The Shortest Innovative Process of Black And Golden Garlic For Enhancing The S-Allylcysteine Content And Antioxidant Activity

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Abstract

Black garlic is a type of heat-treated garlic for which the traditional process is extremely simple but it is very time-consuming, taking more than one month. The purpose of this research was to reduce the processing time of black garlic. A novel processing including soaking and freezing was applied for the pre-treatment of the garlic. The effects of two temperatures (60 and 80 °C) and two relative humidities (65 and 80% RH) were investigated to find the optimum conditions for black garlic production. The optimum conditions for black garlic production were pre-treatment using CaCl₂ soaking followed by freezing before being further incubated at 80 °C and 80% RH for 1 week. This optimal condition produced black garlic with high antioxidant activity as 5,390 and 25,421 mg Trolox/100 g dry weight for DPPH and ABTS assays, respectively. However, the incubation process at 60 °C and 65% RH provided golden garlic with the highest S-allylcysteine (SAC) content by about 1,772 mg/100 g dry weight. This novel process shortened the processing time of black garlic by about four times and provided a new processed garlic product called “golden garlic”.

Introduction

Garlic is widely used as a seasoning for food and as a medicinal agent for the treatment of multiple human diseases and disorders because it has shown antimicrobial, antioxidant, anti-atherosclerotic and anti-cancer properties from the effects of its many active components. Garlic contains various bioactive compounds, such as organosulphur compounds, saponins, phenolic compounds and polysaccharides [1]. Sulphur-containing allicin (diallyl thiosulphinate) is the most well-known biologically active component in freshly crushed garlic cloves or extracts [2]. Allicin is made from the non-protein amino acid alliin by the enzyme alliinase [3]. The amount of thiosulphinates, which can affect garlic odour intensity and antibiotic properties, varies among garlic cultivars [4]. Although garlic has many active components that contribute to its health benefits, including allicin and its derivatives, the consumption of raw garlic has limits, because eating it excessively can cause stomach upset, and some people do not like its pungent flavour. At present, heat treatment is used to help improve the flavour of garlic to eliminate the unpleasant odour. In addition, heat increases the stability of the bioactive compounds in garlic [5].

Black garlic is a processed garlic produced by heat treatment under controlled humidity. Generally, it takes 1–2 months to produce without pre-treatment. During heat treatment, the colour of the garlic changes from white to black due to a browning process, and the total soluble solids content is improved, resulting in a sweet taste and a rubbery texture. It also has increased polyphenol content and antioxidant capacity [6]. On the other hand, the increased antioxidant capacity is also due to the formation of S-allylcysteine (SAC) [7]. SAC is formed by the enzymatic hydrolysis of γ -glutamyl-S-allylcysteine, catalysed by γ -glutamyl transpeptidase (γ -GTP, EC 2.3.2.2). The SAC content is up to 6 times higher after the ageing process [8]. However, heat treatment reduces the activity of γ -GTP, which also results in reduced SAC formation. Therefore, the temperature is one of the most important factors affecting the quality of black garlic during the manufacturing process.

The traditional process is time-consuming because the whole bulb of raw garlic is heated at high temperatures and controlled relative humidity without any pre-treatment. The high temperature destroys the cell structure and causes substances to react with each other [9], as does freezing, which is one of the cell disruption methods causing freezing injury [10]. Li et al. [11] found that freezing for 30 h had the greatest impact on increasing the reducing sugar content, total phenolic content and 5-hydroxymethyl-2-furaldehyde (5HMF) levels. In addition, the amount of SAC is considered an important indicator of the quality of black garlic. An increase in SAC both before and after black garlic production should be considered. In a study on the effect of soaking on the γ -GTP activity and SAC content in garlic, Xu et al. [12] found that endogenous γ -GTP in garlic increased 23 times and the SAC content increased 4 times after soaking in 10 mM CaCl_2 solution at 10°C for 72 h.

However, the two pre-treatment methods consisting of CaCl_2 soaking and freezing have never been used together to produce black garlic and compared with commercial black garlic. Furthermore, the classification of the constituents of black garlic produced by this method has not been studied. Therefore, the objective of this research was to reduce the processing time of black garlic by pre-treatment at different temperature and relative humidity conditions and to summarise the differences of the chemical components of black garlic and also to develop a new “golden garlic” product.

