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Chemical and Physicochemical Contents and Bioactivity of Black Garlic

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Abstract

Black garlic is a garlic product that has been in great demand recently because it has a fruit-like taste and is easy to eat. In this article, it is aimed to obtain black garlic samples using fresh Taşköprü (Kastamonu) garlic and to determine the chemical, physicochemical and biological properties of these samples. Compared to fresh garlic, black garlic samples had significantly increased browning intensity, total acidity, reducing sugar content, total protein, crude oil, ash, and crude fiber in contrast to moisture content and pH value. The total flavonoid and phenolic acid contents of the samples varied based on the aging period and the extract used, but significant increases in the scavenging activities of the samples were obtained with the aging period. No increase in the antimicrobial capacity of the samples was observed. In summary, the biological, chemical, and physicochemical properties of garlic changed during the aging period, with a particularly marked increase in antioxidant capacity.

Keywords: Black garlic, antimicrobial, antioxidant, chemical, physicochemical

Siyah Sarımsağın Kimyasal ve Fizikokimyasal İçerikleri ve Biyoaktivitesi

Öz

Siyah sarımsak, meyvemsi bir tada sahip olması ve kolay tüketilebilmesi nedeniyle son zamanlarda büyük talep gören bir sarımsak ürünüdür. Bu makalede, taze Taşköprü (Kastamonu) sarımsağı kullanılarak siyah sarımsak örneklerinin elde edilmesi ve bu örneklerin kimyasal, fizikokimyasal ve biyolojik özelliklerinin belirlenmesi amaçlanmıştır. Taze sarımsak ile karşılaştırıldığında siyah sarımsak örneklerinin nem içeriği ve pH değeri azalma gösterirken esmerleşme yoğunluğu, toplam asitlik, indirgen şeker içeriği, toplam protein, ham yağ, kül ve ham lif miktarları önemli ölçüde artmıştır. Örneklerin toplam flavonoid ve fenolik asit içerikleri, olgunlaşma periyoduna ve kullanılan özüte bağlı olarak değişmiştir, ancak olgunlaşma periyodu ile örneklerin radikal süpürme aktivitelerinde kayda değer artışlar elde edilmiştir. Örneklerin antimikrobiyal kapasitesinde ise herhangi bir artış gözlenmemiştir. Özetle, olgunlaşma periyodu ile birlikte sarımsağın biyolojik, kimyasal ve fizikokimyasal özelliklerinde değişimler meydana gelmiş ve özellikle antioksidan kapasitedeki artış belirgin olmuştur.

Anahtar Kelimeler: Siyah sarımsak, antimikrobiyal, antioksidan, kimyasal, fizikokimyasal

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1. Introduction

With a total harvested area of 1.437.690 hectares and an annual yield of 24.255.303 tons of dry head, garlic (*Allium sativum* L.) is one of the most essential vegetables in the world (Martins et al., 2016). Apart from being widely used for general culinary purposes, it is of great importance in both traditional and modern medicine due to its therapeutic and medicinal properties (Martins et al., 2016; Kimura et al., 2017; Lu et al., 2017). The unmistakable taste of cloves, which is the outcome of complicated biochemical reactions, is the most important quality characteristic of garlic. The sulfur-containing, non-volatile amino acids like S-allyl cysteine sulfoxide (ACSO) or alliin are the main causes of this flavor. Besides their flavor properties, these compounds are involved in the well-known medicinal properties of garlic, such as antimicrobial, antioxidant, anti-cancer, anti-inflammatory, anti-diabetic, cardio-protective, and immunomodulatory activity. They also promote glutathione biosynthesis, which has strong antioxidant properties. In addition to its volatile compounds, garlic contains vitamins, flavonoids, phenolic compounds, antioxidants, minerals, amides, nitrogen oxides and proteins, and saponins and sapogenins with important therapeutic and medicinal functions (Martins et al., 2016; Kimura et al., 2017).

