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Does Curing Moisture Content Affect Black Garlic Physiochemical Quality?

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Abstract: This research examined the changes of black garlic (BG) quality attributes when raw materials of different initial moisture contents (iMC) were used. Fresh garlic bulbs (cv. Thai) were shade-dried for eight weeks at a controlled condition at 29 °C and relative humidity (RH) of 55% to the desired iMC (ranging from ca. 50–70%). BG processing was at 75 °C, RH = 80% for ten days. After processing, physiological characteristics and chemical properties of garlic were determined. Results illustrated that fresh garlic with higher moisture content (ca. 70%) resulted in BG of a dark brown colour, sloppy texture, and lesser acidity (pH = 4.44), while samples with lower iMCs (<50%) gave products that were completely black, elastic in texture, and with higher acidity (pH = 3.79). The analysed bioactive compounds, as well as their antioxidative potentials, suggested that the longer the curing time, the higher the functional properties of the finished products, possessing a total phenolic, total flavonoid content, and antioxidant activity of 15.54 mg/kg dry matter sample, 1.53 mg/kg dry matter sample, and 95.39%, respectively. Principle component analysis (PCA) of active metabolites confirmed that sulfur, S-allyl-L-cysteine, and flavonoid were among the main phytochemicals found in the BG. In summary, higher quality BG can be achieved by using raw materials of lower iMC.

Keywords: dehydration; non-enzymatic browning; initial drying process; processing

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1. Introduction

Garlic (*Allium sativum*), a bulbil species of the onion genus, has been widely cultivated and used as culinary herb for >5000 years [1–3]. In the historical Egyptian papyrus, as well as medical papers, garlic has been recorded as a medicinal plant that can cure many diseases and aliments such as headache, typhus, diphtheria, fever, and cholera [4]. Today, garlic has been scientifically proven and known as a "super food" due to the organosulfur compounds present, including allicin (diallyl thiosulfinate), alliin (S-allyl-L-cysteine sulfoxide), ajoene, diallyl polysulfides, and other active compounds [5]. They possess long lists of biological functionalities including antibacterial, antibiotic, antifungal, antiviral, anticancer, and antioxidant properties [6–13]. Additionally, garlic has been clinically proven to prevent carcinogenesis, cardiovascular, and age-related diseases [10].

In the culinary world, fresh garlic (FG) bulbs are known for their distinctive taste and pungent aroma, which are not favoured by many people. Processing garlic is therefore an alternative way to minimise the intense flavour, thereby increasing its marketability as well

as retaining its medicinal benefits [1,14]. Black garlic (BG) has recently gained increasing attention in the pharmaceutical market as an expensive exotic medicinal food [15]. It has been consumed in South Korea and Japan for hundreds of years, and its popularity has spread widely in recent years [16]. In BG processing, garlic bulbs are heated at high temperature and humidity for 4–40 days, and the flesh of the raw materials slowly turns from white to black due to the browning of non-enzymatic and enzymatic reactions [12,17,18]. The principal non-enzymatic Maillard reaction is the reaction between amino acids and reducing sugars at high temperatures, resulting in brown pigmentation. In this condition, the reaction alters the physical and chemical properties of the garlic, including colour, flavour, and antioxidant activity [19,20]. BG also has a chewy, jelly-like texture and sweetness due to the degradation of fructan to simple sugars such as fructose and glucose [1,12,15,21]. The sulfur volatiles (i.e., allicin) and cellular-bound components are converted into water soluble antioxidants such as alkaloids and flavonoids which elevate their antioxidative potentials [9,21–24]. Additionally, BG also has a high amount of reducing sugars, amino acids, and many beneficial antioxidative compounds [25]. Nonetheless, the raw materials used for BG processing are usually obtained from local suppliers, where curing processes vary from place to place. This results in raw materials of differing moisture content, which is in fact the major hurdle for the standardisation of BG processing, particularly when sensorial attributes and functional ingredients are held to high standards in markets. While attention is generally focused on its processing technology and medicinal functionality, information on raw material selection is limited. Consequently, in this research we attempted to explore the effects of initial moisture content of the raw materials used in BG processing on the processing qualities of BG. The key findings of this research could be used to establish a criteria for the raw material of premium BG products.

2. Materials and Methods

2.1. Materials and Sample Preparation

FG of Thai variety (50 kg), which were morphologically confirmed according to the report of Sommano et al. [26], were purchased from a Good Agricultural Practices (GAP) certified farmer in the San Pa Tong District, Chiang Mai Thailand (latitude: 18.615903, longitude: 98.910397) [27]. They were freshly harvested in March 2018 at commercial maturity stage (i.e., 90 days after cultivation), and transported to the laboratory immediately. The garlic bulbs with no disease or pest damage were cleaned and bunched (ca. 1 kg) prior to hanging to dry naturally in the shade with controlled atmospheric conditions (5×10 m^2, 29 °C, and 55% relative humidity (RH)). During the curing process, the garlic (ca. 250 g) was sampled every 10 days until a constant weight was reached (ca. 8 weeks). FG samples at each time interval were also used as controls. For each time interval, the initial moisture content (iMC) of the samples was determined by measuring the weight loss percentage after 12 h at 105 °C in a drying oven.

