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Mitigation of Acrylamide and 5-Hydroxymethylfurfural in Black Garlic by Vacuum Aging

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ABSTRACT: Acrylamide and 5-hydroxymethylfurfural (5-HMF) are chemical process contaminants generated in black garlic through the Maillard reaction. Vacuum aging (16 kPa, 70–75 °C for 12 days) was introduced as a new aging technology to mitigate acrylamide and 5-HMF in black garlic. Results showed that vacuum aging suppressed the formation of acrylamide (from 2.86 μg/g to below the detection limit, *i.e.*, 100% reduction) and 5-HMF (from 173.26 μg/g to 7.90 μg/g, *i.e.*, 95.4% reduction) in black garlic due to the reduced thermal exposure. Vacuum aging inhibited the Maillard reaction, and resulted in the higher lightness (L = 22.14) and the lower browning degree (ΔE = 62.76) of black garlic than those (L = 15.16, ΔE = 69.47) prepared by conventional aging. The reduced thermal exposure also restrained the hydrolytic release of fructose, glucose, total polyphenols, and total flavonoids in vacuum-aged black garlic. It reduced them by 95.0%, 100%, 89.9%, and 91.8%, respectively, which in turn diminished its antioxidant capacity by 58.4% and 79.1% based on the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. These limitations of vacuum aging, including decreased antioxidant capacity together with sensorial quality reduction, require further process refinement in future work before this technology can be implemented for industrial-scale production of black garlic.

Keywords: Black garlic; Vacuum aging; Acrylamide; 5-HMF; Mitigation; Maillard reaction

1. Introduction

Black garlic is produced by thermal processing of the raw garlic at high temperature (60–90 °C) and moderately high relative humidity (RH, 70–90%) for one month or more [1,2]. Black garlic exhibits a sweet-sour flavor and loses the pungent smell of raw garlic [3], and enhances the nutritional components and bioactive substances, including reducing sugars, polysaccharides, organic acids, amino acids,

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polyphenols, flavonoids, melanoidins, and organosulphur compounds during the aging process [4–7]. Numerous studies have demonstrated that black garlic has numerous health benefits, including anti-obesity, antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, anti-allergic, and antimicrobial activities [8]. Therefore, black garlic has become increasingly popular worldwide in recent years due to its nutritional, biological, and therapeutic properties.

It is well-known that the transformation from raw garlic to black garlic is driven mainly by the Maillard reaction during the aging process [3,9,10]. Maillard reaction not only drives the browning of raw garlic and increases the levels of nutritional and bioactive components, but also generates certain potentially harmful contaminants such as acrylamide and 5-hydroxymethylfurfural (5-HMF) [11,12]. Acrylamide and 5-HMF are intermediate products of the Maillard reaction and are often present in roasted, baked, or fried foods rich in starch and protein [13–16]. Many studies indicate that acrylamide possesses neurotoxicity, carcinogenicity, reproductive and developmental toxicity, so it has been classified as Group 2A probable carcinogenic substance by the International Agency for Research on Cancer [17,18]. Similarly, 5-HMF displays carcinogenicity, neoplastic transformation, and hepatotoxicity and nephrotoxicity [19]. Therefore, inhibiting the formation or minimizing the residual amounts of acrylamide and 5-HMF in foods has become an important food safety topic in the food industry [20].

Acrylamide and 5-HMF were not detected in raw garlic according to the reported literature [2,11,21]. Our previous study reported the levels of acrylamide at 34.01 μg/g and 5-HMF at 1368.23 μg/g in aged black garlic [11]. Across different ageing strategies, the levels of these two compounds varied widely, with higher 5-HMF concentrations observed under alternative processing conditions [2,11]. The 5-HMF level of 100 μg/g was detected in black garlic aged at 65 °C and 70 % RH for 24 days, while a value of 2820 μg/g was obtained for black garlic aged at 84 °C and 85 % RH for 50 days [22]. The 5-HMF contents of 6300 μg/g, 5800 μg/g, 5000 μg/g, and 3800 μg/g were observed in black garlic subjected to freezing pretreatment, high-pressure pretreatment, ultrasonic pretreatment, and the control group, respectively [23]. 5-HMF levels ranging from 11 μg/g to 1143 μg/g were also determined in black garlic prepared by different processing methods [2]. A 5-HMF content of 260.7 μg/g was reported for black garlic produced from freezing-pretreated garlic [21]. Therefore, acrylamide and 5-HMF coexist in black garlic, and their contents are affected by many factors, including aging temperature and time [16,21,22], relative humidity [22,24], pretreatment method of raw garlic [23], aging technique [2], water content and water activity of raw [16], total acid and pH of raw [7], nutritional components (especially reducing sugars and amino acids) in raw [5], adding antioxidants and acidulants [25], and so on.

Due to their potential toxicity, reducing acrylamide and 5-HMF levels in foods and further decreasing health risks to consumers have become important trends. Many studies have explored strategies to reduce acrylamide and 5-HMF in foods [18–20,26]. The processing strategies for mitigating or removing acrylamide and 5-HMF in foods include optimizing processing parameters (especially processing temperature and time, relative humidity), pretreatment techniques (such as soaking or blanching), ultraviolet irradiation, infrared radiation, vacuum treatment, microwave heating, pulsed electric fields, ultrasound, high hydrostatic pressure, air-jet impingement, supercritical fluids technology, radio-frequency, extrusion, steaming, novel combinations such as pulsed electric fields and blanching, enzymatic interventions with asparaginase and glucose oxidase, yeast or lactic acid bacteria treatment and fermentation, as well as using additives/coatings (such as edible coatings, acidulants, vitamins) and plant extracts (such as polyphenols), and so on [19,27,28].