The most common approach to remove the undesirable taste and odor of garlic is heat treatment. When garlic is heat-treated, it undergoes various physicochemical changes, changing its color, taste, and nutritional content due to non-enzymatic browning reactions such as the Maillard reaction, chemical oxidation of phenols, and caramelization. Some of these reactions are connected with the formation of compounds that exhibit significant antioxidant effects (Bae et al., 2014).

Recently, a garlic product called black garlic has begun to appear on the market. To produce black garlic, fresh garlic is kept at high temperatures and humidity for a certain time. Unlike fresh garlic, black garlic has a fruity sweet taste, and thus it can be consumed easily (Kang, 2016; Ngan et al., 2017). The levels of amino acids, S-allyl-cysteine (SAC), polyphenols, and flavonoids are significantly higher in black garlic. It has stronger radical scavenging activity and exhibits antimicrobial behaviors against pathogens such as *Escherichia coli*, *Candida albicans*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*. It also has superior anti-tumor, anti-cancer, and immunomodulatory effects than fresh garlic (Ngan et al., 2017).

In Türkiye, especially in Taşköprü (Kastamonu), garlic is cultivated intensively, but despite its important properties, the consumption of fresh garlic is still avoided for specific reasons. It has a strong unpleasant taste and odor, causes gastrointestinal diseases in some people, and has side effects such as hemolytic anemia and allergic reactions. Black garlic products, on the other hand, are easier to consume and have important chemical and biological properties compared to fresh garlic. Therefore, the aim of the study is to investigate antimicrobial and antioxidant activity, as well as physicochemical and chemical compositions of black garlic samples obtained from fresh Taşköprü (Kastamonu) garlic, which is an important resource and value for Türkiye.

2. Materials and Methods

2.1. Garlic Samples

Fresh garlic samples were purchased in 2018 from garlic producers in Taşköprü (Kastamonu).

2.2. Preparation of Black Garlic Samples and Extraction

The method used to prepare black garlic samples was reported in the literature (Choi et al., 2014). Fresh unpeeled garlic bulbs were incubated for 14, 21, 28, and 35 days in a climatic chamber at 70°C and 90% relative humidity. Then, the fresh and black garlic cloves were peeled and powdered with a high-speed mixer. The powdered samples were mixed with ethyl acetate (EtOAc), ethanol (EtOH), and methanol (MeOH) at a ratio of 1:3 (solid:liquid), and the extraction was carried out three times for 1 hour in a shaking incubator at room temperature. The supernatants collected after centrifuging the extracts at 4000 rpm for 10 minutes were evaporated using a rotary evaporator (Hei-Vap Precision, Heidolph) and kept at -20°C until used.

2.3. Determination of Physicochemical Properties

The official AOAC method was used to determine the moisture content of garlic samples (AOAC, 2000). 10 g of powdered samples were dried at a temperature of 70±5ºC in an oven until the weight was stabilized and the humidity rate was expressed as %. To measure the pH values, 10 g of powdered samples were homogenized with 100 ml of distilled water, and the pH values were measured with a calibrated pH meter (Starter3000, Ohaus) (Bae et al., 2014). By measuring absorbance at 420 nm, the browning intensity was evaluated spectrophotometrically (Bae et al., 2014). UV-visible spectra were recorded by measuring absorbances in the range of 200-800 nm in a spectrophotometer (UV-61 OOPCS Double Beam Spectrophotometer, Mapada) to detect the browning development (Choi et al., 2014). The total acid contents were evaluated by titration to pH 8.3 with 0.1 N NaOH and reported as a percentage of tartaric acid. The 3,5-dinitrosalicylic acid (DNS) method was used to determine the reducing sugar content (Miller, 1959).

2.4. Determination of Chemical Content and Elemental Analysis

In powdered samples, total protein (TS 1620, 2002), crude fat (TS EN ISO 11085, 2016), total ash (TS 2131 ISO 928, 2001), and crude fiber (TS 6932, 1989) were determined.