For BG processing, the dry leaves and outer skin were removed. The garlic bulbs were weighed individually prior to BG processing. BG was produced by heat treatment in a rice cooker (KS11E, Federal Electric Corporation, Bang Phli, Thailand) in warm mode for 10 days (at a constant 75 °C, RH = 80%), according to methods of Sasaki [15] and Kimura et al. [16]. The BG samples were collected and stored at −20 °C for further analyses.

2.2. Physicochemical Analyses

2.2.1. Texture

The individual cloves of the garlic samples were peeled. Firmness of the garlic sample was measured by a Force Gauge-Tension using a digital force gauge (RS232, Spencer Scientific, Derry, NH, USA) and the set-up condition was according to Nishizawa et al. [28].

2.2.2. pH and Colour

Flesh of the garlic sample (10 g) was blended with 100 mL of distilled water to obtain garlic slurry. The pH of the solution was measured using a pH meter (FiveEasy F20, Mettler

Toledo, Columbus, OH, USA) [12]. Moreover, the browning intensity of the slurry was measured at an absorbance of 420 nm using a spectrophotometer (SPECTROstar Nano; BMG LABTECH, Ortenberg, Germany) [29–31].

2.3. Light Microscope and Electron Microscope Observation

The garlic sample was cross sectioned to 3 mm-thick and fixed with formaldehyde: acetic acid: ethanol at 10:10:80 for 3 days, then dehydrated with a series of t-butanol concentrations (10, 25, 40, 55, 70, 85 and 100%). After that, the dehydrated sample was embedded in paraffin. The solidified sample was sliced into 10 µm-thick sections using a microtome (PR-50, Yamato Light Machine Industry, Saitama, Japan). The sliced samples were placed on glass slides and the paraffin layer was removed by a series of xylene-ethanol solutions (100:0, 50:50, 0:100), followed by ethanol solutions at the concentrations of 90, 75, 50, 35, 0 %. The slices were stained with 0.01% w/v toluidine blue, then washed in distilled water until clear. The glass slides were dried on a paraffin extender (40 °C), and cell structure was observed using an Olympus CX31 light microscope with an Olympus DP21 digital microscopy camera (Olympus Corporation, Shinjuku, Tokyo, Japan).

2.4. Chemical Properties

2.4.1. Extraction of Soluble Metabolites

The extraction method described by Sunanta et al. [32] was used. The flesh of garlic samples was initially frozen at −80 °C for 12 h, then lyophilised at −40 °C under vacuum for 48 h in a freeze dryer (Beta 2-8 LSCbasic, Martin Christ, Osterode am Harz, Germany). The sample (100 mg) was ground and weighed into a test tube, then 5 mL of 80% methanol were added, which was then heated at 70 °C for 30 min. The supernatant was collected after centrifugation (1-16K, Sigma Corporation, Osterode am Harz, Germany) at 6800× g for 12 min. The extraction step was repeated five times and all supernatant was combined. Finally, the total volume was adjusted to 25 mL with 80% methanol. The extract (1.5 mL) was added into a 2 mL microcentrifuge tube then evaporated off to dryness under the vacuum. Prior to the chemical analyses, the extract was re-dissolved with 1.5 mL of deionised water, and mixed well by vortexing and sonicating. The extract was used for sugar, phenolic, flavonoid, and antioxidant potential and the profile of soluble metabolites.

2.4.2. Sugar Content

The solution was filtered through a glass filter (0.25 µm) before use. Glucose, fructose and sucrose were determined by the LabAssay Glucose kit (Glucose C2, Wako Pure Chemical Industries, Chuo-ku, Osaka, Japan), according to the manual instruction.

2.4.3. Total Phenolic Content

The total polyphenol contents of garlic samples were determined using gallic acid as standard, according to the method described by Kang [19] and Zhang et al. [33]. Briefly, sample extract (30 µL) was mixed with 150 µL of Folin–Ciocalteu reagent, followed by the addition of 120 µL of 7.5% w/v of $NaCO_3$ solution. The reaction was initiated in the darkness at room temperature for 60 min. The absorbance at 765 nm was measured using the spectrophotometer and total polyphenols content was expressed as milligrams of gallic acid equivalents (GAE) per kilogram dry matter of garlic sample.

2.4.4. Total Flavonoid Content

The total flavonoids content was analysed using catechin as standard, according to the method described by Kim et al. [23]. The extract (25 µL) was added to 125 µL of distilled water, then 7.5 µL of 5% $NaNO_2$ solution was added. The mixture was allowed to react at room temperature for 5 min, then 15 µL of 10% $AlCl_3 \cdot 6H_2O$ solution was added. After 6 min of the incubation, one molar of NaOH solution (50 µL) and distilled water (27.5 µL) were added. The absorbance was measured at 510 nm using the spectrophotometer. The

total flavonoid content was expressed as mg Catechin Equivalents (CE) per kilogram dry matter of garlic sample.

