International Journal of Food Science + Technology

3580 International Journal of Food Science and Technology 2023, **58**, 3580–3590

Original article

Black garlic production: The influence of ageing temperature and duration on some physicochemical and antioxidant properties, and sugar content

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(Received 28 February 2023; Accepted in revised form 11 April 2023)

Summary Herein, some quality characteristics (water content, pH, antioxidant activity and sugar contents) of fresh garlic (Taşköprü, Türkiye) and black garlic, which were produced under various ageing temperatures (60 °C, 70 °C and 80 °C) and durations (30, 45 and 60 days), and the effect of ageing processes on these quality characteristics were determined. The ageing process caused a reduction ($P < 0.01$) in the water content and pH value of the samples, while enhanced ($P < 0.01$) antioxidant activity and increased ($P < 0.01$) the content of glucose, fructose, sucrose and reducing sugar. Both the water content and pH value of the black garlic declined significantly with increasing ageing temperature, while only pH value of the black garlic declined significantly with increasing ageing duration. Ageing samples at 60 °C and 70 °C exhibited higher antioxidant activity than those aged at 80 °C, which had similar activity to the fresh samples. A similar status was observed when the ageing temperature was prolonged to 60 days. Sucrose concentration of the black garlic samples raised with elevating the ageing temperature and declined with prolonging the duration. While a remarkable decline in the reducing sugars of the black garlic samples was observed with elevating the ageing temperature, the maximum concentration was recorded on the 45th day of the ageing process. However, the black garlic samples produced at 60 °C for 60 days or 70 °C for 30 days could be recommended to get a final product with good antioxidant capacity, adequate amount of free sugars, pH value and water content.

Keywords Antioxidant activity, black garlic, HPLC, process conditions, sugar content, Taşköprü sarımsak.

Introduction

Garlic (*Allium sativum* L.) is a versatile spice from the Alliaceae family with a strong unique flavour that has had extensive culinary and medical uses. Garlic has been reported to contain a broad spectrum of biologically active compounds including polyphenols, organosulfur compounds and polysaccharides, which attracted researchers' interest for centuries (Butt *et al.*, 2009; Shang *et al.*, 2019; Qiu *et al.*, 2020). It has been valued for its high antioxidant characteristics, anti-inflammatory, antibacterial and antifungal, immunomodulatory, antihypertensive, antihyperlipidaemic, anticancer and hepatoprotective properties (Shang *et al.*, 2019; El-Saber Batiha *et al.*, 2020). Taşköprü garlic, which has a geographical indication sign, is a clone of garlic that is native to Taşköprü region in Kastamonu, Türkiye. Taşköprü garlic is commonly used in Turkish cuisine, and it is distinguished by an intense aroma, high levels of total dissolved solids and long storage ability (Ipek *et al.*, 2008; Destanoğlu, 2022; Turfan, 2022).

Black (aged) garlic is a novel processed form of garlic that has gained popularity recently in several traditional and modern cuisine due to its health benefits and

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doi:10.1111/ijfs.16456

organoleptic features (the sweet–sour taste and soft-chewy texture). This unique form of garlic is processed by ageing the raw bulbs at high relative humidity (70%–90%) and temperature (40 °C–90 °C) for a specific duration (10–90 days), in which various changes occur within the garlic constituents particularly organosulfur compounds, carbohydrates and phenolic substances (Ryu & Kang, 2017; Sun & Wang, 2018; Bedrníček *et al.*, 2021). Breaking down the macromolecules and Maillard reaction (non-enzymatic browning reaction) are reported as the key chemical reactions that occur through the ageing process (Ríos-Ríos *et al.*, 2019; Qiu *et al.*, 2020). The thermal decomposition of alliin and allicin to a stable compound with no taste or odour called S-allylmercapto-cysteine (SAMC) is the primary transformation in the organosulfur substances (Zhang *et al.*, 2016; Ríos-Ríos *et al.*, 2019). On the other hand, the high temperature through the ageing process stimulates the hydrolysis of structural polysaccharides, particularly fructan, into smaller sugars (oligo-, di- and monosaccharides) causing a rise in the free and reducing sugars levels in the black garlic samples. This transformation contributes to the sweet taste and soft texture of black garlic (Zhang *et al.*, 2016; Ríos-Ríos *et al.*, 2019; Leng *et al.*, 2020). An enhancement in the antioxidant activity of the black garlic assessed by various assays such as DPPH• and ABTS• scavenging activity, ferric (Fe^{+3}) reducing antioxidant power and reducing power assay was recorded by various studies. This enhancement is likely to be attributed to the increment in the concentration of the total phenolic substances and flavonoids by several folds, in addition to the increase in the levels of many other antioxidant compounds such as 5-hydroxymethyl furfural, S-allylmercapto-cysteine, S-allyl-L-cysteine and lactic acid (Bae *et al.*, 2014; Choi *et al.*, 2014; Liang *et al.*, 2015; Toledano-Medina *et al.*, 2016; Wang *et al.*, 2016; Bedrníček *et al.*, 2021). Numerous research has documented the potential biological effects of black garlic and its extracts in the treatment and/or reducing the risk of various diseases including hypertension, arterial stiffness, inflammation, stroke, cancer and Alzheimer's disease (Borek, 2001; Ried *et al.*, 2016; Kimura *et al.*, 2017). However, the quality characteristics of black garlic and its biological activity are strongly influenced by the raw material properties (e.g. variety, cultivation conditions, etc.) and the parameters of the ageing process (temperature, duration and humidity) (Bae *et al.*, 2014; Qiu *et al.*, 2020; Bedrníček *et al.*, 2021; Karnjanapratum *et al.*, 2021; Najman *et al.*, 2021). Currently, the standard production parameters of black garlic have not been established yet. However, researchers are still trying to define the optimum ageing conditions to maximise the biological, antioxidant and organoleptic properties of the black garlic samples. Moreover, as far as we know, the effect of the ageing process parameters on Taşköprü garlic, a common garlic clone in Türkiye with a geographical indication sign, has not been investigated previously. Therefore, in the current study, the influence of various ageing temperatures (60 °C, 70 °C and 80 °C) and durations (30, 45 and 60 days) on some quality characteristics of Taşköprü garlic was determined by evaluating its water content, pH value, DPPH• scavenging activity and sugar content (glucose, fructose, sucrose and reducing sugar [glucose + fructose]).

