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Changes of Physicochemical Properties in Black Garlic during Fermentation

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Abstract: To investigate the changes of the main ingredients in black garlic (BG) during fermentation, the contents of moisture, total acids and reducing sugars were determined. Allicin, 5-Hydroxy methylfurfural (5-HMF), and total phenols were also determined as bioactive substances. DPPH scavenging capacity was determined to indicate the antioxidant activity of BG. The changes in hardness and color were detected as well. The results showed that the moisture content decreased from 66.13% to 25.8% during the fermentation. The content of total acids, total phenols, and reducing sugars increased from 0.03 g/g to 0.29 g/g, from 0.045 μg/g to 0.117 μg/g, and from 0.016 g/g to 0.406 g/g, respectively. The content of 5-HMF increased from 0 to 4.12 μg/mL continuously, while the content of allicin increased from 0.09 mmol/100 g to 0.30 mmol/100 g and then decayed to 0.00 mmol/100 g. The L*, a*, and b* values of BG were 23.65 ± 0.44, 0.64 ± 0.06, and 0.85 ± 0.05, respectively. There was a higher intensity of dark color in BG than that in fresh garlic. The hardness values decreased first and then increased in later fermentation from 465.47 g to 27,292.38 g. Principal component analysis (PCA) showed that the samples were divided into three clusters, including cluster1 (fresh garlic, S0), cluster2 (S1), and cluster3 (S3−S9). This research effectively clarified the various stage of the BG fermentation process, and it is expected to supply references for reducing production time in industrial BG fermentation.

Keywords: black garlic; physicochemical properties; antioxidant activity; principal components analysis

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1. Introduction

Garlic is widely used as a condiment around the world. According to authoritative data, China is the main garlic-producing area all over the world, and the yield of garlic reaches 2 million tons annually. Previous research has shown that garlic has a series of biologically active functions, e.g., improving cardiovascular function [1], preventing cancer [2], regulating blood sugar [3], and possessing antibacterial effects [4] and anti-hypertension [5]. Generally considered, organosulfur compounds are the main flavor ingredients in garlic, and they are the most important contributor to the biological activity as well [6,7].

Nowadays, black garlic (BG) is becoming more and more popular around the world as a derivative of fresh garlic (FG). BG is made by heat-treating FG at 60–80 °C for 1−3 months under humidity control [8]. With high-temperature treatment, biochemical reactions include the Maillard reaction, caramelization reaction, and enzyme reaction, with the color of garlic turned from white to black. Meanwhile, the composition and taste have also changed tremendously [9].

With the growing popularity of BG, more and more research has been performed on it. A lot of studies have shown that the antioxidant capacity of BG is stronger than that of

FG [10–15]. Most of the current research is devoted to the influence of the total amount of polyphenols and flavonoids on the scavenging ability of free radicals [16–19].

Several studies are devoted to exploring the changes of certain specific compounds during heat treatment in BG. For example, Yuan et al. [20] studied the changes in various sugar contents in FG and BG. Sembiring et al. [21] reviewed the contents of components in BG. Some studies used omics methods to comprehensively determine the ingredients in BG. William [9] used UHPLC-HRMS to quantitatively analyze organic acids, organosulfur compounds, glycerophospholipids, and Maillard reaction products. Ding et al. [11] used NMR to quantitatively analyze the ingredients in BG with different packaging materials.

As has been proved, LCMS is a mighty tool for characterizing the compositions of different plant substrates, and it also can be used to analyze the compositions of FG and BG. For example, UPLC-Q-TOF/MS [22] was used to determine the content in the compositions of glycerophospholipids, flavonoids, organic oxygen compounds, and fatty acids in Pu'er tea. Wang et al. [23] used UPLC-Q-TOF/MS to analyze the changes of bitter metabolites in beer fermentation; 1239 characteristic peaks were obtained, and 32 biochemical markers were further determined.

At present, most studies focus on the differences between BG and FG, and the improvement of biological activity caused by these differences is also interesting. There are relatively few studies on the changes in the content of various substances. In this work, the contents of moisture, total acids, total phenols, reducing sugars, allicin, and 5-Hydroxymethylfurfural (5-HMF) were determined to explore the changes in various physicochemical properties of BG during fermentation. In addition, the DPPH scavenging capacity, hardness, and color of BG were detected.

2. Materials and Methods

2.1. Overall Strategy

The workflow of this study is shown in Figure 1. Samples were taken every other day during the fermentation of BG. The samples were treated with liquid nitrogen first. Then, the samples were ground. Finally, extraction was conducted according to the method mentioned below. We determined the contents of various ingredients in samples. The data we obtained were analyzed statistically. We classified the differences between black garlic and fresh garlic into three aspects: sensory, physicochemical properties, and bioactive functions.