Calcium (Ca), manganese (Mn), copper (Cu), phosphorus (P), selenium (Se), sodium (Na), iron (Fe), magnesium (Mg), potassium (K), and zinc (Zn) contents were determined according to the NMKL 186 standard (NMKL, 2007). The samples were mixed with 7 ml of 65% nitric acid (HNO_3) and 1 ml of 30% hydrogen peroxide (H_2O_2) and dissolved in a microwave burning device (Start D, Milestone) at 180°C and 270 bar pressure (The samples were allowed to burn until they became transparent in liquid form and no residue remained). The samples were then diluted to 50 ml with distilled water and stored at +4ºC. Elemental analysis was performed on an ICP-MS instrument (7700 Series, Agilent).

2.5. Determination of Total Flavonoid and Phenolic Acids

The total flavonoid (TFC) and phenolic acid (TPC) contents of the extracts obtained from fresh and black garlic samples were determined by spectral methods using $AlCl_3$ and Folin-Ciocalteu reagent, respectively. Total flavonoids were expressed as quercetin equivalent and total phenolic acids as gallic acid equivalent (Özcan et al., 2019).

2.6. ABTS and DPPH Radical Scavenging Activity

The 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity was determined by modifying the method described by Re et al. (1999). 80 µl of the extracts were mixed with 160 µl of the ABTS solution (OD_{750} = 0.7) prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) and incubated for 6 minutes at room temperature in the dark. After incubation, absorbances were measured in a spectrophotometer at 750 nm and expressed as Trolox equivalent.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined by modifying the method proposed by Brand-Williams et al. (1995). 125 µl of extracts were mixed with 125 µl of the 0.1 mM DPPH solution prepared in ethanol and incubated in the dark at room temperature for 45 minutes. After incubation, absorbances were measured in a spectrophotometer at 490 nm and expressed as Trolox equivalent.

2.7. Antimicrobial Activity

The disc diffusion (CLSI, 2015a) and minimum inhibitory concentration (MIC) (CLSI, 2015b) methods were used to determine the antimicrobial activity of the extracts. In the experiments, test organisms *Candida albicans* ATCC 14053, *Escherichia coli* ATCC 29998, *Enterococcus faecium* DSMZ 13590, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, and *Pseudomonas aeruginosa* ATCC 27853 were used.

2.8. Statistical Analysis

In this study, the tests were performed three times, and the findings were reported as an average of the experiments.

3. Results and Discussion

Garlic is one of the most important vegetables consumed for centuries. In many studies conducted so far, it has been reported that garlic has antimicrobial, antioxidant, and anti-cancer properties, as well as medicinal effects such as relieving the effects of fatigue, regulating blood sugar and blood pressure, aiding digestion, and increasing appetite (Lu et al., 2017). Due to all these important benefits, it is one of the most widely used plants in many countries for food and medical purposes, but its unpleasant smell and taste limit its consumption. Heat treatment is a method that eliminates the negative aspects of garlic in order to increase its consumption. It can also contribute to the flavor and quality of garlic and provide novel functions (Bae et al., 2014; Zhang et al., 2016). In this study, black garlic samples were obtained by keeping the fresh garlic samples at 70ºC and 90% relative humidity for 14, 21, 28, and 35 days. Figure 1 shows the color changes of the black garlic samples during the aging period. FG label was used for fresh garlic, and BG14, BG21, BG28 and BG35 labels were used for black garlic samples, respectively.