Material and methods

Materials and chemicals

Taşköprü garlic, which has a geographical indication sign, was purchased from Kastamonu city, Türkiye, and used for the production of black (aged) garlic. Special attention was given to the garlic bulbs to be free from disease, insect damage and/or mechanical damage. Purchased garlic was stored in a cool, dark and dry location until production. Reagents and solvents used during the analyses were of analytical or HPLC grade.

Black garlic production (ageing process)

The ageing process was carried out by placing the raw garlic samples in a climatic chamber (KKS 115, Smart Pro) set at a constant relative humidity of 80%. Black garlic samples were produced using temperatures of 60 °C, 70 °C and 80 °C. The samples were collected after specific intervals (30, 45 and 60 days) of the process. After peeling, the samples were stored in polyethylene bags at −18 °C until analyses. Peeled garlic gloves were ground by a small food processor immediately before the analyses.

Determination of water content of fresh and black garlic

The water content of the garlic samples (both fresh and black garlic) was assessed according to the procedures described by Gökalp *et al.* (2010). The reduction in the samples' weight, which resulted from subjecting them to 105 °C overnight in a laboratory oven, was used to calculate the percentage of their water content.

Determination of pH value of fresh and black garlic

The pH value of the garlic samples (both fresh and black garlic) was assessed according to the procedures described by Gökalp *et al.* (2010). A quantity of 10 g of ground garlic was homogenised in distilled water (100 mL) for 1 min by an ULTRA-TURRAX disperser (T18, Germany) and its pH value was measured at 20 °C using a previously calibrated pH metre (Seven Compact-S220, Greifensee, Switzerland).

Determination of the antioxidant activity of fresh and black garlic

The antioxidant activity of the garlic samples (both fresh and black garlic) was assessed by determining their DPPH• scavenging activity (%) following the method stated by Wu *et al.* (2021) with some changes. The ground garlic samples were vortexed with methyl alcohol (1:10, w:v) for 30 s. The mixture was then subjected to an ultrasonic bath (UC-D10H, WiseClean, Korea) for half an hour at moderate intensity, centrifuged (4980 ***g***, 20 min, 25 °C) and the supernatant filtered using Whatman filter paper (No:1). The extraction process was repeated twice (with 1:5, w:v, garlic:solvent). The absorbance at 517 nm of the DPPH solution (6×10^{-5} M) and extracts aliquot mixture was measured after incubation for 30 min in dark at ambient temperature using a spectrophotometer (T60_UV-Visble, Leicestershire, UK). The percentage of the DPPH• scavenging activity was determined and employed to calculate the IC_{50} values (mg/mL) of the garlic samples.

Determination of sugar content of fresh and black garlic

The sugar (glucose, fructose, sucrose and reducing sugar [glucose + fructose]) content of the garlic samples was assessed using an HPLC system (LC-10A HPLC Series; Shimadzu, Kyoto, Japan) connected to a refractive index detector (RID-10A; Shimadzu) following the method stated by Kelebek *et al.* (2018). An Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad, CA, USA) at a temperature of 55 °C was used for the separation. Five millimetre sulphuric acid solution at a flow rate of 0.7 mL/min was used as a mobile phase. For sugar extraction, 10 g of garlic sample was thoroughly mixed with distilled water (10:1, v:w) using ULTRA-TURRAX disperser, then the mixture was centrifuged (5.500 r.p.m, 4 °C, 15 min – Hettich Universal 320R). The obtained supernatant was filtered through a 0.45 μm filter and directly injected into the HPLC system. The external standard method was employed to estimate the sugar content of the garlic samples. For this purpose, five different concentrations (5–500 mg/L) of both glucose, fructose and sucrose standards were employed to create calibration curves, and the sugar content of black garlic samples was determined by using these curves.

Statistical analysis

A full factorial design with two factors at three levels for each one (3 temperatures × 3 durations) was used for the production of black garlic. The variance analysis was carried out to detect the significant differences between the means, and then Duncan analysis was employed to evaluate the differences between the garlic groups at a level of $P < 0.05$ using SPSS-25 software (IBM SPSS). On the other hand, SIMCA.14.1 software (UMETRICS, Umea, Sweden) was utilised to perform the principal component analysis (PCA).