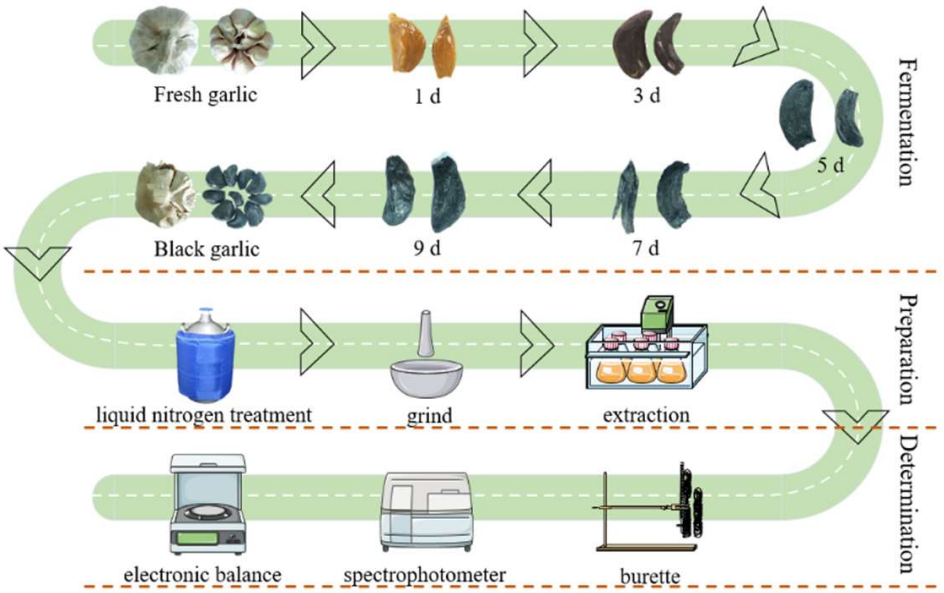


Figure 1. Overall workflow of this study.

2.2. Material

Fresh garlic collected in 2021 at the optimal ripening stage was provided by RT-Mart (Hefei, Anhui, China). The bulbs were stored at −20 °C until used. Cysteine, barbituric acid, and 5-HMF were from Shyuanye (Shanghai, China). Other reagents were supplied by HUSHI (Shanghai, China). Deionized water (18 MΩ cm^{-1}) was obtained through a Milli-Q water purification system from Millipore (Bedford, MA).

2.3. Sample Preparation

In the BG fermentation, samples were taken on 0, 1, 3, 5, 7, and 9 days as S0, S1, S3, S5, S7, and S9, respectively. Sensory evaluation and colorimetric and hardness measurements were performed immediately. Then, every 5 g sample were ground in a mortar. They were transferred into a 100 mL beaker containing 50 mL of deionized water and were ultrasonically extracted at 30 °C for 20 min. Then, the solution was filtered, and the filter residue was discarded. The filtrate was moved into a volumetric flask, followed by dilution with deionized water to 100 mL. It was diluted with deionized water to the appropriate concentration, when it was used.

2.4. Sensory Evaluation

The sensory properties of samples were analyzed by sensory evaluation. Sensory evaluation was conducted according to the method proposed by different researchers [24–27]. Healthy panelists (8 females and 8 males, 20–25 years of age), who had good cognition on the quality attributes on black garlic, were recruited from School of Food and Biological engineering, Hefei University of Technology (Hefei, China). Before sensory evaluation, commercial BG was used to help participants get familiar with sensory characteristics.

For sensory analysis, a whole BG sample was put into a glass container which was marked with a random number. The containers were placed on a plate for each panelist to evaluate them at the same time. The evaluations of taste, color, texture, smell, and dryness were recorded (0 = unsatisfactory and 10 = satisfactory extremely for taste, smell, color, dryness, and texture) by the same panel. The average scores for all descriptors of samples obtained from each panelist were taken. Water was drunk as a taste neutralizer before the evaluation of the next sample.

2.5. Basic Analysis of BG

The sample was dissolved using deionized water to 50 mg/mL. The contents of moisture, total phenolic, total acids, reducing sugars, allicin, and 5-HMF were analyzed. The content of moisture and reducing sugars were determined by the method conducted by Zambrano [28] and Huang [29]. The content of total acids was determined in keeping with the Chinese National Standard (GB). The content of total phenols was determined with the Folin−Ciocalteu method and gallic acid was used as a standard.

In addition, the contents of allicin and 5-HMF were determined by the spectrophotometric method, according to the method conducted by Joan [30] and Feng [31].

2.6. Hardness and Color

The colors of FG and BG surfaces were determined using a CHROMA METER, CR-400 (Minolta, Osaka, Japan). The results were presented in the CIE system, where L*, a*, and b* represent lightness, redness/greenness, and yellowness/blueness, respectively.