Commercially available single-bulb black garlic must retain an intact appearance and physical structure (see Figure 1). Of the strategies mentioned above for controlling acrylamide and 5-HMF, vacuum aging may be the best one to control the acrylamide and 5-HMF contents while maintaining its whole appearance and shape. To date, only a few studies have reported reductions in acrylamide and 5-HMF in biscuits, potato chips, and coffee by vacuum treatment [29–32]. Vacuum baking has been successfully applied to reduce

the acrylamide content in potato chips by 72–98% [29]. A 30% reduction in acrylamide was achieved in cookies using combined conventional-vacuum baking [32]. Negligible amounts of acrylamide were detected in biscuits under vacuum baking at 160 °C, with contents of 11–51 ng/g, which were markedly lower than the values of 39–211 ng/g obtained by conventional baking across all tested temperatures (180–200 °C) [31]. Notably, no 5-HMF was formed in biscuits during vacuum baking over the temperature range of 160 °C to 200 °C [31]. In the same year, a 50% reduction in acrylamide levels was observed in vacuum-processed coffee beans relative to conventionally roasted counterparts [30]. Vacuum treatment led to 43% and 18% acrylamide removal for biscuits and potato chips, respectively [33]. So vacuum processing is an effective technique for mitigating acrylamide and 5-HMF in thin-baked foods, such as biscuits, potato chips, and coffee. However, in this study, single-bulb black garlic requires the preservation of whole-bulb morphological integrity; whether vacuum aging can effectively suppress the formation of acrylamide and 5-HMF has not been experimentally explored to date. Meanwhile, it remains unknown how vacuum aging affects core quality attributes of black garlic, including the degree of browning, accumulation of bioactive components, antioxidant capacity, and sensory acceptability. Therefore, the present study aimed to evaluate the potential of vacuum aging to eliminate acrylamide and 5-HMF in black garlic, and to characterise how this treatment influences the product's physicochemical quality, sensory attributes, and antioxidant capacity.



Figure 1. Appearance of black garlics (**A**) black garlic prepared by conventional aging, (**B**) black garlic prepared by vacuum aging.

2. Materials and Methods

2.1. Materials and Chemicals

Single-bulb raw garlic with a diameter ranging from 30 mm to 35 mm was purchased from the local market in Dali city, Yunnan province, China. Acrylamide, 5-HMF, fructose, glucose, asparagine, rutin, and gallic acid were all standards (purity > 98%) and obtained from Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China. The standards alliin and allicin were provided by Nanjing Yuanzhi Biotech Co., Ltd., Nanjing, China, and Beijing Solaibao Technology Co., Ltd., Beijing, China, respectively. Chromatographic grade methanol and acetonitrile were acquired from Shanghai Sangon Biotech Co., Ltd., Shanghai, China. All other chemicals were analytical grade, with purities greater than 98%.

2.2. Preparation of Black Garlic by Conventional and Vacuum Aging

Black garlic was prepared using two different processes, namely conventional and vacuum aging. For each aging treatment (conventional and vacuum aging), three independent biological batches were prepared. Conventional aging was performed at atmospheric pressure using a 7.5 L of aging oven (Anzhikang, AZK186-80, Zhongshan, China), and the aging procedure was followed, with the temperature set at 75 °C for the first 3 days, and then at 70 °C for the next 9 days with relative humidity ranging from 80% to 90%, keeping a total aging time of 12 days. Vacuum aging was carried out at 16 kPa using a vacuum oven (Yiheng, DZF-6050, Shanghai, China), connected to a rotary vacuum pump (2XZ-2, Linhai, China), and the aging procedure was the same as that of conventional aging. The vacuum oven has no active humidity-

control unit and only provides a real-time relative-humidity display. Under sealed vacuum conditions, moisture evaporated from raw garlic and raised the chamber relative humidity, which stabilised at approximately 85% during the whole aging period. After aging, samples were immediately removed from the oven, cooled to room temperature, and then stored at 4 °C until further analysis. The moisture contents of conventional-aged and vacuum-aged black garlics were 64.8 ± 1.2% and 30.0 ± 1.7%, respectively.