Results and discussion

The water content of fresh and black garlic

Some quality features of fresh and black garlic samples including water content, pH and antioxidant activity (DPPH) are displayed in Table 1. As noted in the table, all studied features were significantly ($P < 0.01$) affected by both ageing temperature and duration. As expected, water content was significantly lower in the black garlic samples compared to that in fresh samples. The ageing process caused a water loss ranging from 46% to 88% depending on the ageing parameters (temperature and duration). The significant reduction in the water content as a result of the ageing process was also confirmed by several authors (Bae *et al.*, 2014; Choi *et al.*, 2014; Zhang *et al.*, 2016; Bedrnĩček *et al.*, 2021). The water content of fresh garlic samples used as material in the present study was comparable to the values stated by Bae *et al.* (2014) (66.1%), Choi *et al.* (2014) (64.21%) and Bedrnĩček *et al.* (2021) (57.04%–65.34%). On the other hand, the water content of the black garlic produced in the current study was lower than the values found by Bae *et al.* (2014) (45.3%–53.4%) and Zhang *et al.* (2016) (46.06%–49.02%), whereas it was compatible with the water content specified by Choi *et al.* (2014) (29.88%–64.21%). However, the observed differences are thought to be due to the disparities in the ageing parameters including temperature, duration and relative humidity. Moreover, our results revealed that the reduction in the black garlic's water values generally increased significantly ($P < 0.01$) as the levels of ageing temperature and duration increased (Table 1). Similarly, Bae *et al.* (2014) and Sun & Wang (2018) reported a lower water content in the garlic samples produced at high ageing temperatures. Additionally, it has been reported that the water content of black garlic decreased with increasing the production duration; however, the water content of the final product was associated with the applied temperature and relative humidity during the process (Zhang *et al.*, 2016; Sun & Wang, 2018). The interaction between the ageing temperature and duration was also noticed to be significant ($P < 0.01$) in terms of water content, in fact, samples aged at 60 °C for 30, 45 and 60 days and those produced at 70 °C for 30 days were found to have soft texture and acceptable elasticity. Whereas the samples produced at 70 °C for 45 days were found to have harder texture and lower elasticity. However,

Table 1 The water content, pH value and IC_{50} values of DPPH• scavenging activity of fresh and black garlic samples aged at various temperatures for different durations ($\bar{x} \pm$ SD)

Ageing parameters	*n*	Water (%)	pH	DPPH (IC_{50}, mg/mL)
Temperature (T)				
Fresh (Raw)	3	61.80 ± 0.11a	6.54 ± 0.02a	13.07 ± 0.31a
60 °C	9	33.44 ± 1.21b	4.07 ± 0.36b	3.85 ± 1.37b
70 °C	9	28.77 ± 1.98c	3.85 ± 0.11c	4.80 ± 0.96b
80 °C	9	7.13 ± 1.40d	3.73 ± 0.04d	14.60 ± 14.23a
Sig.		**	**	**
Duration (D				
Fresh (Raw)	3	61.8 ± 0.11a	6.54 ± 0.02a	13.07 ± 0.31a
30 days	9	23.88 ± 11.48b	4.07 ± 0.37b	4.45 ± 0.92b
45 days	9	23.57 ± 12.36b	3.84 ± 0.1c	5.38 ± 4.14b
60 days	9	21.89 ± 12.78c	3.75 ± 0.03d	13.42 ± 14.57a
Sig.		**	**	**
T × D		**	**	**

**Significant at levels of $P < 0.01$; a–d: values with different letters are significantly different from each other.

samples with moisture content lower than 30% were found to be dry and had a hard texture.

The pH value of fresh and black garlic

As displayed in Table 1, the pH value of fresh garlic (6.54) is significantly higher than that of the black garlic samples with a pH value ranging from 3.73 to 4.07 depending on the ageing temperature and duration. Several studies stated a significant reduction in the pH value of garlic samples as a result of the ageing process (Bae *et al*., 2014; Choi *et al*., 2014; Toledano-Medina *et al*., 2016; Bedrníček *et al*., 2021). The observed decline in the pH value during the ageing process is thought to be associated with organic acids formed through the process. Indeed, Liang *et al*. (2015) stated the presence of various organic acids such as acetic acid, formic acid, succinic acid and 3-hydroxy propionic acid in black garlic samples, whereas they were not detected in the raw samples. The pH value of raw garlic samples used as material in this study was comparable to the values stated by Bae *et al*. (2014) (6.42), Bedrníček *et al*. (2021) (5.99–6.11), Choi *et al*. (2014) (6.33), Nam *et al*. (2023) (6.47) and Toledano-Medina *et al*. (2016) (6.31). Additionally, the pH values of the black garlic produced in the current study were compatible with the values found by Choi *et al*. (2014) (3.74). Regarding the ageing parameters, a significant decline ($P < 0.01$) in the pH value of black garlic was also observed with an increment in the ageing temperature and duration that was applied in the present study (Table 1). Likewise, several authors stated a remarkable decrease in the garlic's pH value with increasing the temperature and/or duration of the ageing process (Bae *et al*., 2014; Choi *et al*., 2014; Toledano-Medina *et al*., 2016). Choi *et al*. (2014) determined the pH values of black garlic samples produced at 70 °C and 90% relative humidity for 7, 14, 21, 28 and 35 days as 5.49, 4.41, 4.22, 4.07 and 3.74 respectively. Similarly, Nam *et al*. (2023) recorded the pH values of black garlic samples produced at similar conditions for 10, 20, 30 and 40 days as 4.57, 4.04, 3.81 and 3.68 respectively. Besides that, the interaction between the ageing temperature and duration was also noticed to significantly ($P < 0.01$) affect the pH value of the samples (Table 1). In this concept, all aged samples, except those aged at 60 °C for 30 days, had a pH value lower than 4.2, which is the recommended value for prohibiting the growth of anaerobic bacteria in the aged samples (Toledano-Medina *et al*., 2016). Additionally, a rapid decline in the pH value of black garlic was noticed when higher ageing temperature was applied especially in the early stage (short ageing duration) of the process, that is, the black garlic aged at 60 °C, 70 °C and 80 °C reached a pH value of 4.53, 4.00 and 3.68, respectively, after 30 days of the process (data not shown). Similar observations were also documented by Bae *et al*. (2014) and Toledano-Medina *et al*. (2016).