The hardness values of FG and BG were determined using a Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK). The maximum force required to break samples individually was presented as gram (g).

2.7. Measurement of Oxidation Resistance

The DPPH scavenging activity assay was conducted in the light of the method reported by Ren et al. [32]. DPPH was dissolved in ethanol to 0.25 mg/mL in an Erlenmeyer flask. To ensure the stability of the DPPH solution, the reagents were prepared 2 h in advance.

To protect the DPPH solution from the light, the flask was covered with aluminum foil and stored in a fridge. During measurement, the DPPH solution was diluted to one-fifth of its original concentration. All experiments were repeated three times. One milliliter of appropriately diluted samples was added to 1 mL DPPH solutions. As a control, 1 mL of ethanol was added instead of 1 mL of appropriately diluted samples. As for the blank, 1 mL samples were mixed with 1 mL ethanol, and the absorbance was measured at 571 nm.

2.8. Statistical Analysis

Statistical analyses of the data were conducted by SPSS Statistics 19.0 (IBM Corporation, Chicago, IL, USA) and Origin 2021b (OriginLab, Northampton, Massachusetts, USA). All experiments were performed in triplicate. All data were expressed as mean ± standard deviation (SD). ANOVA tests, followed by the LSD multiple range test, were conducted for chroma parameters. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Sensory Evaluation

The results of the sensory evaluation are shown in Table 1. In general, the acceptability of the garlic was progressively higher, as fermentation progressed. The quality was at its best on the seventh day, after which there was no significant change in product quality. In terms of color, the garlic turned completely black in the first three days of fermentation. Regarding dryness and texture, there were no significant changes throughout the fermentation process, and the scores were high. In regard to odor, the score gradually increased as the fermentation progressed, but there was no significant difference. In terms of taste, the scores on the first three days of fermentation showed significantly lower scores than those on the fifth day and beyond, reaching a score of 14 on the fifth day and continuing to increase with no significant change thereafter. The product scores improved significantly on the first five days, reaching the maximum score on the seventh day and maintaining it. The final product score stayed around 86 points.

Table 1. Sensory scores of samples during the fermentation.

Samples	Color	Dry Degree	Texture	Smell	Taste	Total Score
S1	6.67 ^{b}	16.67 ^{a}	17.67 ^{a}	13.33 ^{a}	3.67 ^{b}	58.00 ^{d}
S3	17.67 ^{a}	17.00 ^{a}	15.00 ^{a}	13.67 ^{a}	7.00 ^{b}	70.33 ^{c}
S5	17.67 ^{a}	17.00 ^{a}	15.67 ^{a}	15.00 ^{a}	14.67 ^{a}	80.00 ^{b}
S7	18.00 ^{a}	18.33 ^{a}	16.00 ^{a}	17.00 ^{a}	17.33 ^{a}	86.67 ^{a}
S9	18.00 ^{a}	18.33 ^{a}	16.00 ^{a}	16.67 ^{a}	17.33 ^{a}	86.33 ^{ab}

$^{a–d}$ Different superscripts in the same column indicate significant differences in the comparison of sensory parameters at different fermentation times.

3.2. Physicochemical Properties

3.2.1. Moisture

As can be seen from Figure 2A, the moisture content showed a decreasing trend during the production process. The moisture content decreased faster at the beginning of fermentation. The moisture content in fresh garlic (S0) was 66.13%, and after one day of fermentation (S1), the moisture content was 53.52%. The rate of decline was 19.07%. In the subsequent production process, the rates of the moisture content decline slowed down during the nine-day fermentation, which decreased from 53.52% in S1 to 25.8% in S9, with an average decline rate of 8.7%. The average rate of the moisture decline throughout the fermentation process was approximately 9.93%.