2.3. Determination of Acrylamide and 5-HMF

Acrylamide and 5-HMF were determined as previously reported methods with minor modifications [12]. In brief, ground black garlic sample (2.0 g) was mixed with 10.0 mL of 10% (*v*/*v*) of methanol solution and vortexed vigorously for 2 min, followed by ultrasonic extraction for 10 min. The mixed solution was centrifuged at 10,000 rpm for 10 min by a refrigeration centrifuge (at 4 °C). The supernatant was clarified by using the Carrez I (15% potassium ferrocyanide solution, *w*/*v*) and Carrez II (30% zinc sulfate solution, *w*/*v*) solutions to precipitate co-extracted colloids. The clarified solution was centrifuged at 10,000 rpm for 10 min (at 4 °C) again, and the supernatant (1.0 mL) was evaporated to dryness under reduced pressure at 35 °C. The residue was redissolved in 1.0 mL of 10% (*v*/*v*) methanol solution and filtered through a 0.22 μm aqueous membrane for quantitation of acrylamide and 5-HMF. An Agilent LC system (Agilent 1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) coupled to a DAD detector was used to quantify the levels of acrylamide and 5-HMF in black garlic. The separations of acrylamide and 5-HMF were performed on the Kromasil 100-5 C18 column (150 mm × 4.6 mm ID, 5 μm) using methanol and water (10:90, *v*/*v*) as the mobile phase at a flow rate of 0.8 mL/min. The column temperature was controlled at 30 °C, and the wavelengths were set at 210 nm for acrylamide and at 284 nm for 5-HMF. The peaks were identified according to the retention times of the standards acrylamide and 5-HMF, and their contents were calculated using the calibration curves built by work solutions with different concentrations. The limits of detection (LOD) for acrylamide and 5-HMF were 0.11 μg/g and 0.04 μg/g, respectively.

2.4. Measurement of Physicochemical Properties

2.4.1. Color

Color was measured using the method described previously [12] with a chromameter (CR-400, Konica Minolta, Osaka, Japan). The *L* (lightness), *a* (redness), and *b* (yellowness) were measured at different points of black garlic. The color difference (Δ*E*) was used to evaluate the browning degree of black garlic, which was calculated according to the equation: $\Delta E = \sqrt{(L - 84.6)^2 + (a - 0.3155)^2 + (b - 0.3213)^2}$, where *L*, *a*, and *b* represent the color components of black garlic, and 84.6, 0.3155, and 0.3213 were the corresponding values of the white plate for calibration.

2.4.2. Fructose and Glucose

Fructose and glucose were extracted from black garlic (2.0 g) using 30 mL ultrapure water with 2 min sonication; the extract was subsequently brought to a final volume of 100 mL with ultrapure water. The mixed solution was centrifuged at 5000 rpm for 10 min; the supernatant (50 μL) was derived with 100 μL of derivative reagent (0.7 g *p*-aminobenzoic acid, 1.0 g glacial acetic acid, and 0.1 g sodium cyanoborohydride were dissolved with methanol and diluted to 10 mL) at 70 °C for 60 min. The derivant was diluted to 2 mL with ultrapure water and filtered through a 0.22 μm aqueous membrane for quantitation by HPLC-FLD (Agilent 1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA). A previously reported method was used for the analysis of fructose and glucose [12].

2.4.3. Alliin and Allicin

Alliin in black garlic (1.0 g) was extracted with 30 mL of phosphate solution (0.01%, *v*/*v*) for 15 min under ultrasonic assistance. The resulting homogenate was brought to a final volume of 50 mL with the phosphate solution (0.01%, *v*/*v*), and then centrifuged at 6000 rpm for 25 min using a refrigerated centrifuge (at 4 °C). The supernatant was passed through a 0.22 μm aqueous membrane before HPLC analysis. Analysis was carried out using the HPLC-DAD system (Agilent 1260 Infinity II, USA), and the procedure followed the one described previously [11].

Allicin content was quantified based on a modified HPLC–DAD protocol [34]. In brief, black garlic (2.0 g) was homogenized with 10 mL of methanol, and the homogenate was centrifuged at 9000 rpm for 10 min using a refrigeration centrifuge (at 4 °C). Supernatant (1.0 mL) was diluted with an 85% methanol solution (2.5 mL) and filtered through a 0.22 μm membrane prior to chromatographic analysis. The Agilent HPLC-DAD system was used to determine allicin. Allicin was separated on an ODS-3 C18 column (250 mm × 4.6 mm ID, 5 μm) at 35 °C using the mobile phase consisting of methanol and water (85:15, *v*/*v*) at a flow rate of 0.8 mL/min. Detection was carried out at 214 nm with an injection volume of 10 μL.

2.4.4. Asparagine

Asparagine content was measured based on a previously reported procedure [12]. In brief, black garlic (2.0 g) was hydrolyzed by HCl solution (6 mol/L) at 95 °C and in a vacuum state for 60 min, and the hydrolysate was diluted with ultrapure water, then filtered to obtain the filtrate. The filtrate was derived with phenyl isothiocyanate and passed through a 0.22 μm organic membrane for quantitation by HPLC-DAD. The Kromasil 100-5 C18 column (150 mm × 4.6 mm ID, 5 μm) was applied to separate asparagine using the mobile phase comprised of (A) sodium acetate/triethylamine solution (1.64 g/L, pH 6.20) and (B) acetonitrile solution (80%, *v*/*v*) with a gradient elution program (92% A for 2 min, decreased to 81% A within 10 min, and then decreased to 65% A within 9 min) at a flow rate of 1.0 mL/min. The column temperature was controlled at 40 °C, the detector wavelength was set to 254 nm, and the injection volume was 10 μL.

2.4.5. Total Polyphenols (TP) and Total Flavonoids (TF)

TP content was determined by the Folin-Ciocalteu method, and TF content was measured by the aluminum nitrate method, following previously reported procedures [11].