Results And Discussion

Effect of processing conditions on colour of black garlic

The colour results for black garlic produced at various temperatures (60 and 80 °C) and relative humidities (65 and 80% RH) showed that temperature had a significant effect ($p < 0.05$) on colour (Figure 1 and Table 1). L^* decreased, but ΔE increased with increasing temperature. At 80 °C, L^* decreased rapidly within 2 weeks; after that, it began to stabilise; meanwhile, treatment at 60 °C caused a gradual decrease in L^* (Figure 2a). The a^* of garlic produced at 60 °C increased slightly for 2–3 weeks and then showed a gradual decline; meanwhile, after 1 week at 80 °C, a^* dropped sharply and remained stable after week 3 (Figure 2b). Whilst the b^* change is similar to the L^* change (Figure 2c). Thus, the ΔE of garlic produced at 80 °C increased rapidly to more than 80 within 1 week, after which, it began to stabilise; meanwhile, the ΔE of garlic produced at 60 °C was 80 in 4 weeks (Figure 2d). It is well known that black garlic is a processed garlic product formed by heat treatment under controlled humidity. The heating process leads to the Maillard reaction [13] due to the polysaccharides and amino acids in raw garlic. The main constituents of the polysaccharides and amino acids in raw garlic are fructan and arginine [14]. The Maillard reaction causes a colour change, from the white of raw garlic to the dark brown of black garlic [5]. The decrease in a^* and b^* values with an increase in temperature and treatment time affects the colour change from yellow-brown to the red-black of black garlic. This indicates that there is an aggregation of melanoidin particles over time in black garlic produced at high temperatures [15]. Hence, L^* decreases while ΔE increases during heat treatment. The ΔE increases very rapidly with an increase in temperature due to an increase in the reactivity of the Maillard reaction [16].

Effect of processing conditions on moisture content and pH of black garlic

The results from this experiment show that temperature had a significant effect on the colour values, while relative humidity did not affect the colour values. However, it did affect the black garlic's moisture content (Figure 2e). The moisture content of the black garlic produced at 80% RH was higher than that produced at 65% RH at the same temperature. In addition, the black garlic produced at the same relative humidity had a lower moisture content when produced at 80 °C than at 65 °C. This indicates that the moisture content of black garlic is affected by heat treatment conditions. At a higher temperature, the moisture in the product decreases more rapidly, while a higher relative humidity of the process results in a higher moisture content of black garlic. Initially, the decrease in moisture in the processed product proceeds slowly due to the high relative humidity, and then it proceeds very quickly due to evaporation at a high temperature [17,18], after which it can slow down again or even stop due to the degradation of water-retaining compounds, such as fructans. During the garlic ageing process, these compounds decompose into simple sugars or disaccharides [19]. A decrease in moisture content affects the Maillard reaction because moisture is associated with limited molecular mobility and the consequent retarding of the reaction rate [20]. As previously reported by Van Boekel [21], starting the Maillard reaction from a high water activity and then lowering it results in increasing reactant concentrations and makes them react more easily. Therefore, the rate of Maillard reactions increased at the initial stage. However, over time, the system becomes too concentrated, resulting in less diffusion. Therefore, the Maillard reaction rate decreased due to reactants not meeting so easily anymore. In this study, L^* began to stabilise as the sample moisture content dropped below 40%.

During processing, pH was directly related to L^* and b^* but inversely proportional to ΔE because of the browning process. At the end of the process, after the garlic had turned perfectly black (ΔE was 80 within 1 week at 80 °C and within 5 weeks at 60 °C), the pH of black garlic decreased to below 4.6 (Figure 2f). A lower pH was found for black garlic, possibly due to the presence of acetic acid and succinic acid. Liang et al. [22] observed acetic acid and succinic acid in black garlic, but not in fresh garlic samples. This is because, during heat treatment, there is sugar fragmentation from the α -dicarbonyl and β -dicarbonyl of hexose or pentose to the short-chain carboxylic acid, which can also produce formic acid, succinic acid and 3-hydroxypropionic acid [22]. In addition, the decrease in pH can be associated with browning substances formed because of the formation of carboxylic acid substances [23]. A lower pH means that black garlic is more acidic [24].