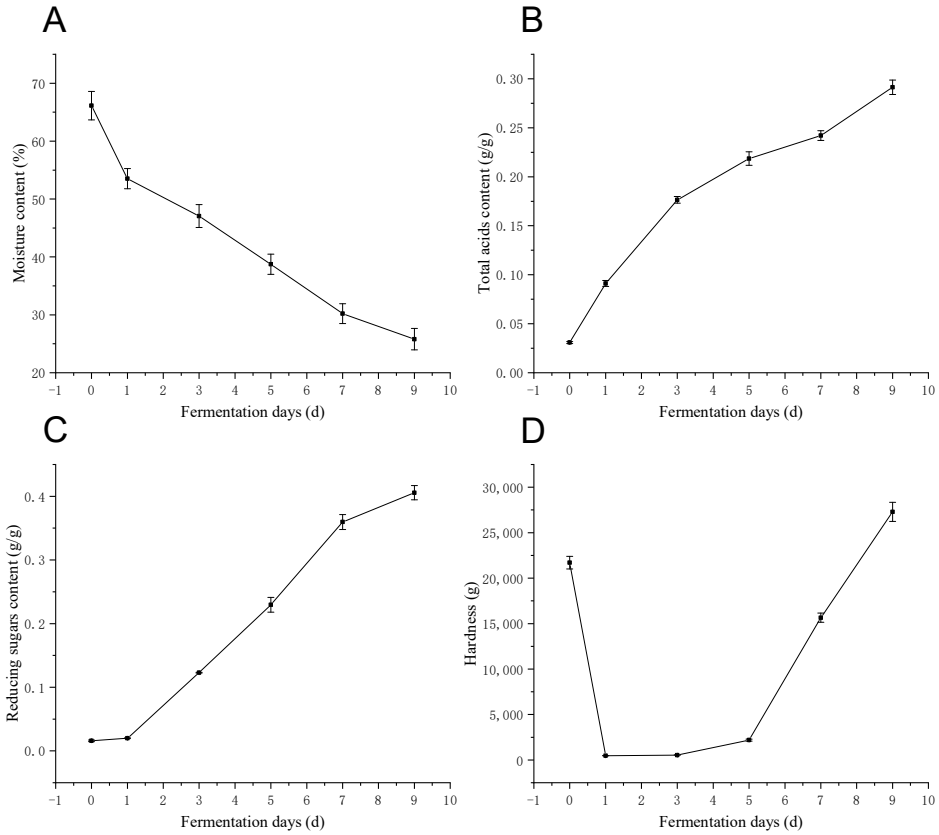


Figure 2. The change of moisture content (**A**), total acids content (**B**), reducing sugars content (**C**), and hardness (**D**) during BG fermentation. All data were expressed as mean ± standard deviation (SD).

3.2.2. Total Acids

During the fermentation process, the total acid content showed a significant upward trend, as shown in Figure 2B. The rate of increase was faster in the first three days. The total acid content in fresh garlic (S0) was low at 0.0309 g/g, and then, it increased to 0.0911 g/g after the first day of fermentation (S1), with a growth rate of 195%. The average growth rate for the first three days of fermentation was about 78.76%. In the following four days, the total acid content increased from 0.1765 g/g (S3) to 0.2420 g/g (S7), with an average growth rate of about 8.21%. The total acid content in the product at the end of fermentation was 0.2914 g/g (S9), which was nine times higher than in fresh garlic (S0), with an average growth rate of 28.32%.

3.2.3. Reducing Sugars

As can be seen from Figure 2C, the reducing sugar content showed a significant increasing trend during the entire fermentation process. The increase in reducing sugar content was not significant at the beginning of fermentation. There was no significant difference in the reducing sugar content for the first two days (1.58% in S0 and 1.99% in S1). From then on, the reducing sugar content increased dramatically, from 1.99% in S1 to 40.57% in S9, with an average growth rate of 45.77%.

3.2.4. Hardness

As can be seen from Figure 2D, the hardness of garlic decreased significantly immediately after the beginning of fermentation. The hardness of the fresh garlic (S0) was 21,711.77 g, and the hardness of the sample at the end of the first day of fermentation (S1) was 465.47 g, a 98% decrease compared to that of S0. During the next four days, the hardness remained at a lower level and increased slightly (465.47 g for S1, 530.41 for S3, and 2182.23 for S5). From the fifth day until the end of fermentation, the hardness showed

a sharp increase, with 2186.23 g for S5, 15,653.56 g for S7, and 27,292.38 g for S9, an 11-fold increase compared to that of S5, with an average growth rate of 87.97%.

3.2.5. Color

As can be seen from Figure S1, the white color of the garlic disappeared after one day of fermentation. By the third day, the garlic changed to dark brown, and after five days the color of the samples became completely black. Table 2 shows the chromaticity values of the products. The L* values of the samples kept decreasing with the extension of the fermentation time. The L* values of S0, S1, and S3 were 63.73, 37.00, and 22.86, respectively, indicating that the colors of the samples became closer to black. The L* of S5 was the lowest at 19.34, after which the L* values of the samples increased slightly but remained stable, indicating that the blackening of the samples was completed and the color stabilized. a* and b* values at the fermentation a* changed from −2.17 in S0 to 12.77 in S1 and then to 1.86 in S3. b* changed from 21.94 in S0 to 19.84 in S1 and then to 2.95 in S3. Correspondingly, the ΔE values of the samples showed an increasing trend in the first three days, from 43.00 in S0 to 72.61 in S3, and remained stable in the subsequent production process, indicating that the total color difference of the samples was increased.

Table 2. Chromaticity of samples with different fermentation days.