2.5. Determination of Antioxidant Capacity

DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,2′-azino-bis-(3-ethylbenzo thiazoline-6-sulfonate)] assays were used to evaluate the antioxidant capacity of black garlic, according to the previously reported procedures with minor modifications [35]. In brief, black garlic (1.5 g) was ground with 50 mL of ethanol solution (70%, *v*/*v*) to obtain the homogenate; then it was extracted by ultrasound for 60 min at room temperature. The extract was centrifuged at 8000 rpm for 5 min using a refrigeration centrifuge (at 4 °C), and the supernatant was collected and stored at 4 °C until analysis.

2.5.1. DPPH Assay

The extracted supernatant (about 20 mg/mL) was diluted by 70% ethanol solution (*v*/*v*) to obtain the work solutions with different concentrations (100, 200, 400, 600, 1000, and 2000 μg/mL). Each of the work solutions (1.5 mL) was mixed with DPPH solution (1.5 mL, 0.1 mmol/L) and kept in the dark for 30 min at room temperature. The blank control consisted of the work solution at different concentrations (1.5 mL) and anhydrous ethanol (1.5 mL). The negative control was comprised of DPPH solution (1.5 mL) and anhydrous ethanol (1.5 mL). After incubation in the dark, the absorbance values were measured at 517 nm

using a spectrophotometer (UV-VIS T6 NewCentury, PUXI, Beijing, China). The DPPH radical scavenging rate (K, %) was calculated using the following Equation (1):

$$K\ (\%)\ =\ (1\ -\ \frac{A_1 - A_2}{A_3}) \times 100\% \quad (1)$$

where A_1, A_2, and A_3 are the absorbance values of the sample, blank control, and negative control, respectively. The half-maximal inhibitory concentration (IC_{50}) of the sample was computed using the SPSS 25.0 software based on the DPPH radical scavenging rates (K, %) versus the concentrations.

2.5.2. ABTS Assay

The extracted supernatant (about 20 mg/mL) was diluted by 70% ethanol solution (*v*/*v*) to the work solutions with different concentrations (50, 100, 150, 200, 250, and 300 μg/mL). The diluted supernatant of each sample (1.5 mL) was mixed with the $ABTS^{\bullet+}$ solution (1.5 mL, 7 mmol/L). Blank control was composed of the work solution with different concentrations (1.5 mL) and anhydrous ethanol (1.5 mL). The negative control was comprised of $ABTS^{\bullet+}$ solution (1.5 mL) and anhydrous ethanol (1.5 mL). After incubation in the dark for 30 min at room temperature, the absorbance was measured at 734 nm. The ABTS radical scavenging rate (K, %) was calculated using Equation (1), and the IC_{50} of the sample was computed using the above-mentioned method.

2.6. Sensory Evaluation

A panel of 10 assessors, trained in sensory analysis, was convened to conduct the sensory evaluation. It was performed using a paired-comparison test with a 9-point scale to determine differences in appearance, taste, odor, and acceptability of black garlic, where 9 represents “like extremely”, and 1 represents “dislike extremely”. The sensory evaluation standards of black garlic are presented in Table S1 [23].

2.7. Statistical Analysis

All measurements were performed in triplicate, except for sensory evaluation (n = 10), and the results were presented as means ± standard deviations (SD). One-way ANOVA and Duncan’s tests (SPSS 25.0, USA) were used to assess the significant differences at the level of $p < 0.05$.

3. Results and Discussion

3.1. Changes of Acrylamide and 5-HMF Contents in Black Garlic

Acrylamide can be spontaneously produced in rich-starch and protein foods when subjected to temperatures higher than 120 °C based on the Maillard reaction between the asparagine and the reducing sugars [15]. 5-HMF is also a Maillard reaction product, formed by the degradation of 3-deoxyglucosone (3-DG) and the acid-catalyzed decomposition of hexoses [5,36]. Their formations are closely correlated with sugars, amino acids, free acid content, pH, total acidity, water content, water activity of the raw material, processing temperature and time, and relative humidity [5,37].

The changes in acrylamide and 5-HMF contents were monitored in black garlics prepared by conventional and vacuum aging (Figure 2). The acrylamide and 5-HMF contents in the black garlic processed by conventional aging were 2.86 ± 0.97 μg/g and 173.26 ± 12.29 μg/g, respectively. These values fall within the broad literature ranges summarized in the Introduction, yet are lower than several previously-reported maximum values. Such inter-study discrepancies can be ascribed to multiple sources of variability: (1) biological variation of raw-garlic materials, including garlic cultivar, growing region, harvest season, and initial contents of fructans, free amino acids, especially asparagine, and moisture; (2) differences in

aging parameters such as aging temperature and time, and processing relative humidity applied in different aging techniques.