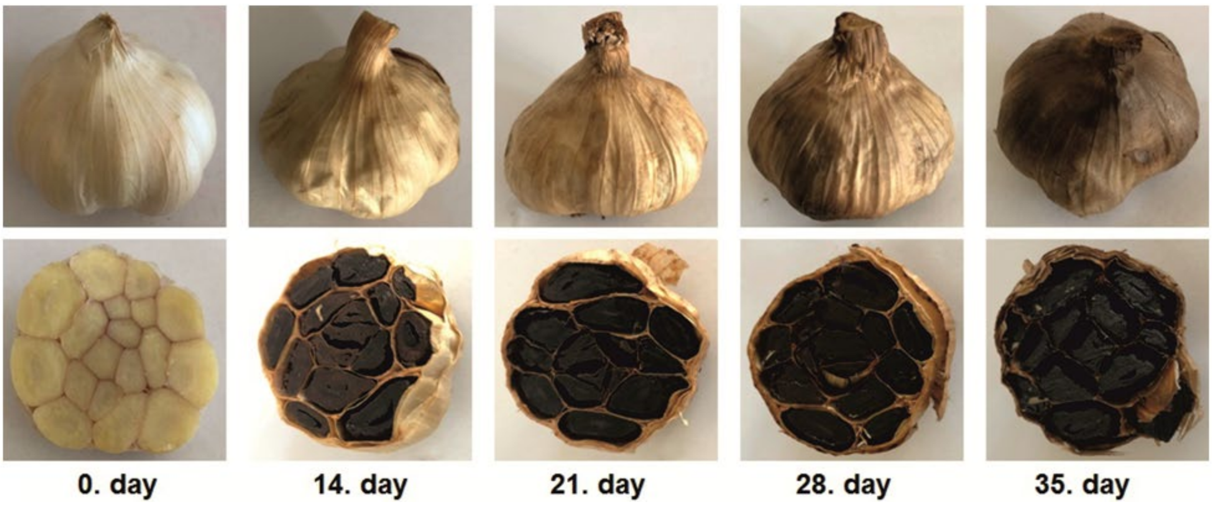

Figure 1. Color changes of black garlic samples during the aging period

Table 1 shows the physicochemical characteristics of FG and BGs. As shown in the table, the aging period increased total acid and reducing sugar contents and it decreased humidity and shifted pH values to acidity. It was also noted that the browning intensity of the samples gradually increased with increasing incubation time. In a research conducted by Choi et al. (2014), fresh garlic samples from the South Korean agricultural market were aged at 70ºC and 90% relative humidity for up to 35 days to determine the physicochemical properties of FG and BG samples, and the authors found similar results consistent with this study. The UV-visible absorbance spectra of FG and BG samples are shown in Figure 2. The fresh garlic gave the highest absorbance at 240 nm, and BG35 displayed the highest absorbance at 370 nm. The changes in color and spectral patterns are due to the products formed as a result of the Maillard reaction, and the aging conditions such as temperature and time have an effect on the formation of the products (Choi et al., 2014).

Table 1. Physicochemical properties of FG and BG samples during the aging period

Samples	Moisture (%)	pH	Browning intensity (420 nm)	Total acid content (% tartaric acid)	Reducing sugar content (g glucose kg^{-1})
FG	63.47	6.89	0.163	0.06	9.55
BG14	59.40	5.36	0.410	0.12	142.64
BG21	57.80	5.10	0.580	0.15	202.01
BG28	55.05	4.63	1.133	0.24	301.33
BG35	42.04	4.60	1.899	0.31	316.93