2.4.5. DPPH Scavenging Radical Activity

The free radical scavenging activity of BG based on the scavenging activity of the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was determined using the method described by Kang [19]. DPPH (0.2 mM in ethanol) solution was prepared by dissolving 0.005 g of DPPH in 100 mL ethanol. Methanol extract (25 µL) was added to 250 µL of 0.2 mM DPPH in ethanol solution. After incubating the solution at room temperature in the dark for 30 min, the absorbance was measured at 550 nm using the spectrophotometer. The anti-radical activity was calculated as a percentage of DPPH decolourisation.

2.4.6. Thiosulfinate Content

The amount of water-soluble thiosulfinate in both of the FG and BG samples was determined by the method of Kang [19]. Garlic sample (0.5 g) was weighed into a 100 mL Erlenmeyer flask and 25 mL of distilled water was added. The sample was reconstituted for 10 min by shaking gently. The mixture was filtered through Whatman No.1 filter paper. The clear supernatant was added into a new 100 mL Erlenmeyer flask. The thiosulfinate was extracted from the water by using 10 mL of hexane, swirling the mixture gently till the hexane layer was separated. The aqueous layer was decanted into the flask, and the remaining thiosulfinate was extracted with 5 mL of hexane. The first and second extracts were combined, and the absorbance of the solution was measured at 254 nm. The thiosulfinate content of the hexane solution was calculated using the following equation:

$$C = A/(\varepsilon \times b), \quad (1)$$

where A is the absorbance,
b is the path length (cm),
C is the solution concentration (µmoL/g), and
ε is the molar absorptivity of thiosulfinate solution at 254 nm (0.014 g/µmoL·cm).

2.4.7. Identification of Soluble Metabolites Using LCMS and FTIR

The FTIR spectra of the extracts were measured (Alpha II, Bruker, Germany). The identification of the soluble metabolites in the BG samples was carried out using an Agilent 6545 QTOF-LC/MS system consisting of an Agilent 1260 LC with a 6545 UHD accurate-mass QTOF mass spectrometer. The methanol-extract was re-dissolved with 1.5 mL of LCMS-grade methanol containing 0.1% formic acid (*v*/*v*) and filtered through a nylon syringe membrane filter (0.25 µm) prior to the analysis. Separation of the chemical compounds was performed using the Agilent Poroshell 120 EC-C18 column (2.1 mm × 100 mm, 2.7 µm) at 25 °C. The mobile phase consisting of 0.1% of formic acid in water (phase A) and 0.1% of formic acid in methanol (phase B) was used. Gradient elution was as following: 5% phase B for 1 min, then increased to 95% up to 10 min and held for 19 min. The total run time was 30 min. The applied flow rate was 0.4 mL/min and the injection volume was 10 µL. MS and MS/MS analysis were carried out using a 6545 Agilent Ultra-High-Definition Accurate-Mass QTOF-MS coupled to the LC, equipped with an Agilent Dual Jet Stream electrospray ionisation (Dual AJS ESI) interface in positive ionisation mode, and data elaboration was performed using Mass Hunter software (Agilent Technologies, Santa Clara, CA, USA). The accurate mass and molecular formula prediction were screened for putative molecules in the database (Agilent METLIN PCDL) [34].

2.5. Statistical Analysis

The experiment was conducted using a completely randomised design (CRD) with 5 replicates. All experiment data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the significance of the difference among the samples within each type of

garlic (FG and BG) with a significance level of 0.05, using SPSS 24.0 version (SPSS Institute, Armonk, NY, USA). Principal component analysis (PCA) was used to summarise the visual differences of the chemical components of methanolic extracts and garlic samples using XLSTAT ver. 2018.5 (Suite NY, New York, NY, USA).

3. Result and Discussion

3.1. Physicochemical Properties

Garlics were cured in the shaded condition, then the raw materials at each time interval were first determined for the moisture content and also processed to BG. During which, the moisture content dropped initially from 72.60% to 56.44% from the initial to 40 days, and became more stable beyond this point. The moisture contents of the FG were 72.60%, 66.78%, 59.99%, 56.44% 51.15% and 49.95% after curing for 10, 20, 30, 40, 50 and 60 days, respectively, as shown in Figure 1A. The FG samples with different curing duration were taken for BG processing in the same condition. The result showed that an approximate 13% decrease in the BG moisture content (bMC) was noticed in all treatments (61.20, 50.56, 45.72, 40.40, 40.04 and 39.82% in BG1, BG2, BG3, BG4, BG5 and BG6, respectively). Figure 1A illustrates the relationship of iMC and bMC, which is approximately 10% different, and the morphological appearances of each corresponding BG are illustrated in Figure 1B. It was clear that the colour of BG 1, 2 and 3 with a bMC of 61.20%, 50.56% and 45.72%, respectively, were dark brown and sloppy, while BG 4, 5 and 6 possessed a bMC of 40% 40.04% and 39.82% bMC, respectively.