Our team had verified that acrylamide and 5-HMF were not detected in raw garlic [12]. Their formation in black garlic contributed to many factors, including the high aging temperature (70–75 °C) and long aging time (12 days), as well as physicochemical changes during aging, such as the decreases in water activity and pH [11,38–40], and the increases in fructose content (Table 1) [39,41]. However, acrylamide was not detected, and 5-HMF was significantly decreased to 7.90 ± 1.13 μg/g in the black garlic prepared by vacuum aging, and reduced by 100% and 95.4% ($p < 0.01$), respectively. In conventional aging, three modes of heat transfer, namely conduction, convection, and radiation, took place to heat raw garlic [31]. During vacuum aging, partial removal of air from the oven chamber suppresses convective heat transfer, while conductive and radiative heating still occur. This gives rise to reduced thermal exposure of garlic tissue, which in turn restricts acrylamide and 5-HMF formation [29]. It was also reported that the convective heat transfer coefficient depends on the pressure of the environment, and it gradually decreases under vacuum [42]. In addition, vacuum baking (1 kPa, 120–160 °C for 6–12 min) achieved a 72–98% reduction in acrylamide formation in potato chips [29]. A 98% reduction in acrylamide content was also observed for vacuum-fried potato chips (10 kPa, 125 °C for 180–360 s) [43]. These results are consistent with the ones in this study. Several studies have reported that high relative humidity in the processing environment can inhibit the Maillard reaction and reduce the formation of acrylamide and 5-HMF in thermally treated foods [16,24]. In this study, the low-pressure vacuum lowered the boiling point of water in raw garlic, thus accelerating the evaporation of water from raw garlic to the vacuum oven during aging, which might have caused the increase of relative humidity in the vacuum oven, and then reduced the formation of acrylamide and 5-HMF by inhibiting the Maillard reaction. In addition, acrylamide was not detected in black garlic prepared by vacuum aging. A non-detectable acrylamide level does not mean that no acrylamide was formed under vacuum aging. Instead, trace quantities of acrylamide may be generated and subsequently consumed through reactions with reactive constituents, such as melanoidins, present in black garlic [29,30]. Overall, vacuum aging as a new aging technology is highly effective in mitigating acrylamide and 5-HMF in black garlic.

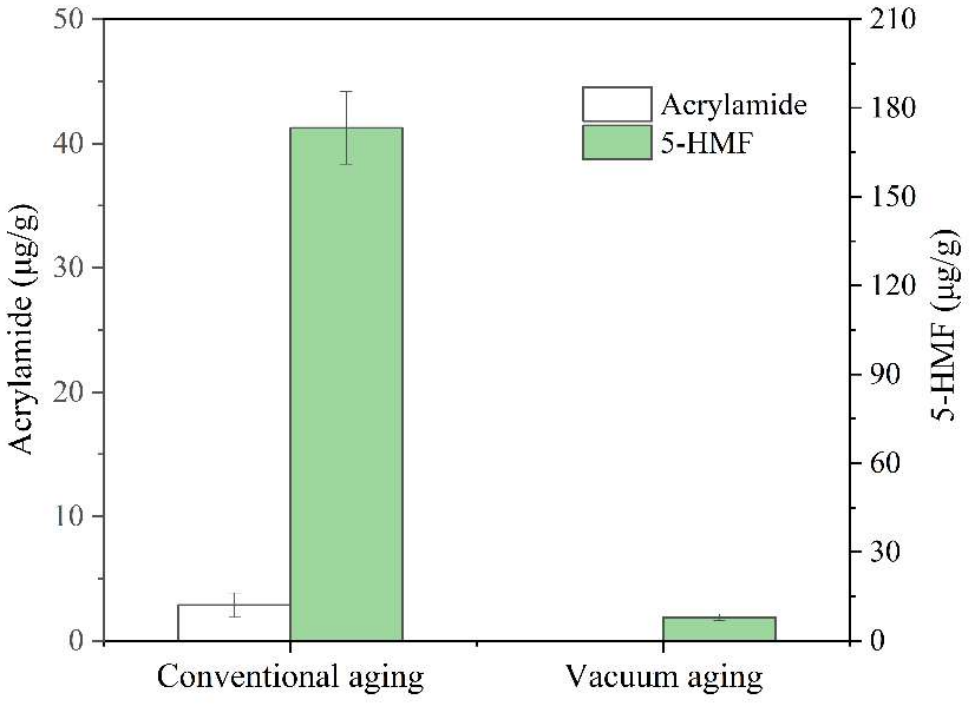


Figure 2. Acrylamide and 5-HMF contents in black garlics prepared by conventional and vacuum aging.

Table 1. Comparison of physicochemical properties of black garlics prepared by conventional and vacuum aging.

Physicochemical Properties of Black Garlic	Conventional Aging	Vacuum Aging
Lightness (*L*)	15.16 ± 1.41 [a]	22.14 ± 0.72 [b]
Redness (*a*)	1.19 ± 0.16 [a]	3.30 ± 1.03 [b]
Yellowness (*b*)	2.42 ± 0.03 [a]	5.53 ± 0.91 [b]
Browning degree (Δ*E*)	69.47 ± 1.41 [a]	62.76 ± 0.71 [b]
Fructose (mg/g)	333.67 ± 28.24 [a]	16.70 ± 1.81 [b]
Glucose (mg/g)	8.39 ± 0.76	Non-detected
Alliin (μg/g)	423.72 ± 1.92 [a]	9.23 ± 0.55 [b]
Allicin (μg/g)	Non-detected	Non-detected
Asparagine (μg/g)	386.58 ± 47.17 [a]	180.88 ± 6.23 [b]
Total polyphenol content (TP, mg GAE/g)	12.93 ± 0.55 [a]	1.30 ± 0.07 [b]
Total flavonoid content (TF, mg RE/g)	3.05 ± 0.14 [a]	0.25 ± 0.01 [b]

Note: The results are expressed as the mean ± standard deviation ($n = 3$); Different letters in the same column represent significant differences at the level of $p < 0.05$.