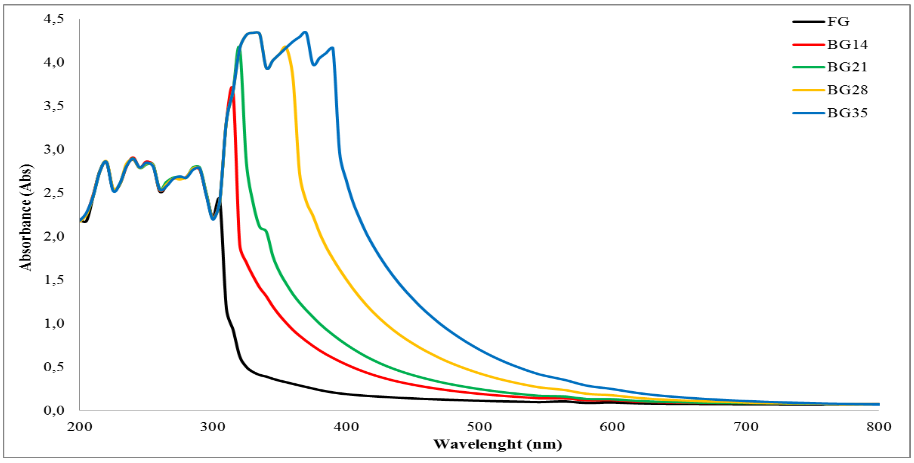

Figure 2. UV-visible absorbance spectra of FG and BG samples

Table 2 summarizes the total protein, crude fat, total ash, and crude fiber values for FG and BG samples. Upon examination of the results, it was generally observed that the longer the aging time, the higher the chemical contents in the samples. Briefly, in FG, the total protein, crude fat, total ash, and crude fiber values were found to be 5.61%, 0.04496%, 0.8753%, and 1.12%, respectively, while in BG35, these values were found to be 12.95%, 0.07393%, 2.2592%, and 2.21%, respectively. Since the studies on black garlic are quite new and insufficient, some of the chemical contents determined in this study could not be compared to the literature data. In a study by Kang (2016), black garlic samples were obtained from fresh garlic harvested in Korea with a stepwise heating schedule. The author examined the ash content of fresh and black garlic samples and found that the fresh garlic had the lowest amount of ash (73.59±0.89 mg 100 g^{-1}) and the black garlic labeled as BG5 had the highest amount (114.36±8.65 mg 100 g^{-1}). There are several types of research in the literature on the chemical content of fresh garlic. In a study by Hacıseferoğulları et al. (2005), some technological and nutritional characteristics of Taşköprü (Kastamonu) garlic were analyzed. The researchers have found that the fresh garlic had a total protein content of 9.26%, crude oil content of 0.34%, crude fiber content of 2.17%, and ash content of 2.3%. In another study, Chekki et al. (2014) investigated the antioxidant and antibacterial activities, and chemical composition of Tunisian garlic, and they found that the garlic contained 1.4% and 5.2% protein and ash contents, respectively.

Table 2. Total protein, crude fat, total ash, and crude fiber values of FG and BG samples

Samples	Total protein (%)	Crude fat (%)	Total ash (%)	Crude fiber (%)
FG	5.61	0.04496	0.8753	1.12
BG14	9.88	0.06495	1.5910	1.50
BG21	8.50	0.07395	1.6561	1.41
BG28	18.05	0.06697	1.6988	2.05
BG35	12.95	0.07393	2.2592	2.21

The mineral contents of FG and BG samples were also investigated in this study (Table 3). K and S elements were detected in the highest amounts in all samples. Along with these two elements, most of the others gradually increased with increasing the aging period. The elements Na, Al, and Se did not increase or decrease gradually. In the study by Kang (2016), the mineral contents of black garlic samples were also determined. Similar to this study, the most abundant element in fresh and black garlic samples was K, followed by S. These two elements are followed by the elements Na, Mg, and Ca. In another study, Gulfraz et al. (2014) determined the concentrations of the elements Mg, Zn, and Cu in fresh garlic samples obtained from different local markets in Pakistan and showed that Mg was the most abundant element in the samples, followed by Zn and Cu elements, respectively. The findings were in agreement with the data from this study.