The antioxidant activity of raw and black garlic

DPPH (1,1-diphenyl-2-picrylhydrazyl radical) assay, a frequently used method to determine the antioxidant activities of food items (Pyrzynska & Pękal, 2013), was used to determine the free radical scavenging capability of fresh and black garlic samples produced in the present study. As illustrated in Fig. 1, the DPPH• scavenging activity of fresh garlic increased from 26.68% to 50.89% in an equivalent way to increasing the extract concentration. Moreover, the DPPH• scavenging activity of black garlic samples aged at various temperatures and duration in our study also increased in an equivalent way with increasing the extract concentration with results ranging from 17.93% to 63.33% (Fig. 1). Regarding the black garlic samples, the maximum DPPH• scavenging activity was observed in the samples aged at 60 °C for 45 days. Bedrníček *et al*. (2021) reported a value ranging from 11.41% to 14.25% for garlic samples belonging to various varieties aged for 15 days. A wide range (22.5%–44.77%) of DPPH• scavenging activity was stated by Bae *et al*. (2014) in samples aged at different temperatures (40 °C, 55 °C, 70 °C and 85 °C) for 45 days. It is worth to mention that garlic variety, ageing parameters (temperature, humidity and duration) and extraction conditions (solvent, method, extract concentration, etc.) are likely to influence the antioxidant activity of black garlic.

On the other hand, the IC_{50} values of fresh garlic were found to be 13.07 mg/mL (Table 1) and altered

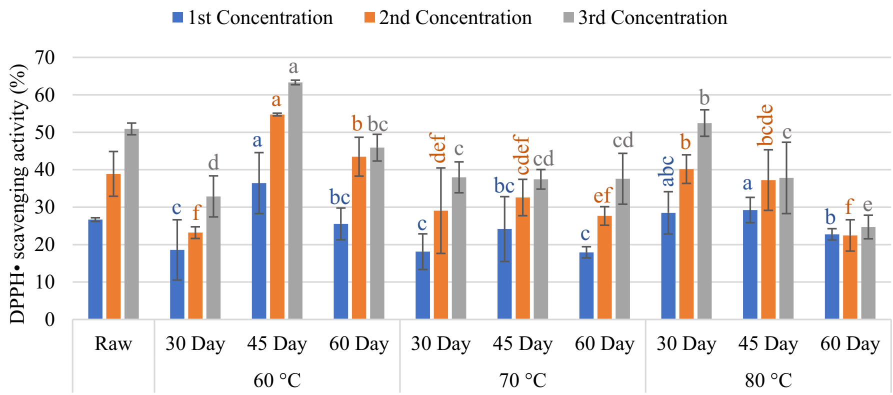

Figure 1 The DPPH• scavenging activity (%) of fresh (raw) and black garlic samples aged at various temperatures for different durations. First, second and third concentrations: 6.67, 10.00 and 13.33 mg/mL for fresh garlic and 1.69, 2.54 and 3.38 mg/mL for black garlic. a–f: columns with different letters (within the same concentration – same colour) are significantly different ($P < 0.05$).