3.2. Changes in Physicochemical Properties of Black Garlic

The changes in physicochemical properties (color, reducing sugars, alliin, allicin, asparagine, TP, and TF contents) of black garlic were monitored (Table 1). Color is one of the most important occurrences during the aging of black garlic. Conventional aging of black garlic can be divided into two stages, including the dehydrating stage and the coloring stage [31]. In the dehydrating stage, the temperature of raw garlic begins to increase, causing a reduction in water content, hydrolysis of oligo- and polysaccharides, tissue softening, and sugar gelatinization. When the temperature and water activity become favorable for non-enzymatic browning reactions in the second stage, they are responsible for the browning of black garlic. The changes in the color parameters (*L*, *a,* and *b*) of black garlic are presented in Table 1. There were obvious differences in lightness (*L*), chroma (*a* and *b*), and browning degree (Δ*E*) of black garlics prepared by conventional and vacuum aging. The higher lightness ($L = 22.14 \pm 0.72$) and lower browning degree ($\Delta E = 62.76 \pm 0.71$) of black garlic prepared by vacuum aging than those ($L = 15.16 \pm 1.41$, $\Delta E = 69.47 \pm 1.41$) of one prepared by conventional aging ($p < 0.05$), showed that vacuum aging inhibited the non-enzymatic browning reactions, especially the Maillard reaction [30]. This observation can also be attributed to vacuum-diminished convective heat-transfer coefficients, resulting in lower thermal exposure relative to conventional aging [29]. In addition, both the redness (*a*) and yellowness (*b*) values of black garlic prepared by vacuum aging were significantly greater than those prepared by conventional aging ($p < 0.05$), which also showed a relatively low browning. So the color change can also indicate that vacuum aging is effective in controlling acrylamide and 5-HMF formation in black garlic by inhibiting the Maillard reaction. Additionally, a close correlation was observed between the degree of browning and acrylamide accumulation (Figure 2) and asparagine content (Table 1) in black garlic. This finding also accounts for the distinct differences between black garlic prepared by conventional and vacuum aging [12].

Fructose and glucose are the important monosaccharides in black garlic, influencing its color, flavor, taste, and even the formation of acrylamide and 5-HMF [5,12]. These monosaccharides in black garlic mainly come from the decomposition of fructans in raw garlic during the thermal process [44]. Seen from the Table 1, the fructose content is significantly higher than the glucose content in black garlic ($p < 0.01$), and it is mainly attributed to the degradation of fructans in raw garlic during the thermal processing, which is comprised of a 14:1 ratio of fructose and glucose [9]. Meanwhile, the fructose (333.67 ± 28.24 mg/g) and glucose (8.39 ± 0.76 mg/g) contents in black garlic prepared by conventional aging are much higher than those (16.70 ± 1.81 mg/g and non-detected) in the one prepared by vacuum aging ($p < 0.01$), which were reduced by 95.0% and 100%, respectively. The result suggests that vacuum aging reduced the degradation of fructans in raw garlic due to the low thermal load on black garlic. It has also been reported that HMF is

more selectively produced from keto-hexoses such as fructose, so the low fructose content in black garlic prepared by vacuum aging is the second reason for the less production of 5-HMF [45].

It is well-known that the most prominent route of acrylamide formation is the Maillard reaction between asparagine and reducing sugars at temperatures typically above 120 °C [27,46]. However, it had been shown that substantial amounts of acrylamide were generated at temperatures below 100 °C with prolonged processing time (24 h) under conditions simulating plum drying (in the presence of asparagine and reducing sugars) [47]. In this study, the asparagine content (386.58 ± 47.17 μg/g) in black garlic prepared by conventional aging was about 2.14 times higher than that (180.88 ± 6.23 μg/g) in the one prepared by vacuum aging ($p < 0.01$), and the more reducing sugars (fructose and glucose) present in conventionally aged black garlic, which are more conducive to the production of acrylamide. It is the second reason that acrylamide content in black garlic prepared by conventional aging is higher than that in the one prepared by vacuum aging. Meanwhile, 5-HMF has been regarded as a key reactant for acrylamide formation, yielding acrylamide at higher yields than hexoses [48]. It may be the third reason that black garlic prepared by conventional aging has higher acrylamide levels than that prepared by vacuum aging. In addition, significant positive correlations have been observed between 5-HMF formation and fructose content, as well as between acrylamide formation and asparagine content, in black garlic. These findings further account for the marked differences in acrylamide and 5-HMF contents in black garlic prepared by conventional and vacuum aging [12].