Chroma	S0	S1	S3	S5	S7	S9
L*	63.73 ± 0.67 ^{a}	37.00 ± 0.40 ^{b}	22.86 ± 0.32 ^{c}	19.34 ± 0.24 ^{e}	21.33 ± 0.32 ^{d}	23.65 ± 0.44 ^{c}
a*	−2.17 ± 0.18 ^{e}	12.77 ± 0.17 ^{a}	1.86 ± 0.09 ^{b}	1.11 ± 0.06 ^{c}	1.01 ± 0.05 ^{c}	0.64 ± 0.06 ^{d}
b*	21.94 ± 0.39 ^{a}	19.84 ± 0.41 ^{b}	2.95 ± 0.17 ^{c}	1.79 ± 0.03 ^{d}	1.46 ± 0.06 ^{d}	0.85 ± 0.05 ^{e}
ΔL	37.28 ± 0.62 ^{a}	−55.70 ± 1.00 ^{b}	−72.56 ± 1.09 ^{d}	−78.74 ± 1.00 ^{e}	−72.45 ± 0.77 ^{d}	−70.39 ± 0.88 ^{c}
Δa	−2.28 ± 0.20 ^{e}	12.44 ± 0.44 ^{a}	2.15 ± 0.20 ^{b}	1.17 ± 0.06 ^{cd}	1.26 ± 0.08 ^{c}	0.77 ± 0.06 ^{d}
Δb	21.31 ± 0.28 ^{a}	15.23 ± 0.41 ^{b}	−1.69 ± 0.15 ^{c}	−2.66 ± 0.15 ^{d}	−3.10 ± 0.18 ^{d}	−3.62 ± 0.10 ^{e}
ΔE	43.00 ± 0.63 ^{d}	59.08 ± 0.93 ^{c}	72.61 ± 1.09 ^{b}	77.13 ± 1.89 ^{a}	72.52 ± 0.77 ^{b}	70.48 ± 0.89 ^{b}

* All data were expressed as mean ± standard deviation (SD). L*, lightness; a*, redness/greenness; b*, blueness/yellowness. Different superscripts (a–e) within the row indicate significant differences of each color parameter compared for the same fermentation time.

3.3. Bioactive Functions

3.3.1. Allicin

As shown in Figure 3A, the fresh garlic (S0) contained a very small amount of allicin, with a specific content of 0.09 mmol/100 g. On the first day of fermentation, the allicin content increased significantly, with 0.30 mmol/100 g in S1. After the first day, the allicin content decreased sharply, with the allicin contents from S3 to S9 decreasing to nearly 0.

3.3.2. 5-HMF

The change of 5-HMF content during fermentation is shown in Figure 3B. In the early stage of fermentation, no 5-HMF was detected in S0, S1, and S3. From S5, 5-HMF started to be produced gradually and accumulated rapidly. 5-HMF contents were found to be 0.14 μg/mL in S5 and 4.12 μg/mL in S9, with an average growth rate of 133%.

3.3.3. Polyphenols

Overall, the polyphenol content showed a clear increasing trend during the fermentation process, as shown in Figure 3C. The total phenolic content in fresh garlic (S0) was 0.045 μg/g and the content increased extremely rapidly in the first three days, while the phenolic content in S3 was 0.10 μg/g with an average growth rate of 30.50%. At the later stage of fermentation, the growth rate of the polyphenol content slowed down but still showed an obvious increasing trend. For S9, the polyphenol content was 0.12 μg/g, with an average growth rate of 11.51% throughout the fermentation stage, which exceeded the polyphenol content in fresh garlic by 25 times.