Effect of processing conditions on bioactive compounds of black garlic

In addition to the Maillard reaction, SAC formation from GSAC also occurs during the production of black garlic. The GSAC content (Figure 3a) of black garlic produced at 80 °C decreased rapidly from 1,104 to 250 mg/100 g dry weight in 1 week; meanwhile, for garlic produced at 60 °C, the decrease in GSAC content was continuous and approximately linear. When GSAC content decreased, SAC content increased in 1 week (Figure 3b). The SAC content of garlic produced at 60 °C was approximately 2-fold higher than that of garlic produced at 80 °C. The increase of SAC content was related to the increase of γ -GTP activity

in the garlic, which is affected by the processing temperature [11]. Garlic samples heated at low temperatures had more SAC content than at high temperatures because it was previously reported that the optimum temperature for γ -GTP activity was 40 °C [25]. In addition, SAC formation is also affected by the water-facilitated reaction between GSAC and γ -GTP [8] because the water in garlic assists the γ -glutamyl transferase reaction, assisting hydrolysis, and enough water supports the transformation of GSAC into SAC [26]. Garlic samples heated at a higher temperature showed greater water loss. Therefore, the garlic sample heated at 60 °C had a higher SAC content than that heated at 80 °C.

After 2 weeks, the SAC content of garlic produced at 60 °C decreased 4-fold; meanwhile, that of garlic produced at 80 °C decreased 6–7-fold when compared to week 1. The SAC gradually decreased until it could not be detected due to the acceleration of Maillard-type reactions between SAC and D-glucose [27]. Kimura, et al. [28] reported that the main volatile compounds formed by the Maillard reaction from the equimolar mixture of SAC and D-glucose were dimethyl disulphide, dimethyl trisulphide, dimethyl tetrasulphide, allyl methyl sulphide and an unknown compound with a molecular weight of m/z 162. However, in the presence of excess glucose, dimethyl disulphide, dimethyl trisulphide and dimethyl tetrasulphide were not formed from SAC.

The results for 5-HMF content presented in Figure 3c show that HMF was not detected at week 0, but there was an increase for HMF after heat treatment. In garlic produced at 80 °C, HMF increased rapidly during weeks 1–3; meanwhile, HMF was detected in week 3 in garlic produced at 60 °C. HMF is considered the most important intermediate product that occurs during two reactions: the acid-catalysed degradation of hexose and the decomposition of 3-deoxyosone in the Maillard reaction [29]. 5-HMF is produced under acid conditions [30]. Therefore, when the pH decreased with increasing incubation time, 5-HMF increased. This is consistent with the research of Nakagawa et al. [31], who found that the 5-HMF level at the 70 °C heating of garlic increased as the number of heating days increased. However, it is noted that there was a continued increase of HMF in garlic produced at 60 °C; meanwhile, in garlic produced at 80 °C, there was a continuous decrease in HMF after week 3, possibly due to moisture content [21].

Effect of processing conditions on antioxidant activity of black garlic

In terms of antioxidant activity (Table 2), both DPPH and ABTS assays showed a similar trend of changes in antioxidant activity (Figure 3e and 3d). The antioxidant activity of garlic produced at 60 °C tended to increase with curing duration, possibly due to the representative antioxidant compounds in garlic: phenolics, flavonoids and sulphur-containing compounds [32,33]. The presence of the browning reaction products (i.e. melanins, 5-HMF) also showed antioxidant abilities [34,35]. While the antioxidant activity of garlic produced at 80 °C was increased dramatically in 1 week and was highest at 2 weeks, after that, it declined steadily. These effects can be primarily caused by temperature. Although the SAC and browning reaction products were formed during the production of black garlic, high temperatures can destroy the bioactive compounds contained in black garlic [36, 37]. Therefore, the production of black garlic at 80 °C should only take 1-2 weeks to incubate for its high antioxidant activity.

Principal component analysis of black garlic qualities during heat treatment

In this study, PCA was used to the explained relationship of the sample based on black garlic qualities. The sample data was used to check the suitability of the preliminary data in the PCA (Kaiser-Meyer-Olkin: KMO) and Bartlett's Test. The KMO was 0.731, indicating that 73.1% of the model could be described. In addition, Bartlett's Test had an approximate distribution in Chi-Square = 805.800, Significance = 0.000, which is less than 0.05, thus rejecting H_0 , indicating that the 15 quality variables are related enough to be used for PCA.