Table 4 shows the total flavonoid and phenolic acid contents (TFC and TPC, respectively) and antioxidant activities of EtOAc, EtOH, and MeOH extracts obtained from FG and BG samples. In the EtOAc extracts, BG14 gave the highest amount of TFC (20.55±2.50 mg quercetin g^{-1} extract), and BG21 gave the highest TPC amount (34.25±1.75 mg gallic acid g^{-1} extract). In the EtOH extracts, FG had the highest amounts of TFC (4.38±0.11 mg quercetin g^{-1} extract) and TPC (49.70±2.53 mg gallic acid g^{-1} extract). From the 14th day of the aging period, significant decreases in the amounts of TFC and TPC were observed, and the decrease was limited in the rest. In the MeOH extracts, the amounts of TFC and TPC gradually increased with increasing the aging period, and BG35 had the highest amounts of TFC (1.39±0.06 mg quercetin g^{-1} extract) and TPC (27.17±1.82 mg gallic acid g^{-1} extract). In the research performed by Choi et al. (2014), they also examined the total polyphenol and flavonoid contents of samples. The authors found the highest amount of total polyphenol (58.33±0.70 mg GAE g^{-1}) in the black garlic sample obtained after a 21-day aging period, while the highest amount of total flavonoids (16.26±1.69 mg RE g^{-1}) was determined in the black garlic sample obtained after a 28-day aging period. In a study conducted by Kim et al. (2013), black garlic samples were prepared from fresh garlic harvested in Korea, similar to the stepwise heating schedule performed by Kang (2016), and the flavonoid and phenolic acid contents of these samples were compared. The authors found that black garlic sample labeled as BG5 had the highest flavonoid and total phenolic contents, followed by black garlic samples labeled as BG4, BG3, BG2, BG1, and FG, respectively. In a research performed by Jang et al. (2018), black garlic sample obtained from extraction with distilled water had the highest total phenol (147.58± 5.27 mg GAE g^{-1}) and flavonoid (338.04±1.60 µg QE g^{-1}) contents.

Table 3. Mineral contents of FG and BG samples

Minerals (mg Kg^{-1})	Samples				
	FG	BG14	BG21	BG28	BG35
Na	132.35±2.625	55.60±0.151	62.41±0.682	35.69±0.653	97.91±2.152
Mg	236.88±4.009	270.20±2.839	309.31±2.421	312.73±0.679	423.58±10.595
Al	3.67±0.036	0.30±0.058	0.99±0.022	0.57±0.050	1.63±0.089
K	3984.98±115.373	4705.80±71.288	5111.90±73.147	5927.71±26.830	7603.60±89.912
Ca	221.26±2.513	289.75±6.815	393.35±5.272	331.31±8.883	387.36±8.353
Mn	3.16±0.063	3.72±0.037	4.52±0.028	4.93±0.029	6.07±0.141
Fe	6.86±0.408	6.87±0.120	8.60±0.177	10.27±0.056	13.63±0.374
Cu	21.38±0.235	33.30±0.239	39.27±0.317	45.60±0.260	48.19±3.259
Zn	155.11±3.034	174.09±2.595	276.80±2.921	259.86±1.308	310.79±6.382
P	1262.43±5.222	1898.36±49.000	2003.45±73.114	2112.75±75.108	2608.55±24.078
S	3857.28±23.658	4914.68±107.836	5446.03±247.589	5447.11±131.225	6723.02±65.647
Se	0.04±0.003	0.02±0.002	0.03±0.001	0.04±0.002	0.04±0.002

After evaluating the results obtained from ABTS and DPPH methods, it was determined that the results were in correlation with each other. In all the extracts, the radical scavenging activity increased as the aging period increased in both methods, with the highest scavenging activities in the EtOAc extract of BG35. The differences observed in the fresh and black garlic samples were impressive. Choi et al. (2014) found that free radical scavenging activities of the extracts increased with the aging period. In contrast to this study, the authors reported a gradual decrease in antioxidant activity in ABTS and DPPH methods after 21 days of aging period. In another study, Bae et al. (2014) obtained black garlic samples from fresh garlic supplied from local markets in Korea using different temperatures (40, 55, 70, and 85ºC) and incubation periods (up to 45 days). The authors stated that the DPPH scavenging efficiencies of the samples increased significantly with increasing incubation time and temperature. Jang et al. (2018) studied the antioxidant activities of distilled water, ethanol, and chloroform extracts of fresh and black garlic samples. They found that the black garlic sample had higher radical scavenging activity than fresh garlic for all three solvents.