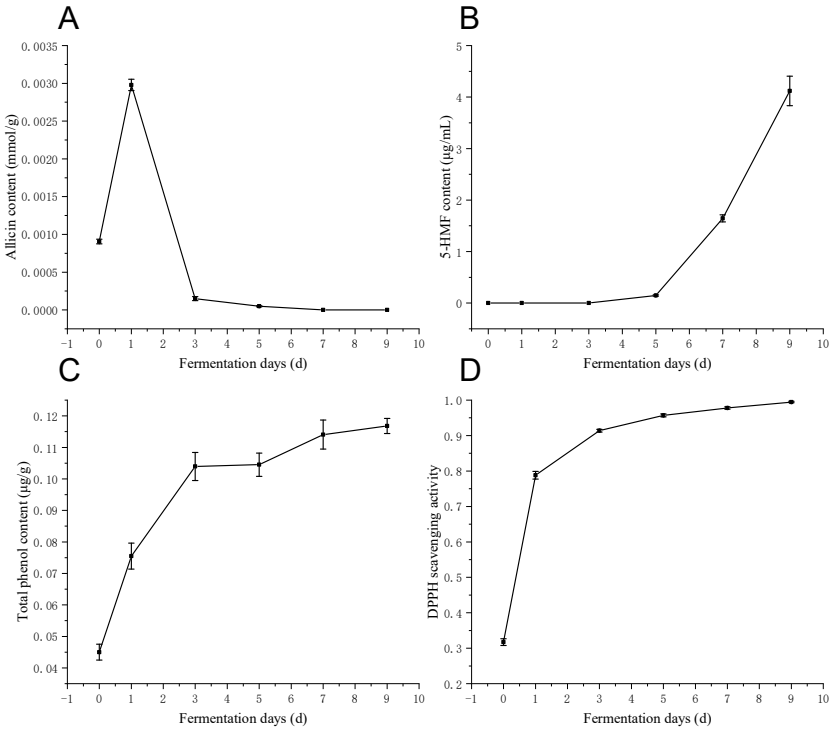


Figure 3. The change of allicin content (**A**), 5-HMF (5-Hydroxymethylfurfural) content (**B**), total phenols content (**C**), and antioxidant properties of black garlic (DPPH scavenging activity) (**D**) during the fermentation. All data were expressed as mean ± standard deviation (SD).

3.3.4. Antioxidation Property

The antioxidant capacity can be characterized by DPPH scavenging activity. Figure 3D shows the changes in the DPPH scavenging activity of BG during the fermentation. The DPPH scavenging rate of fresh garlic was relatively low at 31.72%. The DPPH scavenging rate increased to a relatively high level after the first day of fermentation, and the DPPH scavenging rate of S1 was 78.79%, which was 2.5 times higher than that of fresh garlic. From S3 to S9, the DPPH scavenging rate increased slowly. At the end of fermentation, the DPPH clearance rate of S9 was 99.47%, which was about three times that of fresh garlic (S0).

3.4. Principal Components Analysis

The obtained PCA biplot is shown in Figure 4. It can be observed from the figure that the two principal components explained 88.4% of the total variability, of which PC1 was the most significant, explaining 77.7% of the total variability. A significant separation caused by the fermentation time was observed along PC1, especially the separation of the samples at the beginning of the fermentation from the later stages of the fermentation. What influenced PC1 positively were DPPH scavenging activity, total phenol, total acids, reducing sugars, and 5-HMF. Their concentrations discriminated BG in the later stages of production from the rest of the fermentation times. PC2 also affected the separation of FG and samples at the beginning of fermentation. In this case, allicin and moisture scored the highest, indicating that the separation of S0 and S1 samples was mainly caused by changes in allicin and moisture content. In contrast, these two compounds were the only compounds with negative values in PC1. Obviously, the samples can be grouped into three clusters, including cluster1 (fresh garlic, S0), cluster2 (S1), and cluster3 (S3−S9), respectively. This showed that the BG during fermentation was mainly divided into three stages by the changes of physicochemical properties, which included FG (unfermented), initial fermentation, and late fermentation. It is conducive to the formation of a better quality of BG by proper adjustment of production conditions.

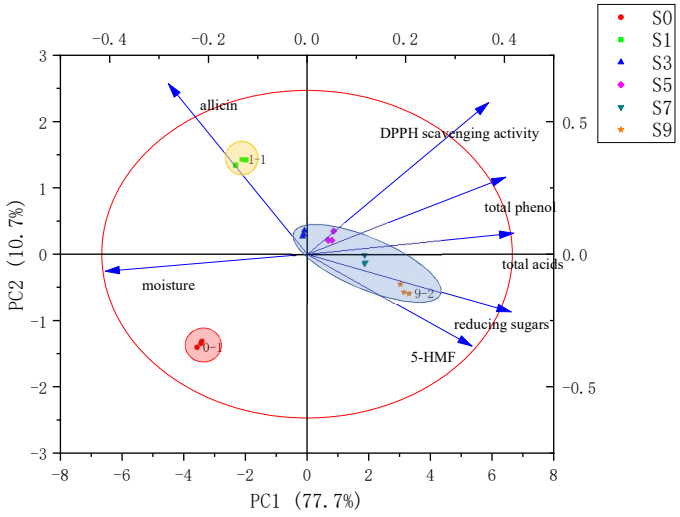


Figure 4. Principal component analysis based on the contents of main components in black garlic at different processing times. The biplot describes the evolution of garlic during fermentation and the contribution of compounds to the variability among samples.

The data obtained in the fermentation experiment were statistically analyzed by PCA to discriminate among fermentation times and establish the physicochemical factors with the largest contribution to separation.

4. Discussion

Black garlic is produced from fresh garlic in a long-time fermentation with a high-temperature and high-humidity environment. Compared with fresh garlic, black garlic not only has no harsh odor, but also has improved texture and taste, especially some special physicochemical properties, and it is well received by consumers [33].