As key organosulfur compounds, alliin and allicin are predominantly found in raw garlic. Physical damage to garlic tissue enables vacuolar alliinase to hydrolyze alliin into allicin, which generates the characteristic pungent flavor and odour of raw garlic [5]. Alliin exhibits relatively high thermal stability; drying at 60 °C exerts little influence on alliin content, and alliin can still be recovered from heat-processed cooked garlic [49,50]. In contrast, allicin is thermally labile and readily decomposes into other stable organosulfur derivatives at temperatures above 35 °C [49]. As shown in Table 1, vacuum-aged black garlic contained only 9.23 ± 0.55 μg/g alliin, which was substantially lower than the value of 423.72 ± 1.92 μg/g measured in the conventional-aged sample. This observation may be attributed to low-pressure-triggered cell rupture in raw garlic, which activates vacuolar alliinase and accelerates the rapid hydrolysis of alliin to allicin. Nevertheless, allicin was undetectable in black garlic from both aging treatments. This finding indicates that allicin underwent extensive thermal decomposition into other organosulfur products throughout the aging process, consistent with its poor thermal stability [49]. In addition, Yang, Song, Wang & Jing reported that the sulfur-containing compounds generated from allicin decomposition—primarily diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS)—are the key flavor constituents of black garlic and the primary contributors to its characteristic cooked garlic aroma. These compounds are inherently volatile, so we deduced that the reduced pressure environment might significantly accelerate their evaporation or dissipation, leading to substantial loss of flavor-active substances during vacuum aging. This is consistent with the lower odor score of vacuum-aged black garlic in the subsequent sensory evaluation [51].

Polyphenols and flavonoids are important antioxidant substances in black garlic, and they are much higher than those in raw garlic, which is one of the main reasons that black garlic possesses a higher antioxidant capacity than raw garlic [52]. The final TP and TF contents in black garlic are governed by a dynamic equilibrium between accumulation and degradation during thermal processing. Maillard reaction promotes the formation of polyphenols and flavonoids [53] by increasing their release from glycoside- and ester-bound fractions of raw garlic [54]. In addition, high aging temperature (70–75 °C) also inactivates the activity of polyphenol-oxidases in raw garlic [55]. So these conditions promoted the accumulation of polyphenols and flavonoids in black garlic. As shown in Table 1, the TP (12.93 ± 0.55 mg GAE/g) and TF (3.05 ± 0.14 mg RE/g) contents in black garlic prepared by conventional aging are 9.95 and 12.20 times higher than those (1.30 mg GAE/g and 0.25 mg RE/g) in the one prepared by vacuum aging ($p < 0.01$),

respectively, which can be explained by the fact that vacuum aging inhibited the Maillard reaction and reduced their release from glycoside- and ester-bound fractions of raw garlic due to the low thermal load to black garlic compared to conventional aging. Their reduction in vacuum-aged black garlic inevitably led to a marked decline in antioxidant capacity. This result is consistent with the subsequent findings from the DPPH and ABTS assays.

3.3. Antioxidant Capacity

Figure 3 shows the IC_{50} values of DPPH and ABTS radical scavenging of the black garlics prepared by conventional and vacuum aging. The IC_{50} value is the concentration of black garlic extract that results in 50% loss of the DPPH or ABTS activity, which is obtained based on the radical scavenging rates (*K*, %) versus the concentrations. A smaller IC50 value indicates stronger antioxidant activity. Compared with vacuum-aged sample, extract from conventionally aged black garlic exhibited significantly stronger radical-scavenging activity: 1.89–6.17 times higher for DPPH and 3.78–5.19 folds higher for ABTS ($p < 0.01$), respectively. The IC_{50} values for DPPH and ABTS radical scavenging of black garlic extract prepared by vacuum aging were 343.50 ± 3.62 μg/mL and 573.10 ± 47.28 μg/mL, whereas the corresponding IC_{50} values achieved from conventional aging were 142.80 ± 15.30 μg/mL and 119.67 ± 6.05 μg/mL ($p < 0.01$), respectively. The antioxidant capacities were reduced by 58.4% and 79.1% based on the DPPH and ABTS assays, indicating obviously low antioxidant capacities for all black garlics prepared by vacuum aging. These results were consistent with the lower TP and TF contents in black garlic prepared by vacuum aging.

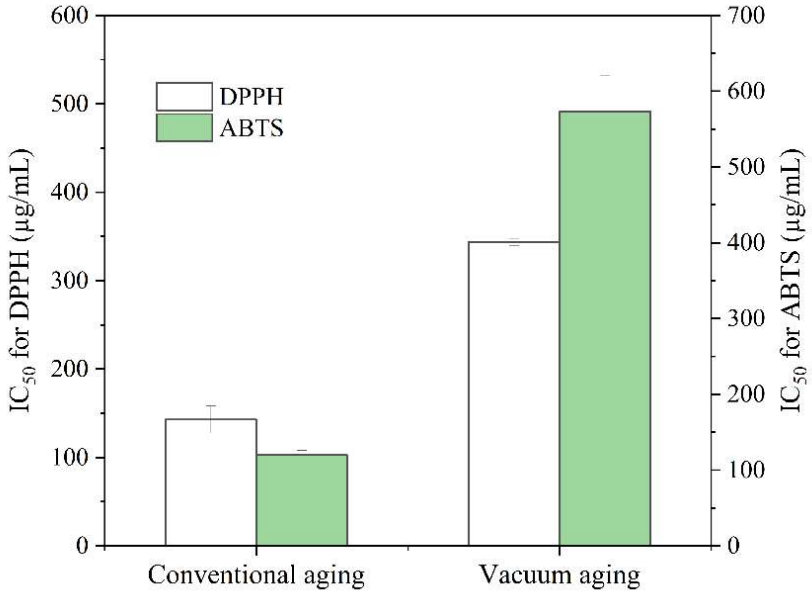


Figure 3. Antioxidant capacity (IC_{50}) of black garlic extracts prepared by conventional and vacuum aging.