This result was in-agreement with the reports of Bae et al. [1] and Kang [19] who illustrated that the moisture content of BG decreased to approximately 10–13% during the BG processing. Moisture content apparently affected the texture of BGas shown in the experiment of Zhang et al. [33]. They also suggested that, after processing, BG with high moisture content (50–70%) gave products with a soft and mushy texture, while when MC of BG was lower (40–50%), the texture was elastic. Based on these findings, BG3–BG6 with approximately 40% iMC, at which the final product became completely black and the texture was soft and elastic, were theoretically acceptable for BG processing [34].

Result of the texture analysis of the processed garlic is shown in Table 1. The firmness of FG was the hardest at approximately 1.65 N. The firmness of BG depended on the iMC of the raw materials; sample BG6 was 10-fold higher in value than that of BG1, giving the iMC of 49.95% and 72.60%, respectively. BG processing is recognised also as a dehydrating process in which the moisture from the garlic cell is dried off, hence the collapse of the cell wall structure weakens the cell structure of the garlic. Moreover, the heat processing induces the degradation of pectic polysaccharides of the cell wall. For these reasons, the texture of BG had become softer than that of the FG [19]. The pH of the FG remained stable throughout the curing process (6.46), while the pH of BG decreased to 4.44, 4.37, 4.04, 4.02, 3.79 and 4.35 for BG1, BG2, BG3, BG4, BG5 and BG6, respectively. The decrease in pH was associated with browning mechanisms during heat processing [1]. Heat treatment during BG processing leads to pH reduction by non-enzymatic reactions including those of the Maillard reaction, and possibly caramelisation as the result of carboxylic acid formation and chemical oxidation of phenols to phenolic acids [12,19]. High acidity due to these biochemical changes also advances to the unique balsamic flavour characteristic of BG [35,36].

The UV absorbance at 420 nm is regularly used to detect the water-soluble product of the Maillard reaction between glucose and fructose with amino acids [36,37]. Table 1 showed that the browning intensity of BG increased continuously when the iMC of raw material decreased at the same processing condition using the UV–visible spectroscopy. The browning intensity of BG was as high as 68-fold compared to that of the FG. The browning intensity of the BG samples was higher than that of FG. The results were comparable to the observation of Zhang et al. [33], who addressed three stages of BG colour development initially from white to light brown, then thereafter to dark brown during the heating process. It was found that high moisture content inhibited the activation energy of Maillard reactions,

thereby the heat was accelerated the rate of moisture removal [1]. Kim et al. [38] indicated that BG processing required a relatively high activation energy for browning based upon the iMC. Anywise, water activity (a_w) is an important factor to control the stability of dry and dehydrated products during the processing and storage. In the BG processing, the rate of Maillard reaction during dehydration at high a_w was slower due to the diluted reactant by water, resulting in the lower intensity of the blackening products [39,40].

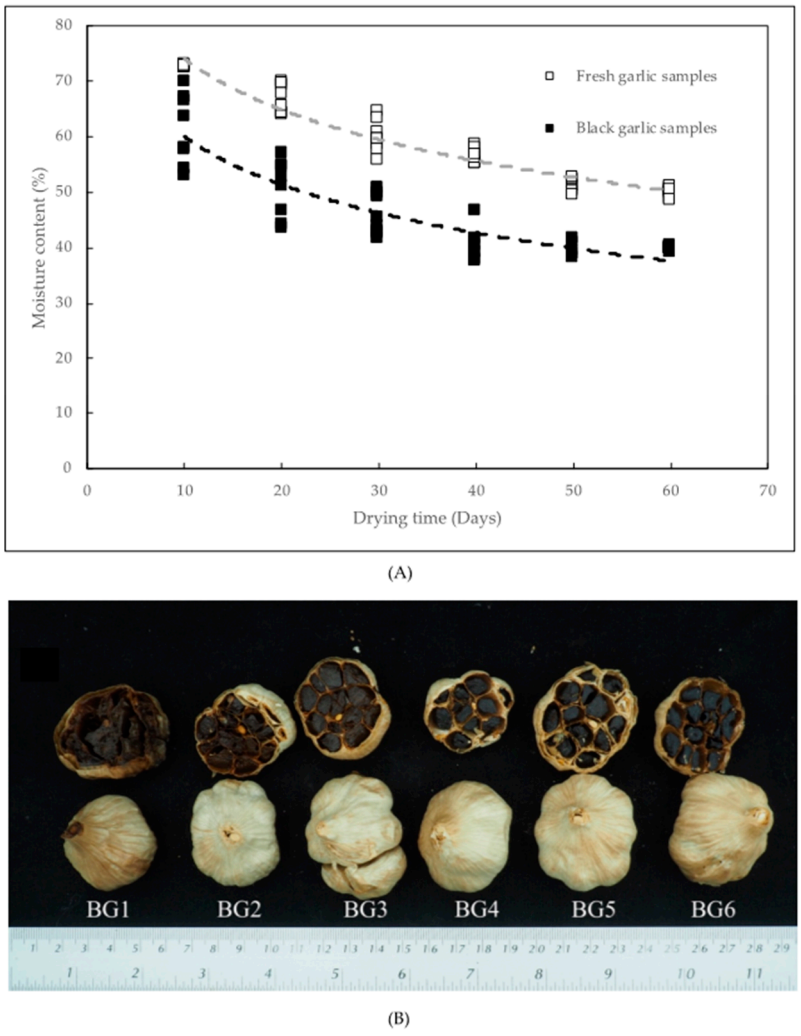

Figure 1. (**A**) Differences of moisture contents of fresh garlic (FG) as reported as initial moisture content (iMC) and black garlic BG as reported as BG moisture content (bMc); (**B**) the cross section of BG samples (BG1, BG2, BG3, BG4, BG5 and BG6 with the iMCs of 72.60%, 66.78%, 59.99%, 56.44% 51.15% and 49.95%, respectively).