The black garlic obtained from the treatment differs significantly from the fresh garlic in all dimensions, and all the differences are ultimately reflected in the increase in consumer acceptance. According to the results of sensory evaluation, the most significant changes in the sensory evaluation of each dimension during the fermentation process were taste and color. The production process of black garlic is macroscopically a dynamic combination of various biochemical reactions, and the final effect reflected in the product is the browning reaction, including enzymatic browning and the Maillard reaction. The Maillard reaction is the most important reaction in black garlic production. The Maillard reaction, also known as a non-enzymatic browning reaction, is a widespread non-enzymatic browning in food processing. The Maillard reaction is an important reaction in the formation of flavor and color in foods [34]. The end product of the Maillard reaction is melanoidin, a high-molecular-weight brown compound, which is the reason why black garlic turns black [15]. During the heat treatment of black garlic, the cells are broken under high temperature. Enzymes and substrates come into full contact, and various enzymatic reactions occur at an accelerated rate. Alliin is degraded to allicin, which is the origin of the pungent taste of garlic. Allicin continues to degrade under the action of enzymes and high temperature to various organic sulfides, and the pungency disappears [9]. Likewise, polysaccharides are degraded by enzymes and high temperatures to the corresponding monosaccharides and oligosaccharides, producing a sweet taste. In this way, the spiciness of the garlic disappear, and a sweetness emerge. Therefore, the taste of black garlic is better and more acceptable than that of fresh garlic.

During the production of black garlic, the physicochemical properties change considerably. The production process of black garlic is a high-temperature process in which the moisture of the BG continued to evaporate, which means that the moisture content continues to decrease. The change in moisture content can have both physical and chemical effects on black garlic. Physically, the decrease in moisture causes a change in texture. BG

becomes sticky and waxy rather than crunchy, as the moisture content decreases, providing the consumer with a completely different taste than fresh garlic. Chemically, the moisture content is one of the most important factors affecting the rate of the Maillard reaction. The strength of the Maillard reaction depends heavily on the hydration of the medium. Firstly, studies have shown that too low moisture content inhibits the Maillard reaction, and it is difficult for the Maillard reaction to occur in completely dry foods. Within a certain range of moisture content, the higher the moisture content, the faster the Maillard reaction. Secondly, too high moisture content also inhibits the Maillard reaction [35]. In our research, 5-HMF, a marker of Maillard reaction, started to be produced in large quantities on the fifth day. We speculated that the moisture content above 40% is not conducive to the Maillard reaction. As the moisture content decreased, the texture of the sample inevitably changed. Hardness is relevant to the strength of the sample, which is expressed as the maximum force of the first compression [36]. The maximum compressive force during crushing was used to explain the texture of samples from the point of hardness. The hardness of fresh garlic suddenly decreased at the beginning of production and gradually increased during the fermentation process. The results are in agreement with the study reported by Karnjanapratum [12], which found a significant increase in hardness during the fermentation process. Several studies have reported that steam pretreatment softens the garlic matrix and controls color production in BG at a higher rate during fermentation [12,37,38]. As the Maillard reaction proceeds, the alkaline amino groups in the BG decrease gradually [35]. Acids are also produced because of the degradation of free amino acids, peptides, and sugars [39]. This results in a significant increase in the total acid content of black garlic compared to that of fresh garlic, giving it a sour taste as well. Fructans are the most abundant polysaccharides in garlic [24]. During the heat treatment of garlic, both the total polysaccharide and fructan contents decrease, and the fructan content increases. Fructan hydrolases are easily inactivated at high temperatures [40], so it is speculated that heating is the main cause of polysaccharide degradation. It has been shown that fructose is the main reducing sugar in black garlic [24], and glucose is not detected in black garlic. Therefore, it is the Heyns rearrangement rather than the Amadori rearrangement that occurs in the Murad reaction in black garlic. During the fermentation of BG, the content of reducing sugars continuously increases. According to the changing trend of reducing sugar content, Li et al. proposed the concept of the reducing sugar balance point (RSBP) to characterize the degree of the Maillard reaction [24]. The generation rate of reducing sugar was greater than the consumption rate before RSBP. Oppositely, the generation rateis less than the consumption rate after RSBP. The key point of RSBP indicates that the reducing sugar required for the Maillard reaction is gradually reduced. The Maillard reaction may be restricted after RSBP, and the reaction rate decreases accordingly. The end product of the Maillard reaction is melanoidin, which causes the garlic to turn black [41]. Moreover, blackening is an important indicator of black garlic ripening. According to our observations, the garlic turned dark brown on the third day and was completely black by the fifth day, indicating that the product entered the ripening stage. The observation of the product was consistent with the colorimetric measurements. The blackening of BG was significantly and positively correlated with 5-HMF content [42]. BG started to darken, when 5-HMF started to accumulate in large amounts. In addition to the Maillard reaction, enzymatic browning is also a cause of black formation.