3.4. Sensory Evaluation

Table 2 summarizes the results of sensory evaluation for black garlics prepared by conventional and vacuum aging. The sensory evaluation of black garlic was performed based on the standards presented in Table S1. There were significant differences between taste, odor, acceptability, and total score of black garlics prepared by conventional and vacuum aging ($p < 0.05$). While no significant difference was observed between their appearances ($p > 0.05$). Overall, vacuum-aged black garlic obtained markedly poorer sensory quality compared with conventionally aged one, characterized by a hard and dry texture, weaker sweetness, and lack of soft, sticky mouthfeel and caramel-like aroma of conventional black garlic,

resulting in considerably lower overall acceptability. Specifically, the fructose content in the vacuum-aged group (16.70 mg/g) was only 5% of that of the conventional group (333.67 mg/g), indicating insufficient polysaccharide degradation, which accounted for the deficiency in sweetness and the hardened texture. In addition, sulfur-containing compounds derived from allicin decomposition rapidly volatilized under low vacuum conditions, which explains the weak aroma and the absence of caramel-like flavor [51].

Table 2. Sensory evaluation of black garlics prepared by conventional and vacuum aging.

Aging Method	Appearance	Taste	Odor	Acceptability	Total Score
Conventional aging	7.9 ± 0.7 [a]	8.0 ± 0.8 [a]	7.2 ± 1.0 [a]	5.8 ± 0.9 [a]	28.9 ± 2.1 [a]
Vacuum aging	7.6 ± 0.8 [a]	2.1 ± 0.7 [b]	7.1 ± 1.0 [b]	2.3 ± 0.8 [b]	19.1 ± 1.8 [b]

Note: The results are expressed as the mean ± standard deviation (n = 10); Different letters in the same column represent significant differences at the level of $p < 0.05$.

Our findings deliver two important practical implications for black garlic industrial manufacture. First, vacuum aging (16 kPa, 70–75 °C for 12 days) effectively inhibits the Maillard reaction by reducing the thermal load during processing, thereby effectively suppressing the formation of acrylamide (100% reduction) and 5-HMF (95.4% reduction) in black garlic, which provides a potential route for producing black garlic with lower levels of acrylamide and 5-HMF. However, this benefit comes with obvious quality trade-offs: vacuum-aged black garlic exhibits a lighter color and insufficient browning, along with marked reductions in reducing sugars, total polyphenols, total flavonoids, and alliin contents, leading to a pronounced decline in antioxidant capacity. Moreover, its flavor, taste, and overall acceptability are substantially inferior to those of conventionally aged black garlic. Therefore, current parameters and conditions of vacuum aging are not suitable for direct large-scale industrial adoption. Future work will focus on improving the overall quality of black garlic—including sensory, nutritional, and functional properties—while maintaining the high inhibition of acrylamide and 5-HMF by optimizing the processing parameters and conditions of vacuum aging.

4. Conclusions

Black garlic is produced from raw garlic that is thermally processed at high temperature for a long time, and the Maillard reaction is the main mechanism that resulted in the browning of garlic. It not only enhances the color, taste, odor, texture, and acceptability of black garlic, but also leads to the formation of chemical process contaminants, especially acrylamide and 5-HMF. Due to their potential harm to human health, mitigation of acrylamide and 5-HMF in black garlic is a critical issue for consumers, health authorities, and the food industry. Many efforts were performed to make thermally processed foods safe by inhibiting the chemical reactions responsible for the formation of acrylamide and 5-HMF. In this study, vacuum aging (16 kPa, 70–75 °C for 12 days) was used to control the production of acrylamide and 5-HMF in black garlic during aging. Compared with conventional aging, vacuum aging significantly mitigated the acrylamide and 5-HMF contents in black garlic by 100% and 95.4%, respectively, since it lowered the thermal load without increasing the total aging time. However, it simultaneously causes notable quality deterioration, including insufficient browning, and marked reductions in reducing sugars, polyphenols, flavonoids, and alliin, as well as antioxidant capacity and sensory acceptability. So, current parameters and conditions of vacuum aging are not suitable for direct large-scale industrial adoption.

This study confirmed the potential of vacuum aging for mitigating acrylamide and 5-HMF in black garlic. However, the poor quality of black garlic must be improved by optimizing the vacuum aging process and its parameters to maintain inhibitory efficacy against these contaminants while preserving the physicochemical properties, antioxidant capacity, and sensory quality of the final product in our future work. Based on our professional expertise and the research achievements of predecessors, we propose the

following improvement strategies: (i) two-stage aging (vacuum aging followed by short-term normal-pressure post-curing); (ii) gradient pressure aging; and (iii) combined pretreatments (microwave, freezing, or high pressure). Each strategy offers distinct advantages in contaminant control, sensory quality, nutritional retention, and industrial feasibility; however, their effectiveness will require systematic experimental validation in our future work.

Supplementary Materials

The following supporting information can be found at: https://www.sciepublish.com/article/pii/1244, Table S1: Sensory evaluation standards of black garlic samples.

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Author Contributions

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Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The authors state that the data supporting the findings of this study are included within the paper and its supporting information. Raw data files can be obtained from the corresponding author upon request.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest, and all funding partners and collaborators have been acknowledged.

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