The relationship between BG samples and their physicochemical properties is shown in Figure 2. The variables of texture (as determined by firmness and post-process moisture content), pH and colour (as determined by the colour intensity at 420 nm) and BG samples account for the total of 94.04 % in the PC1 that deposited 79.33%, and PC2 at 14.72%. Across the PCA axes, the BG samples distinguished into two major groups: the BG of higher iMC (BG1–3), and the BG from lower iMC (BG4–6). The PCA-biplot also advised that less acidity as described by higher pH values and moisture content of BG were correspondent with those of higher iMC (BG1–BG3), but the browning intensity was higher when the iMC of garlic was low. This incidence was described by the reduction of rate constants in the kinetic of Maillard browning with the access amount of moisture content [41]. Moreover, the firmness of the BG product also related to iMC. The BG texture was harder when lower iMC material was used.

Table 1. Physiochemical properties of fresh garlic and black garlic with different curing durations.

Components	Samples	Curing Duration (Days)						
		0	**10**	**20**	**30**	**40**	**50**	**60**
Moisture content (%)	FG	80.07 ± 0.13 a	72.60 ± 0.00 b	66.78 ± 2.53 c	59.99 ± 2.88 d	56.44 ± 1.23 e	51.15 ± 1.15 f	49.95 ± 0.85 f
	BG	-	61.20 ± 6.38 A	50.56 ± 5.15 B	45.72 ± 3.58 C	40.40 ± 2.94 D	40.04 ± 1.38 D	39.82 ± 0.36 D
Firmness (N)	FG	1.77 ± 0.08 a	1.76 ± 0.05 a	1.73 ± 0.04 ab	1.68 ± 0.02 ab	1.61 ± 0.01 bc	1.53 ± 0.02 cd	1.47 ± 0.01 d
	BG	-	0.05 ± 0.01 A	0.16 ± 0.02 B	0.22 ± 0.02 C	0.27 ± 0.02 D	0.35 ± 0.03 D	0.56 ± 0.01 E
pH	FG	6.47 ± 0.01 a	6.48 ± 0.01 a	6.47 ± 0.01 a	6.27 ± 0.20 a	6.47 ± 0.01 a	6.46 ± 0.00 a	6.46 ± 0.00 a
	BG	-	4.44 ± 0.01 A	4.37 ± 0.01 B	4.35 ± 0.00 C	4.04 ± 0.00 D	4.01 ± 0.00 E	3.79 ± 0.00 F
Browning pigments (OD420)	FG	0.05 ± 0.00 a	0.05 ± 0.00 a	0.05 ± 0.00 a	0.05 ± 0.00 a	0.05 ± 0.00 a	0.05 ± 0.00 a	0.05 ± 0.00 a
	BG		0.05 ± 0.00 D	0.05 ± 0.00 D	0.06 ± 0.00 D	1.52 ± 0.07 C	3.02 ± 0.04 B	3.41 ± 0.09 A
Free sugar (g/kg dry weight)								
Glucose	FG	0.61 ± 0.02 a	0.65 ± 0.04 a	0.59 ± 0.00 b	0.57 ± 0.00 bc	0.55 ± 0.00 bc	0.51 ± 0.01 c	0.45 ± 0.01 d
	BG	-	1.54 ± 0.11 a	0.57 ± 0.03 c	0.59 ± 0.03 c	0.92 ± 0.04 b	0.82 ± 0.07 b	0.82 ± 0.07 b
Fructose	FG	0.04 ± 0.01 a	0.02 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a
	BG	-	3.71 ± 0.25 A	3.22 ± 0.08 AB	3.14 ± 0.12 B	3.67 ± 0.13 A	3.46 ± 0.06 AB	3.58 ± 0.22 AB
Sucrose	FG	0.32 ± 0.01 a	0.32 ± 0.00 a	3.22 ± 0.08 ab	0.29 ± 0.00 c	0.29 ± 0.00 c	0.27 ± 0.00 d	0.26 ± 0.00 e
	BG	-	0.49 ± 0.01 A	3.22 ± 0.08 AB	0.44 ± 0.01 B	0.40 ± 0.01 C	0.32 ± 0.01 D	0.42 ± 0.02 BC

Values are mean ± SD; values followed by different letter in the same row are significantly different ($p < 0.05$) by Duncan's multiple range test (DMRT).