Allicin is an important characteristic flavor substance in fresh garlic, and it is the source of the pungent flavor of garlic. In fresh garlic, allicin is produced by the degradation of alliin in the presence of alliinase. Under the function of relevant enzymes and high temperature, allicin further generated a series of organosulfur compounds such as S-allyl-L-cysteine (SAC), S-propenyl-L-cysteine (SPC), γ-glutamyl-S-allyl-L-cysteine (GSAC), and γ-glutamyl-S-1-propenyl-L-cysteine (GSPC), among which SAC is considered as the main biologically active substance in BG [9]. This is the reason why the allicin content rose sharply at the beginning of black garlic production and then fell sharply. 5-HMF in black garlic is formed by fructose through the Maillard reaction, which is one of the important

intermediate products of the Maillard reaction and an important indicator to mark the stage of the Maillard reaction [43]. It is also an important indicator to characterize the maturation of BG and is closely related to the blackening of BG. Our results revealed that 5-HMF was not detected in the early stage of black garlic fermentation but accelerated accumulation from the fifth day, indicating that the Maillard reaction was in the primary stage in the first four days, entered the intermediate stage and started to accelerate from the fifth day. Polyphenols are an important class of bioactive substances in black garlic. It is reported that polyphenols have several biologically active functions such as improving cardiovascular and cerebrovascular function, kidney function, and eye health [44]. Some researchers have performed in vitro gastrointestinal digestion simulations on black garlic [45]. They found that polyphenols decreased at the beginning of the digestion process and were mainly affected in gastrointestinal digestion. The Folin−Ciocalteu method is currently the classical method for the detection of polyphenols, although its selectivity and specificity are questionable [46]. In the above experiments of in vitro digestion, researchers used UHPLC-HRMS to analyze phenolics in black garlic [45]. Caeic acid and gallic acid are the main compounds in the fresh garlic, while these compounds plus coumaric acid are the main ones found in the black garlic. In our study, the polyphenol content in black garlic at the end of fermentation was 2.45 times higher than that in fresh garlic. In some other studies, the polyphenol content was increased by 1.5 to 10 times [16,47]. We assumed that it is the difference in raw materials that causes the difference in polyphenol content. The phenolic content of black garlic is greatly influenced by the raw material variety, cultivation, and environment [48]. There are several explanations for why the polyphenol content increases. First, bound forms are broken under high temperature, leading to the increase of free forms. Second, enzymatic oxidation involving the antioxidant compounds may be inhibited in the raw plant material. Third, at the later phase of the browning reaction, the levels of the complex polyphenols increase [17]. It is generally believed that the antioxidant properties of BG come from polyphenols, in which sulfhydryl and electrophilic groups have the effects of scavenging oxygen and free radicals. Our research found that black garlic had three times the DPPH scavenging capacity of fresh garlic, approaching 100%. Relevant studies have shown that polysaccharides are also partly responsible for excellent antioxidant properties. Research by Cheng et al. [49] showed that the chemical modification of polysaccharides is an effective method to improve antioxidant capacity.

Overall, compared to fresh garlic, black garlic has a significant improvement in organoleptic, physicochemical properties, and bioactive functions, which leads to a higher acceptance of black garlic in certain groups of consumers. Although there is a general understanding of the content changes of various substances in the BG production process, the mutual transformation mechanism is still unclear and further research is needed. Currently, metabolomics and proteomics are widely used to analyze the changes of metabolites in BG. Further research on BG metabolites will help clarify the formation mechanism of BG, shorten the production cycle and improve the quality.

5. Conclusions

This work studied the changes in the physicochemical properties and the contents of various characteristic substances of homemade BG during the fermentation with statistical methods. The results showed that during the whole heating production process, the moisture content slightly reduced, and the total acid and the reducing sugar content continued to increase, endowing the product with a sweet and sour taste. Polyphenols and 5-HMF were used as biologically active substances in BG, whose contents presented a trend of continuous increase. The content of allicin increased drastically and then decreased sharply, so the pungent smell disappeared. In terms of color, because of the Maillard reaction, 5-HMF concentration gradually accumulated, and the samples started to turn black on the 3rd day and completely black on the 5th day. The beginning of maturity of BG was marked by the formation of black color, which was consistent with experimental results. The results of PCA showed that the samples at each fermentation stage were

quite different. The contributions of the two main components were 71.5% and 14.1%, respectively. The samples were obviously separated along time. The products on the 3rd to 9th days were clustered together, indicating that the product began to mature after three days of fermentation.

Although there is general understanding of the content changes of various substances in the BG production process, the mutual transformation mechanism is still unclear and further research is needed. Currently, metabolomics and proteomics are widely used to analyze the changes of metabolites in BG. Further research on BG metabolites will help clarify the formation mechanism of BG, shorten the production cycle and improve the quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8110653/s1, Figure S1: Changes of the colors of the samples (S0–S9) during the fermentation.

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