significantly in the aged samples, indicating that the ageing process generally caused a significant variation in the antioxidant of garlic samples depending on the applied parameters during the process. However, both studied parameters (temperature and duration) significantly ($P < 0.01$) influenced the DPPH• scavenging activity of garlic samples expressed as IC_{50} value (Table 1). As can be noticed from the table, subjecting garlic to a temperature of 60 °C or 70 °C caused a significant decline in their IC_{50} values compared to the fresh sample, indicating a remarkable increment in their antioxidant activities. During the ageing process, a rise in the concentration of many compounds with antioxidant characteristics such as phenolic substances, ascorbic acid, thiosulfinates and S-allyl cysteine has been recorded in various studies (Choi *et al.*, 2014; Zhang *et al.*, 2016; Sun & Wang, 2018; Bedrníček *et al.*, 2021). This rise is likely to be associated with the increment in the antioxidant activity of the aged garlic samples. Otherwise, using a higher temperature (80 °C) during the ageing process resulted in a final product with an IC_{50} value statistically similar to that assessed in the raw samples and higher than those found in samples obtained using lower (60 °C and 70 °C) ageing temperatures (Table 1). In brief, our results revealed that increasing the ageing temperature from 60 °C to 70 °C did not significantly impact the IC_{50} values of black garlic samples, whereas increasing the ageing temperature to 80 °C adversely influenced the IC_{50} value of the final product. In contrast, Bae *et al.* (2014) and Sun & Wang (2018) recorded an enhancement in the antioxidant activity of black garlic with increasing the production temperature, which disagrees with our findings. This disparity is likely to be attributed to the differences in the ageing duration and humidity applied in their studies. In a similar manner, increasing the ageing duration from 30 to 45 days did not significantly influence the IC_{50} values of the aged samples, whereas increasing the duration to 60 days adversely influenced the IC_{50} value of the samples, resulting in a final product with IC_{50} value similar to the value determined in the raw garlic (Table 1). Ageing garlic for a long duration can cause a loss in the concentration of the substances that exhibit antioxidant features. Indeed, several authors reported a decrease in the antioxidant activity of aged garlic after a specific duration which varied remarkably depending on the applied parameters in their studies (Choi *et al.*, 2014; Zhang *et al.*, 2016; Sun & Wang, 2018). Besides that, the interaction between the ageing temperature and duration was also noticed to significantly ($P < 0.01$) affect the IC_{50} values of the garlic samples (Table 1). In this concept, all aged garlic exhibited lower IC_{50} values than the values of raw ones, except for the garlic aged at 80 °C for 60 days. Garlic samples aged at 60 °C for 45 days had the lowest IC_{50} value, indicating the highest radical scavenging capability among other samples, whereas the highest IC_{50} value was found in the samples aged at severe conditions of both temperature and duration (80 °C for 60 days) (data not shown). This reduction could be explained by the thermal degradation of various antioxidant substances under these severe conditions. However, the phenolic compounds can be influenced diversely by the thermal treatment according to their structure and thermal stability, the processing method and conditions and the physicochemical characteristics of food (Ioannou *et al.*, 2012; Ríos-Ríos *et al.*, 2019). It is worth mentioning that, the antioxidant activity of the aged garlic produced in the current study has been evaluated by only the DPPH assay. Thus, other assays such as ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) and CUPRAC (cupric reducing antioxidant power) assays (Capanoglu *et al.*, 2018) could be also employed for better representing the best ageing–duration combination that provides the best antioxidant activity.

The sugar content of fresh and black garlic

The concentration of glucose, fructose, sucrose and reducing sugar (glucose + fructose) in both fresh and black garlic samples is displayed in Table 2. As noticed in the table, the ageing process caused a significant increment in the sugar contents (glucose, fructose, sucrose and reducing sugar) of garlic samples. Similarly, many studies reported a rise in the concentration of these sugars in aged garlic in comparison with raw garlic (Choi *et al*., 2014; Kang, 2016; Zhang *et al*., 2016). The thermal breakdown of polysaccharides during the ageing process is considered the key factor for this increment resulting in smaller sugars, particularly fructose and glucose due to the degradation of fructans (the primary sugar in fresh garlic) (Toledano-Medina *et al*., 2016; Lu *et al*., 2018; Ríos-Ríos *et al*., 2019). Besides that, fructose was found to be the predominant sugar in the aged garlic with a remarkably high concentration in comparison with glucose and sucrose (Table 1). Similarly, Choi *et al*. (2008) and Kang (2016) stated the dominance of fructose over other sugars (glucose and sucrose) in black garlic. The thermal hydrolysis of glucan, a polyfructosylsucrose molecule, is associated with the recorded large amount of fructose since its hydrolysis yields more fructose molecules than glucose. The presence of fructose in a large amount in aged garlic coupled with its high sweetness power makes it the key contributor to the sweet taste of black garlic (Yuan *et al*., 2018; Ríos-Ríos *et al*., 2019; Qiu *et al*., 2020).

Regarding the ageing parameters, all studied sugars were significantly ($P < 0.01$) affected by both ageing temperature and duration and the interaction between these two factors (Table 2). As can be noticed in Table 2, the highest concentration of glucose and sucrose was determined in garlic aged at 80 °C. Moreover, increasing the ageing temperature caused a significant increase in the sucrose concentration, whereas the lowest levels in the concentration of fructose and reducing sugar were found in the samples aged at 80 °C. Increasing the ageing temperature from 65 °C to 75 °C resulted in a final product with a larger quantity of reducing sugar as recorded by Sun & Wang (2018). Similar results were also recorded by Zhang *et al*. (2016) when the ageing temperature increased from 60 °C to 70 °C. Lu *et al*. (2018) reported that ageing garlic at high temperatures quickened the thermal degradation of polysaccharides into smaller sugars such as oligo-, di- and monosaccharides. However, the ageing duration in these studies was relatively low compared to ours. Moreover, a significant amount of reducing sugars is likely to be depleted by the caramelisation and Maillard reaction when high ageing temperatures are used (Corzo-Martínez *et al*., 2012; Zhang *et al*., 2016).