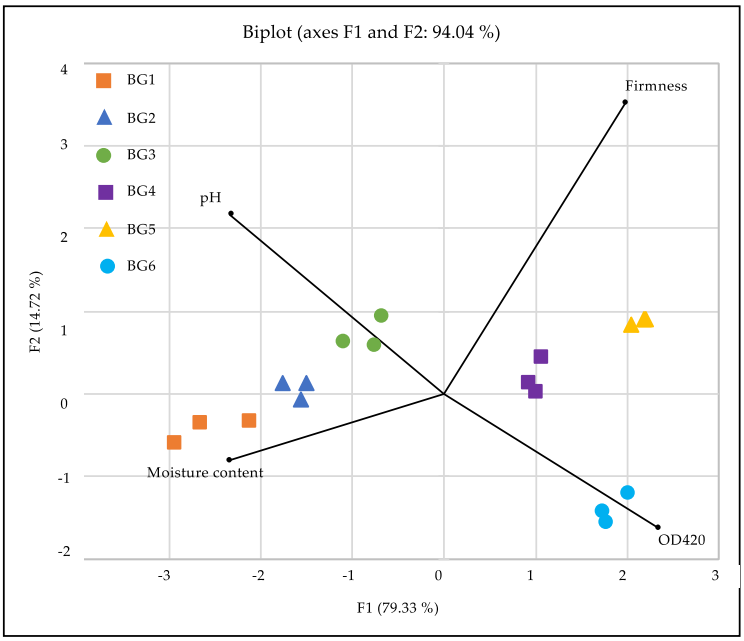

Figure 2. Principal component analyses of physicochemical properties; BG1, BG2, BG3, BG4, BG5 and BG6 were processed from FG with different iMC namely 72.60%, 66.78%, 59.99%, 56.44% 51.15% and 49.95%, respectively.

3.2. Light Microscope and Electron Microscope Observation

Figure 3A illustrates the cell structure of garlic raw material prior to the processing. The epidermis cells were thin and delicate, radially elongated and covered by a cuticle layer at the outer surface, and could not be stripped from the clove; the vascular bundles were widely distributed near the outer epidermis and around the central opening. The phloem and the xylem were in contact with each other directly, and most of the storage parenchyma were quite uniformly filled with finely granular materials. These structures were described in the common FG as mentioned in Mann [42] and Miryeganeh and Movafeghi [43]. In our BG samples, the cell structure showed black pigments spread all over the cells, which was possibly due to the formation of melanin by-products generated from an enzymatic reaction that may have happened at the initial stage of BG processing [20] (Figure 3B–G). The overall structure of BG1, BG2 and BG3 were collapsed with separated epidermis, and the shapes of the parenchyma and vascular bundle were not detected (Figure 3B–D). In addition, from the cell structure of BG4, BG5 and BG6, almost all of the epidermis structures were detected and the shape of the parenchyma and vascular bundles were still intact (Figure 3E–G). Dehydration with high temperature in the presence of moisture could ideally enhance the separation of cells and the loss of cell wall rigidity [44]. In addition, the elasticity also depends on the polysaccharide composition of the cell wall, as well as the water-holding capacity [45]. Hence, the iMC of raw material could indeed affect the cell structure of the BG.