The PCA extracted two components with Eigenvalues greater than 1, explaining 80.09% of the total variance in the data set. The first and second components explained 48.68% and 31.41%, respectively, of all variations (Figure 4a). This implies that the PCA explained 48.68% of the black garlic qualities during heat treatment summarised as two underlying dimensions coined from the black garlic qualities loaded significantly in the two extracted components. The loadings express how well the new abstract principal components correlate with the old variables. The first new abstract principal component, PC1, correlated well with L^* , pH and GSAC content of black garlic qualities. The ΔE , DPPH and ABTS were negatively correlated with the new PC. The second component, PC2, correlated well with a^* , b^* , Moisture content and SAC content. HMF was negatively correlated with the new PC.

The factor scores of each garlic sample on PC1 and PC2 were plotted on the regions defined by PC1 and PC2, resulting in a biplot showing the position of each garlic sample on PC1 and PC2 as shown in Figure 4b. The data were categorised into four groups; the first group consisted of samples treated with low temperature (60 °C) between 1–4 weeks that was distinct from the others by a^* , b^* , moisture and SAC contents. The second group consisted of samples treated with low temperature (60 °C) between 6–8 weeks that was distinct from the others by E and antioxidant activity, and the third group consisted of samples treated with high temperature (80 °C) that was distinct from the others by 5HMF contents. Meanwhile, fresh garlic was separated and distinct from the others by L^* and pH.

The fresh garlic sample was separated by L^* and GSAC because it had white colour and high GSAC content. GSAC is often found in fresh garlic because it is a precursor to SAC formed during heat treatment [8]. Therefore, after ageing, SAC increases but GSAC decreases, resulting in high SAC contents of samples treated with low temperature (60 °C) between 1–4 weeks. However, high temperature and long-time process resulted in a decrease in SAC; meanwhile, 5HMF was increased due to the Maillard reaction. Therefore, samples treated at low temperature (60 °C) between 6–8 weeks changed in colour from white to black, resulting in high E and high antioxidant activity due to Maillard products, but low SAC contents. In addition, samples treated with high temperatures (80 °C) had higher 5HMF contents but lower moisture contents than samples treated with low temperatures, because high temperatures increase the rate of Maillard reactions [16] and accelerate the evaporation of water more than low temperatures [19]. On the contrary, an increase in the acidity during the process due to the browning reaction also reduced the pH of the black garlic. It dropped below 4.2 after the first week of aging due to carboxylic acids that formed during thermal processing by browning reaction [38]. The effect of high

temperature and acidity may inhibit γ -GTP activity because the optimum pH and temperature for γ -GTP activity are pH 6 and 70 °C [39]. Therefore, samples treated with high temperatures (80 °C) have less SAC content than at low temperatures. For this reason, the samples can be divided into four main groups, namely the fresh garlic group, samples treated at low temperature (60 °C) between 1–4 weeks, samples treated at low temperature (60 °C) between 1–4 weeks and samples treated with high temperature (80 °C).

Comparison of bioactive compounds and antioxidant activity of various processed garlic

In this study, the optimum conditions for producing black garlic were 80 °C and 80% RH because this condition had a short processing time of within 1 week, which produced soft and elastic black garlic with a final moisture content of 66%. The SAC content was 874.26 ± 57.27 mg/100 g dry weight. In addition, the black garlic produced under these conditions had high antioxidant activity: $5,390.02 \pm 180.03$ and $25,421.11 \pm 262.39$ mg Trolox/100 g dry weight when analysed with DPPH and ABTS, respectively. However, processing at 60 °C and 65% RH for 1 week produced golden garlic with the highest SAC content ($1,772.15 \pm 48.98$ mg/100 g dry weight), about 2 times more than black garlic. Therefore, it can be said that in this study, two garlic-processing processes were obtained: black garlic processing and golden garlic processing. Moreover, the comparison of the amount of SAC, 5HMF and antioxidant activity in Table 3 ($P \leq 0.05$) found that golden garlic had the highest SAC content, more than 11 times higher than commercial black garlic, but the lowest antioxidant activity compared to other products. Our black garlic contained 5 times the SAC content of commercial black garlic and had the highest 5HMF content and antioxidant activity.