Table 4. Total flavonoid and phenolic acid contents of extracts from FG and BG samples

Samples	Extracts	Total flavonoid (mg quercetin g^{-1} extract)	Total phenolic acid (mg gallic acid g^{-1} extract)	ABTS (µg Trolox g^{-1} extract)	DPPH (µg Trolox g^{-1} extract)
	EtOAc	15.16±1.84	14.12±2.53	4.00±0.06	3.12±0.06
FG	EtOH	4.38±0.11	49.70±2.53	6.99±0.44	0.52±0.02
	MeOH	0.46±0.05	12.56±0.84	1.04±0.01	0.35±0.01
	EtOAc	20.55±2.50	28.47±0.13	36.17±0.26	13.49±0.33
BG14	EtOH	1.94±0.10	31.13±0.97	7.18±0.25	0.79±0.04
	MeOH	0.84±0.06	18.60±0.39	3.65±0.10	0.76±0.03
	EtOAc	9.37±0.92	31.13±0.97	48.12±0.44	21.48±0.54
BG21	EtOH	1.67±0.16	32.69±1.36	7.44±0.93	1.64±0.05
	MeOH	1.16±0.05	24.18±0.39	5.44±0.23	1.98±0.07
	EtOAc	12.46±1.78	34.25±1.75	53.39±3.78	24.28±0.85
BG28	EtOH	0.98±0.11	30.94±0.52	7.88±0.07	2.58±0.06
	MeOH	1.15±0.08	26.65±1.17	7.83±0.09	2.32±0.07
	EtOAc	13.18±0.66	30.74±0.58	55.41±0.35	26.10±0.78
BG35	EtOH	1.23±0.05	31.58±1.04	8.97±0.95	3.00±0.10
	MeOH	1.39±0.06	27.17±1.82	8.59±0.70	2.65±0.07

The antimicrobial activities of EtOAc, EtOH, and MeOH extracts obtained from FG and BG samples in this study were investigated using disc diffusion and broth dilution methods. When the results of both methods were evaluated together, it was seen that the antimicrobial activity of black garlic samples was lower than that of fresh garlic. The EtOAc extract of FG exhibited inhibition zones against *C. albicans* (12 mm), *E. faecium* (7 mm), MRSA (9 mm), and *P. aeruginosa* (9 mm), while the MIC values of this extract were 256 µg ml^{-1} against *E. faecium*, MRSA and *P. aeruginosa*, and 128 µg ml^{-1} against *C. albicans*. However, the black garlic samples showed activity only against *C. albicans*. In the research carried out by Jang et al. (2018), the antimicrobial activities of fresh and black garlic extracts were determined using the disc diffusion method. The authors reported that only chloroform extract from the fresh garlic sample had an inhibitory zone (9.67^{b} mm) against *Bacillus cereus*. From the black garlic samples, distilled water extract had antimicrobial activity against *E. coli* and *S. aureus*, ethanol extract had activity only against *E. coli*, and chloroform extract showed antimicrobial activity only against *B. cereus*.

4. Conclusions

This research aimed to determine the changes in the chemical and physicochemical contents and the in vitro antimicrobial and antioxidant properties that occur during the aging process of garlic samples to produce black garlic. Compared to FG, the browning intensity, total acid and reducing sugar contents, total protein, crude fat, total ash, and crude fiber contents improved substantially in BGs, even as the moisture contents and pH values decreased. The radical scavenging capacities have been significantly better than that of FG. However, no significant differences have been observed in the total flavonoid and phenolic acid contents and antimicrobial activities of the samples during the aging period. In light of the findings obtained from this study, it is possible to say that the biological, chemical, and physicochemical properties of garlic change during the aging process. Therefore, the aging period should be optimized for the desired target in biotechnological processes.

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Statement of Conflicts of Interest

The author declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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