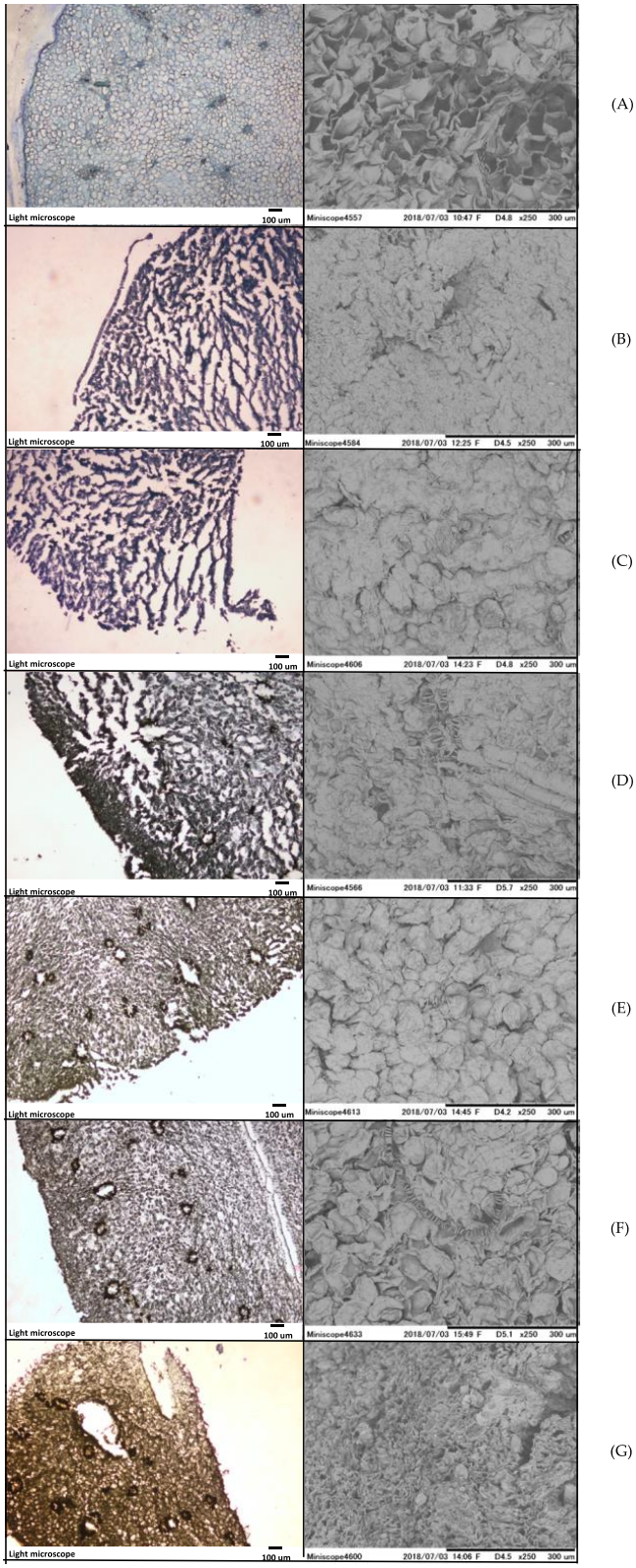

Figure 3. The cross-section of garlic scape under light microscope and electron microscope observation: (**A**) fresh garlic; (**B**) BG1, (**C**) BG2, (**D**) BG3, (**E**) BG4, (**F**) BG5, and (**G**) BG6, which were processed from FG of different iMC (72.60%, 66.78%, 59.99%, 56.44% 51.15% and 49.95%, respectively).

3.3. Chemical Properties

Free sugar contents in the BG depends on the degree of polysaccharide degradation along with the rate of sugar consumption through Maillard reaction and caramelisation.

In the processing of the BG at 60–70 °C, the rate of the reducing sugar formation was faster than the rate of sugar consumption, disposing the accumulation of the free sugars, thus making products with a relatively sweet taste [33]. The free sugar contents of the BG with different iMC were presented in Table 1. It is apparent that in the BG samples, the glucose, fructose and sucrose are higher than those of the FG. Sucrose was the major sugar composition in the FG; while in the BG, fructose was the major sugar, followed by sucrose and glucose. These results were compatible with the finding of Atashi et al. [46], who reported that sucrose was the largest quantity followed by fructose and glucose in the FG. In our study, there was a 10-fold enhancement of the total free sugars (from 4.22 g/kg in the FG to 43.5 g/kg of the BG1), and this result was similar to the discovery of Qiu et al. [47].

The total phenolic content represents the mean response of all major phenolic compounds found in fruits and vegetables [48]. In this experiment, total phenolic content of the FG at the harvesting state (day 0) was 0.87 ± 0.03 mg/kg dry matter of sample, and decreased along with curing duration to 0.67 ± 0.01 mg/kg dry matter of sample at day 60. After BG processing, the total phenolic content increased as compared with the FG samples. Total phenolic contents in the BG samples were higher at low iMC, and the BG6 with iMC of 49.95% gave the highest total phenolic contents (BG1 4.16 ± 0.71 and BG6 15.54 ± 0.14 mg/kg dry matter of sample) as shown in Figure 4A. Kim et al. [23] and Kang [19] reported that the total phenolic contents in BG increased more than 10-fold compared with the FG, because the high temperature processing released the bound forms of phenolic compounds (i.e., glycosylate) to the phenolic acids and the complex polyphenols from the browning reaction to phenolic acids [49]. Jastrzebski et al. [50] suggested that accumulation of phenolics could also improve the total antioxidant capacity and acidity of BG.

