Microchemical Journal, 91(2), 187-192. https:// doi.org/10.1016/j.microc.2008.10.008
- Kim, D., Jung, S. J., Cheon, S. Y., Cho, Y. J., Tae, M. H., Kim, K. H., & Yook, H. S. (2012). Antioxidant activity of giant black garlic. Proceedings of the Nutrition Society, 72(OCE4), E230. https://doi.org/10.1017/S0029665113002553
- Kim, J. H., Nam, S. H., Rico, C. W., & Kang, M. Y. (2012). A comparative study on the antioxidative and anti-allergic activities of fresh and aged black garlic extracts. International Journal of Food Science & Technology, 47(6), 1176-1182.
- Kim, I., Kim, J. Y., Hwang, Y. J., Hwang, K. A., Om, A. S., Kim, J. H., & Cho, K. J. (2011). The beneficial effects of aged black garlic extract on obesity and hyperlipidemia in rats fed a high-fat diet. J. Med. Plants Res, 5(14), 3159-3168.
- Kim, J. W., Kim, Y. S., & Kyung, K. H. (2004). Inhibitory activity of essential oils of garlic and onion against bacteria and yeasts. Journal of Food Protection, 67(3), 499-504. https://doi.org/10.4315/0362-028X-67.3.499
- Lee, H. S., & Coates, G. A. (2000). Quantitative study of free sugars and myo-inositol in citrus juices by HPLC and a literature compilation. https://doi.org/10.1081/JLC-100100476
- Li, M., Yan, Y. X., Yu, Q. T., Deng, Y., Wu, D. T., Wang, Y., ... & Zhao, J. (2017). Comparison of immunomodulatory effects of fresh garlic and black garlic polysaccharides on RAW 264.7 macrophages. Journal of Food Science, 82(3), 765-771. https://doi.org/10.1111/1750-3841.13589
- Liu, J., Zhang, G., Cong, X., & Wen, C. (2018). Black garlic improves heart function in patients with coronary heart disease by improving circulating antioxidant levels. Frontiers in physiology, 9, 1435. https://doi.org/10.3389/fphys.2018.01435
- Martínez-Casas, L., Lage-Yusty, M., & López-Hernández, J. (2017). Changes in the aromatic profile, sugars, and bioactive compounds when purple garlic is transformed into black garlic. Journal of agricultural and food chemistry, 65(49), 10804-10811. https://doi.org/10.1021/acs.jafc.7b04423
- Moutia, M., Habti, N., & Badou, A. (2018). In vitro and in vivo immunomodulatory activities of Allium sativum L. Evidence-Based Complementary and Alternative Medicine. https://doi.org/10.1155/2018/4984659
- Nassur, R. D. C. M. R., Boas, E. V. D. B. V., & Resende, F. V. (2017). Black garlic: transformation effects, characterization, and consumer purchase intention. Comunicata Scientiae, 8(3), 444-451. https://doi.org/10.14295/cs.v8i3.2251
- Oyawoye, O. M., Olotu, T. M., Nzekwe, S. C., Idowu, J. A., Abdullahi, T. A., Babatunde, S. O., ... & Faidah, H. (2022). Antioxidant potential and antibacterial activities of Allium cepa (onion) and Allium sativum (garlic) against the multidrug resistance bacteria. Bulletin of the National Research Centre, 46(1), 1-7. https://doi.org/10.1186/s42269-022-00908-8
- Qiu, Z., Zheng, Z., Zhang, B., Sun-Waterhouse, D., & Qiao, X. (2020). Formation, nutritional value, and enhancement of characteristic components in black garlic: A review for maximizing the goodness to humans. Comprehensive reviews in food science and food safety, 19(2), 801-834. https://doi.org/10.1111/1541-4337.12529
- Queiroz, Y. S., Ishimoto, E. Y., Bastos, D. H., Sampaio, G. R., & Torres, E. A. (2009). Garlic (Allium sativum L.) and ready-to-eat garlic products: In vitro antioxidant activity. Food Chemistry, 115(1), 371-374. https://doi.org/10.1016/j.foodchem.2008.11.105
- Raghu, G., Collard, H. R., Anstrom, K. J., Flaherty, K. R., Fleming, T. R., King Jr, T. E., ... & Brown, K. K. (2012). Idiopathic pulmonary fibrosis: clinically meaningful primary endpoints in phase 3 clinical trials. American journal of respiratory and critical care medicine, 185(10), 1044-1048. https://doi.org/10.1164/rccm.201201-0006PP
- Rahman, M. M., Rahaman, M. S., Islam, M. R., Rahman, F., Mithi, F. M., Alqahtani, T., Almikhlafi, M. A., Alghamdi, S. Q., Alruwaili, A. S., Hossain, M. S., Ahmed, M., Das, R., Emran, T. B., & Uddin, M. S. (2021). Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules (Basel, Switzerland), 27(1), 233. https://doi.org/10.3390/molecules27010233
- Sato, E., Kohno, M., Hamano, H., & Niwano, Y. (2006). Increased anti-oxidative potency of garlic by spontaneous short-term fermentation. Plant Foods For Human Nutrition, 61, 157-160.
- Saafi, E.B., El Arem, A., Issaoui, M., Hammami, M., Achour, L. (2009). Phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. International Journal of Food Science & Technology 2009, 44, 2314–2319. https://doi. org/10.1111/j.1365-2621.2009.02075.x
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16(3), 144-158. https://doi.org/10.5344/ajev.1965.16.3.144
- Tao, D., Zhou, B., Zhang, L., Hu, X., Liao, X., & Zhang, Y. (2016). 'Laba'garlic processed by dense phase carbon dioxide: the relation between green colour generation and cellular structure, alliin consumption and alliinase activity. Journal of the Science of Food and Agriculture, 96(9), 2969-2975. https://doi.org/10.1002/jsfa.7463
- Yuan, H., Sun, L., Chen, M., & Wang, J. (2016). The comparison of the contents of sugar, Amadori, and Heyns compounds in fresh and black garlic. Journal of Food Science, 81(7), C1662-C1668. https://doi.org/10.1111/1750-3841.13365
- Zhang, X., Li, N., Lu, X., Liu, P., & Qiao, X. (2016). Effects of temperature on the quality of black garlic. Journal of the Science of Food and Agriculture, 96(7), 2366-2372. https://doi.org/10.1002/jsfa.7351
- Zhu, Q., Kakino, K., Nogami, C., Ohnuki, K., & Shimizu, K. (2016). An LC-MS/MS-SRM method for simultaneous quantification of four representative organosulfur compounds in garlic products. Food Analytical Methods, 9, 3378-3384. https://doi.org/10.1007/s12161-016-0535-1