Concerning the ageing duration, the glucose, fructose and reducing sugar contents reached the highest concentration on the 45th day of the ageing process, whereas garlic samples aged for 60 days exhibited a significantly lower concentration of these sugars (Table 2). A similar rise in the reducing sugars content of black garlic followed by a marked decrease was recorded by Ahmed Sun & Wang (2018) and Zhang *et al*. (2016) when high ageing temperatures (80 °C–90 °C) were applied. Similarly, Nam *et al*. (2023) recorded a rise in the concentration of reducing sugar until the 20th day of the ageing process performed at a temperature of 70 °C and 90% humidity, samples then experienced a reduction in their reducing sugar content. However, Yuan *et al*. (2018)

Table 2 The sugar contents of fresh and black garlic samples aged at various temperatures for different durations (g/kg) ($\bar{x} \pm$ SD)

Ageing Parameters	*n*	Glucose	Fructose	Sucrose	Reducing sugar◊
Temperature (T)					
Fresh (Raw)	3	7.14 ± 0.02d	40.00 ± 0.05d	3.60 ± 0.05d	47.13 ± 0.03d
60 °C	9	12.52 ± 5.52b	237.09 ± 76.31a	26.94 ± 8.98c	249.61 ± 81.82a
70 °C	9	11.70 ± 2.52c	225.52 ± 60.21b	34.90 ± 5.88b	237.22 ± 62.6b
80 °C	9	16.05 ± 8.63a	45.01 ± 34.56c	37.35 ± 13.81a	61.06 ± 42.97c
Sig.		**	**	**	**
Duration (D)					
Fresh (Raw)	3	7.14 ± 0.02d	40.00 ± 0.05d	3.60 ± 0.05d	47.13 ± 0.03d
30 days	9	14.50 ± 8.11b	172.97 ± 92.59b	36.89 ± 12.95a	187.47 ± 90.18b
45 days	9	15.33 ± 2.77a	176.46 ± 98.84a	33.77 ± 9.62b	191.79 ± 96.16a
60 days	9	10.44 ± 5.87c	158.20 ± 134.88c	28.52 ± 8.50c	168.64 ± 140.59c
Sig.		**	**	**	**
T × D		**	**	**	**

**Significant at levels of $P < 0.01$; a–d: values with different letters are significantly different from each other, ◊: reducing sugars: refers to the total glucose and fructose content of the garlic.

recorded a rise in fructose concentration through 90 days of the ageing process at 55 °C and 80% humidity. On the other hand, sucrose declined gradually through the ageing process and the lowest concentration was found in samples aged for 60 days (Table 2). The observed decline is likely to be associated with the breaking down of sucrose into its constituents (glucose and fructose) under the conditions of the ageing process (high temperature and low pH) (Kang, 2016). In contrast, a stable level of sucrose and glucose during the whole ageing process was recorded by Yuan *et al.* (2018). However, the concentration of various sugars in the final product is strongly attributed to the applied parameters through the ageing process, since ageing conditions can induce the formation of small sugars by polysaccharides' degradation, particularly fructan and the dissipation of reducing sugars by Maillard reaction. Consequently, the decline in the reducing sugars in the later stage of the ageing process could be observed after the complete hydrolysis of fructan, when the consumption rate of reducing sugar exceeds its production rate. In terms of each ageing temperature (60 °C, 70 °C and 80 °C) individually, extending the ageing duration caused an increase in the concentration of all studied sugars when garlic aged at low temperatures (60 °C), and a decline in their amounts at high ageing temperatures (70 °C and 80 °C). Moreover, subjecting garlic to 80 °C caused a marked drop in the levels of fructose, which is the primary contributor to aged garlic sweetness, in contrast, the highest concentration was detected in samples aged at 60 °C for 60 days (data not shown).

Correlation between some chemical, physicochemical and biological characteristics of fresh and black garlic

The relationship among water content, pH, antioxidant activity and sugar contents of garlic samples is displayed in Fig. 2. As can be noticed in the Figure, the water content of garlic samples positively correlated with their pH values ($r = 0.788$; $P < 0.01$), whereas a negative relation was recorded with their sucrose content ($r = -0.611$; $P < 0.01$). A negative relationship between the pH value of garlic samples was found with their content of sucrose and glucose ($r = -0.746$; $P < 0.01$ and $r = -0.441$; $P < 0.05$, respectively). Reducing the pH of garlic samples could induce the hydrolysis of polysaccharides and sucrose into their constituents, especially when coupled with high temperature, causing a rise in the levels of di- and monosaccharides. A similar relation was also observed between the IC_{50} value and the samples content of sugars (glucose, fructose, reducing sugar and sucrose, $r = -0.502$, $r = -0.618$, $r = -0.637$; $P < 0.01$ and $r = -0.416$; $P < 0.05$, respectively), in other words, increasing the antioxidant activity of garlic samples positively related to their content of sugars. The positive correlation between the antioxidant activity of garlic samples and their sugar content could be explained by the degradation of the structural polysaccharides which can lead to the release of the bound phenolic compounds, consequently increasing the antioxidant activity of the aged garlic. Indeed, food processing (especially those coupled with high temperatures) was reported to enhance the release of bound phenolic compounds, consequently increasing the antioxidant properties of foods (Acosta-Estrada *et al*., 2014) including garlic (Alide *et al*., 2020). Interestingly sucrose content of black garlic was positively correlated ($r = -0.829$; $P < 0.01$) with glucose content. Moreover, a strong significant correlation between fructose and reducing sugar content of garlic samples was found, since fructose is considered the predominant free sugar in aged garlic.