Conclusion

Garlic was processed into black garlic at different temperatures and relative humidity conditions, resulting in significantly different biological activities and contents of various substances in garlic. The temperature had a remarkable impact on the quality of black garlic. The results showed that good qualities occur under processing at 80 °C and 80% RH within 1 week for black garlic and at 60 °C and 65% RH within 1 week for golden garlic. However, the quality of black garlic and golden garlic is determined by a very complex process that results from other factors besides temperature, such as the garlic cultivar. More efforts should be made to explain the effects of these factors on the quality of black garlic and golden garlic products in future studies.

Materials And Chemicals

Garlic (*Allium sativum* L.) was purchased from local markets in Srisaket province (Jae Mam Phanit), Thailand. Identification was done according to an Alliaceae expert and the literature [40]. A voucher specimen (GA55001) was referenced by the Srisaket Horticultural Research Centre, Srisaket, Thailand. The garlic samples were kept at room temperature until use. The use of plants in the present study complies with international, national and institutional guidelines. 2,2-Diphenyl-1-picrylhydrazyl (DPPH),

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), S-allyl-L-cysteine (SAC) and 5-(hydroxymethyl)furfural (5HMF) were purchased from Sigma-Aldrich (Massachusetts, USA). L-γ-Glutamyl-(S)-allyl-cysteine (GSAC) was purchased from Toronto Research Chemicals (Toronto, Canada). Anhydrous sodium carbonate, aluminium chloride and potassium acetate were purchased from Loba Chemie (Mumbai, India). Ethanol was purchased from RCI Labscan Ltd. (Bangkok, Thailand). All other chemicals and reagents used in the experiments were analytical grade.

Processing conditions of black garlic

The fresh garlic was peeled and sized, and only clean whole cloves were selected. Selected fresh garlic bulbs were pre-treated by soaking with CaCl_2 at 10 °C for 72 h, and then they were frozen at –18 °C for 30 h. After that, pre-treated garlic was heated at two temperatures (60 and 80 °C) in two relative humidities (65 and 80% RH) for 8 weeks in a drying oven (UF110, Memmert, Germany). Colour, pH, moisture content, GSAC, SAC, 5HMF and antioxidant activity were determined every week for up to 8 weeks.

Preparation of black garlic extracts

The black garlic was peeled and then ground in a blender (HR2115, Philips, Indonesia) and mixed with 50% ethanol at a ratio of 1:10 (w/v). The mixture was extracted using the condition of Pakakaew et al. [41] at 60 °C for 90 min in a water bath shaker (Memmert, Schwabach, Germany), filtered through Whatman® no. 4 filter paper, and the filtrate was kept at –18 °C for analysis.

Colour and pH measurements

The colour values of the garlic samples were measured using a MiniScan EZ 4500L spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) calibrated with black and white porcelain reference plates following the method of Phimolsiripol et al. [42]. The CIE L^* , a^* and b^* colour spaces were measured. L^* indicates lightness, and a^* and b^* indicate colour directions; $+a^*$ is redness, $-a^*$ is greenness, $+b^*$ is yellowness and $-b^*$ is blueness. The pH was measured using an electronic pH meter (Mettler Toledo FE20, Küsnacht, Switzerland).

GSAC, SAC and 5-HMF content measurements

The extracts were filtered through a 0.45-μL syringe filter before being injected into a high-performance liquid chromatograph (HPLC) with a UV detector (1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) to analyse the GSAC, SAC and 5-HMF contents. The HPLC column was an Inertsil ODS-3 (C18 column, 5 μm, 4.6 × 250 mm, GL Sciences Inc., Japan). The isocratic HPLC system was used for analysis. The mobile phase was prepared by mixing 50 mM phosphate buffer (pH 2.8) and methanol in a ratio of 85:15 v/v, respectively, all HPLC grade. The flow rate of the mobile phase was 1 mL/min, with a sample injection volume of 10 μL and column temperature of 25 °C. The detection wavelength was 205 nm for GSAC and SAC detection [43] and 285 nm for 5-HMF detection [44].