The principal component analysis (PCA)

The principal component analysis (PCA) was conducted to recognise the variation in several quality parameters (water content, pH, %DPPH (concentration dependent), IC_{50}, sucrose, glucose, fructose and reducing sugar [Glu + Fru]) of fresh (raw) garlic and black garlic produced under different ageing conditions. In the biplot for garlic samples classified into ten groups [one control (fresh garlic) and nine (3 ageing temperatures × 3 ageing durations) black garlic samples are represented in orange, and the dependent variables in green (Fig. 3a)]. Herein the first two principal components (PC1 = 41.5% and PC2 = 28.5%) could explain 70% of the total variation among the garlic samples. As noticed in the figure, the garlic samples which are represented by orange colour are spread over the four regions indicating the variation among the garlic samples based on the dependent variables. Garlic samples that are close to each other are similar to each other in terms of the examined features and differ from other samples (separated ones). Moreover, the water content and pH values of the garlic samples were found to be close to each other representing a strong positive correlation between them. On the other hand, a strong negative relationship between water and pH values with the sucrose and glucose content was also determined. In addition, samples that were found near the dependent variable (represented in different colours) indicate higher levels of this variable in the considered sample. In this context, the fresh sample was located in the upper left of PCA biplot near the pH variable, indicating a higher pH value in fresh garlic, similar situation can be seen for the IC_{50} and the sample aged at 80 °C for 60 days; and DPPH•% scavenging activity and the sample aged at 60 °C for 45 days.

Principal components analysis was also conducted to reveal the differences between fresh and black garlic aged at different temperatures (Fig. 3b). In the biplot of garlic

	Water	pH	IC_{50}	%DPPH_ 1st Conc.	%DPPH 2nd Conc.	%DPPH 3rd Conc.	Sucrose	Glucose	Fructose
pH	0.788**								
IC_{50}	-0.259	0.131							
%DPPH_ 1st Conc.	-0.008	0.019	-0.109						
%DPPH 2nd Conc.	0.229	0.043	-0.443*	0.679**					
%DPPH 3rd Conc.	0.381*	0.211	-0.540**	0.650**	0.804**				
Sucrose	-0.611**	-0.746**	-0.416*	0.012	0.085	-0.014			
Glucose	-0.358	-0.441*	-0.502**	0.375*	0.516**	0.451*	0.829**		
Fructose	0.301	-0.318	-0.618**	-0.036	0.293	0.270	0.318	0.242	
Reducing sugar	0.277	-0.338	-0.637**	-0.015	0.317	0.291	0.359	0.294	0.999**

Figure 2 The Person's correlation between the various chemical and physiochemical features of black garlic. *$P < 0.05$; **$P < 0.01$.

samples classified into four groups according to the ageing temperature [one control (raw garlic) and three different temperatures], the first two principal components (PC1 = 56.2% and PC2 = 29.4%) could explain 85.6% of the total variation among the garlic samples. As a result of the general evaluation, it was observed that the fresh garlic was on the right side of the graph, and the black garlic samples were on the left side of the graph indicating a significant difference between them. Besides that, the fresh garlic had higher water content and pH value compared to the black garlic samples, while it had lower sucrose content. On the other hand, black garlic produced at 60 °C and 70 °C had the highest fructose and reducing sugars contents and the lowest IC_{50} values.

The principal component analysis was conducted again to reveal the differences between fresh and black garlic aged for different durations (Fig. 3c). In the biplot for garlic samples, which were classified into four groups according to the ageing duration [one control (fresh garlic) and three different durations] and the first two principal components (PC1 = 68.4% and PC2 = 25.4%) could explain 93.8% of the total variation among the garlic samples. Like the previous biplot (according to temperature), the fresh garlic (right side) was also separated from the samples aged for different durations (left side). As noticed in the figure, the fresh garlic had higher water content and pH value compared to the aged samples, while it had lower sucrose and reducing sugars contents. On the other hand, the black garlic aged for 45 days exhibited the highest glucose, fructose and reducing sugar contents, whereas the lowest IC_{50} value and the highest sucrose content were determined in black garlic samples produced for 30 days.

Conclusion

The ageing process significantly influenced many quality features of Taşköprü garlic, Türkiye. However, ageing temperatures (60 °C, 70 °C and 80 °C), duration (30, 45 and 60 days) and the interaction between them were found to have significant ($P < 0.01$) impacts on the water, pH, antioxidant activity and the sugar content of black garlic samples. An important decline was recorded in both water content and pH value in parallel with increasing the ageing temperature and duration. On the other hand, ageing garlic at 60 °C and 70 °C resulted in a final product with higher antioxidant activity than both fresh samples and those aged at 80 °C. A similar manner was found in samples aged for 30 and 40 days in comparison to the fresh garlic and those aged for 60 days. Increasing the ageing temperature caused a rise in the sucrose concentration but a decline in the concentration of reducing sugar. On the other hand, prolonging the ageing duration caused a reduction in sucrose levels, whereas the glucose and fructose levels reached the maximum levels after 45 days, followed by an important drop. It is worth mentioning that garlic samples with water content lower than 30% (samples aged at harsher conditions than 70 °C for 45 days) tend to have hard and unfavourable textures. However, all black samples, except those aged at 60 °C for 30 days, had a pH value lower than 4.2, the recommended

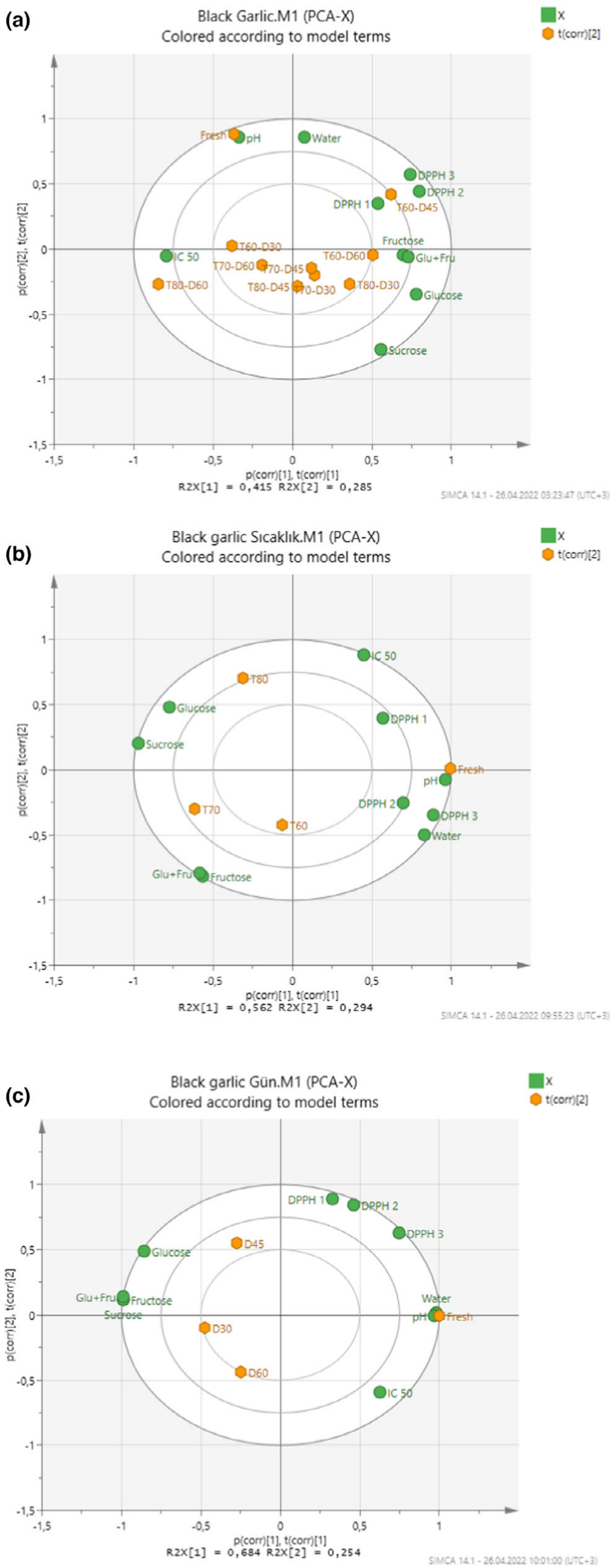

Figure 3 The principal components analysis (PCA) of garlic samples, (a) biplot of ten groups (one fresh and nine black garlic samples aged at three different temperatures × 3 different durations); (b) biplot of four groups (one fresh and three different ageing temperatures); biplot of four groups (one fresh and three different ageing durations).

value for prohibiting the growth of anaerobic bacteria. The highest antioxidant activity was recorded in garlic aged at 60 °C for 45 days, whereas the highest levels of fructose, the primary contributor to aged garlic's sweet taste, were found in samples aged at 60 °C for 60 days. In conclusion, depending on our findings, ageing Taşköprü garlic at either 60 °C for 60 days or 70 °C for 30 days could be recommended to get a final product with good antioxidant capacity, adequate amount of free sugars, pH value and water content which contribute to favourable features of the aged garlic (sweet and sour taste, storage stability, soft texture and high antioxidant activity). Future research can focus on identifying the specific bioactive compounds responsible for the antioxidant activity of aged garlic, evaluating the antioxidant activity of hydrophilic and lipophilic extracts of aged garlic, exploring sensory attributes and examining the antimicrobial activity of aged garlic.

Acknowledgments

This research was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) with project no: 220O087. The authors gratefully acknowledge financial support from TÜBİTAK. The authors also would like to thank the support provided by Ataturk University (Türkiye), Adana Alparslan Turkes Science and Technology University (Türkiye), *RMIT University (Australia)*, *Riddet Institute (New Zealand)*, the Researchers Supporting Project number (RSD-2023R668), King Saud University (Riyadh, Saudi Arabia) and Qatar University (Qatar) for the preparation of this manuscript. This research was presented in part at the 9th International GAP Summit Scientific Research Congress, Adıyaman, Turkey, July, 1–2, 2022.

Author contributions

Eyad Aoudeh: Formal analysis (equal); investigation (equal); writing – original draft (equal). **emel Oz:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Hasim Kelebek:** Investigation (equal); validation (equal); writing – review and editing (equal). **Charles**

Stephen Brennan: Writing – review and editing (equal). **Naushad Ahmad:** Writing – review and editing (equal). **Tahra El Obeid:** Writing – review and editing (equal). **Fatih ÖZ:** Methodology (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

Conflict of interest

The authors have declared no conflicts of interest.

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ijfs.16456.

Data availability statement

The data that support the findings of this study are available from the authors upon reasonable request.

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