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined using the method of Lu et al. [45] and Surin et al. [46] with minor modifications. For detection, 300 μL of garlic extract was mixed with 3000 μL of 0.208 mM DPPH solution. The mixture was then incubated for 30 min in the dark at room temperature followed by the measurement of its absorbance at 515 nm. Trolox was used as a standard at concentrations of 0–500 $\mu\text{mol/L}$. The antioxidant capacity of the sample was expressed as mg Trolox equivalent antioxidant capacity (TEAC) per 100 g dry weight. The measurements were carried out in triplicate.

ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was modified from the method of Surin et al. [47,48]. Briefly, 7 mM ABTS was dissolved in distilled water with 2.45 mM potassium persulfate. The mixture was incubated 12–16 h in the dark at 30 °C to obtain ABTS radical cations ($\text{ABTS}^{\bullet+}$). The $\text{ABTS}^{\bullet+}$ solution was adjusted with sodium phosphate buffer (0.1 M, pH 7.4) to an initial absorbance of 0.75 ± 0.005 at 734 nm. For determination, 2900 μL of the $\text{ABTS}^{\bullet+}$ solution was well mixed with 100 μL of garlic extract. The mixture was then incubated for 10 min in the dark at 25 °C followed by the measurement of its absorbance at 734 nm. Trolox was used as the calibrating standard. The antioxidant capacity of the sample was expressed as mg Trolox equivalent antioxidant capacity (TEAC) per 100 g dry weight. The measurements were carried out in triplicate.

Statistical analysis

All experiments were conducted in triplicate. The mean and standard error of the mean were determined using SPSS Statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences among the means of treatment were determined using analysis of variance (ANOVA) with Duncan's multiple range post hoc analysis at the 95% confidence level. Principal component analysis (PCA) and biplots were also generated to analyse the correlation structure of a group of multivariate observations and provide the axis along which the maximum variability in the data occurred [49].

Declarations

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Author contributions statement

P.P., Y.P. and N.U. conceived the idea of the study and designed the experiment. N.U. was supervisor and project administration. Y.P. and S.K. suggested the project. P.P. carried out the data collection and data analysis and wrote the original draft. S.T., W.K. and Y.P. reviewed and edited the manuscript. All authors were involved in reviewing the article and approved the manuscript.

Competing interests

The authors declare no competing interests

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Tables

Due to technical limitations, tables 1 and 2 are only available as a download in the Supplemental Files section.

Table 3 Comparison on bioactive compounds and antioxidant activity of various processed garlicks

Treatment	SAC content (mg/100 g dry weight)	5-HMF content (mg/100 g dry weight)	Antioxidant activity (mg TEAC/100 g dry weight)	
			DPPH	ABTS
Golden garlic (60 °C 65 %RH; 1 week)	1772.15±48.98 ^a	-	146.291±25.72 ^c	4681.05±338.07 ^c
Black garlic (80 °C 80 %RH; 1 week)	874.26±57.27 ^b	50.03±0.01 ^a	5390.02±180.03 ^a	25421.11±262.39 ^a
Commercial black garlic	150.86±8.14 ^c	9.85±0.67 ^b	3550.15±65.72 ^b	17184.27±436.30 ^b

^{a-c} Different superscripts within the same column indicate significant differences ($p \leq 0.05$).

Figures

Figure 1

Black garlic prepared using CaCl_2 soaking followed freezing (SF) pre-treatment methods at various temperatures and relative humidities

Figure 2

Colour of black garlic after different thermal processes at various temperatures and relative humidities: a) L^* , b) a^* , c) b^* , d) ΔE , e) moisture content and f) pH; error bars represent standard deviations of the mean ($n = 3$).

Figure 3

GSAC content (a), SAC content (b), 5HMF content (c) and antioxidant activities by DPPH assay (d) and ABTS assay (e) of black garlic after different thermal processes at various temperatures and relative humidity; error bars represent standard deviations of the mean (n = 3)

Figure 4

Principal component (PC) analysis of garlic qualities (a) and biplot analysis of garlic samples and their physiochemical properties (b); T1 = 60 °C and 65% RH, T2 = 60 °C and 80% RH, T3 = 80 °C and 65% RH, T4 = 80 °C and 80% RH and w0–w8 = weeks

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables.docx](#)