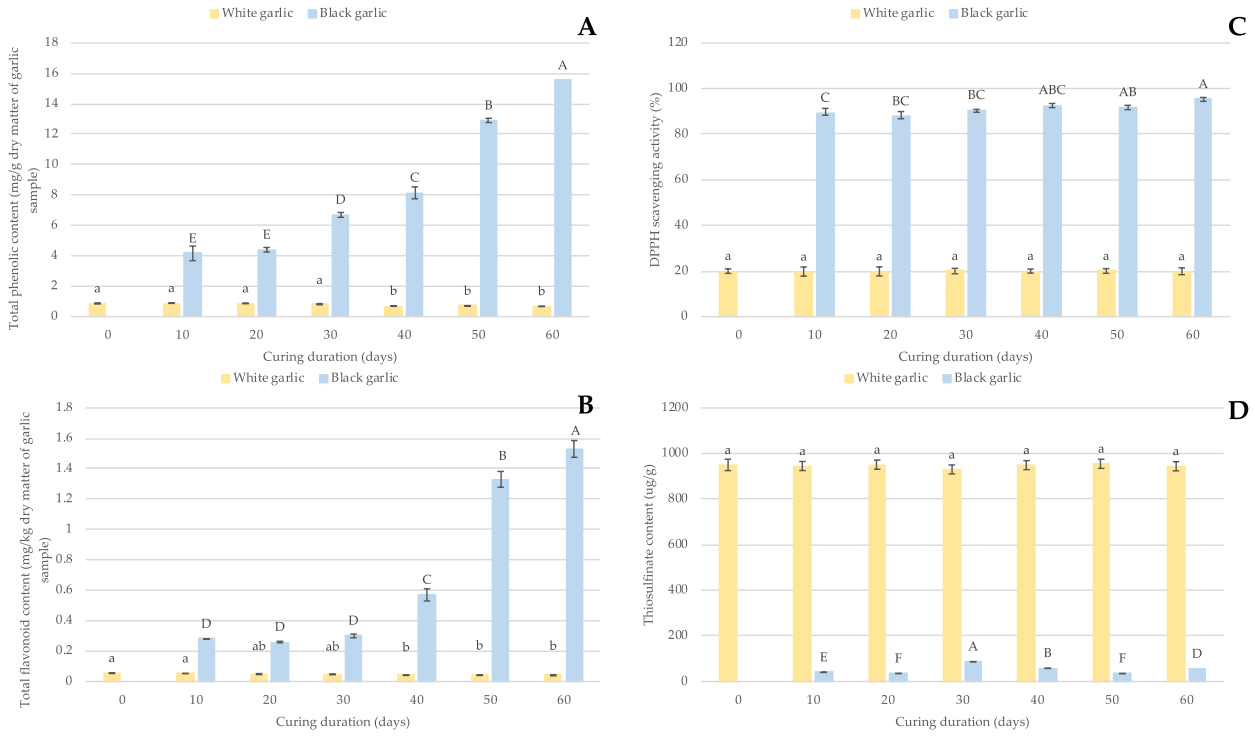

Figure 4. The chemical analysis of fresh garlic and black garlic samples at different curing durations. The different letter on the top of the bar is significantly different ($p < 0.05$) by DMRT; the comparison is within two groups of samples (yellow bar represents FG and blue bar represents BG); the initial moisture content of FG was 72.60%, 66.78%, 59.99%, 56.44% 51.15% and 49.95% in 0, 10, 20, 30, 40, 50 and 60 days of cured process, respectively. Bars indicated the mean of five replicates with standard error. (**A**) Total phenolic content; (**B**) total flavonoid content; (**C**) DPPH scavenging activity; (**D**) thiosulfinate content.

Flavonoids are the bioactive compounds belonging to the phenolic group. Kim et al. [23] demonstrated that among the subgroups of flavonoids, the flavanol was the main flavonoid in BG, followed by flavanones and flavones; however, in FG, the flavanones were the major metabolites. These metabolites contribute many biological functions in plants, for example, as defensive and antioxidant compounds against stresses [51]. High temperature processing affected flavonoid availability depending on the temperature and duration of heat processing and their sensitivity. However, depending on the processing conditions, the flavonoid contents are variable [52]. Similarly, the total flavonoid content in FG decreased after curing for a long time (total flavonoid content at day 0 was 0.05 ± 0.002, and at day 60 was 0.04 ± 0.003 mg/kg dry matter of sample). After heat processing, total flavonoid content was higher than the FG. The total flavonoid content in the BG was significantly increased when the material used had low initial moisture content (BG6: iMC = 49.95%), as shown in Figure 4B. Total flavonoid content of BG1 was 0.28 ± 0.01 and escalated to 1.53 ± 0.05 mg/kg dry matter of sample in BG6. Commonly, thermal processing of vegetables resulted in the breakdown of the cellulose structure from the plant cell and thus the availability of bioactive compounds can be improved [53].

The antioxidant activities of FG and BG samples are shown in Figure 4C. The DPPH scavenging activity is characterised by the ability of the substrates to obtain electrons from the reaction mixture. The curing duration was not related to the antioxidant activity of FG (approximately 20%); nevertheless, BG processing can improve the antioxidant activity of BG. The DPPH free radical scavenging activity was increased to 95.39% in BG6. Bae et al. [1] also found that the DPPH radical scavenging ability of the heated garlic samples was significantly higher than that of FG, and also increased with increasing temperature and time. Leelarungrayub et al. [54] suggested that the representative antioxidant compounds in garlic were phenolics, flavonoids and sulfur-containing compounds. In addition, the products of the enzymatic browning reaction (i.e., melanins) also showed antioxidant abilities [55]. In this study, the browning intensity of BG increased with the decrease of iMC of raw material, and it presented a trend analogous to that of DPPH radical scavenging activity; the increase in the contents of total phenolic acids and flavonoids in the BG compared with those in the FG were reported. The result of this study suggested that the iMC of FG was correlated well with the antioxidant activity of the BG.

Thiosulfinate is the main flavour substance of FG and is rapidly decreased during thermal processing [19]. The thiosulfinate contents of garlic samples were presented in Figure 4D. FG had higher thiosulfinate content (approximately 950 μg/g dry matter of sample) than that of the BG, and the curing duration had not influenced the thiosulfinate content in FG. The contents decreased in all BG samples due to the conversion of thiosulfinate to S-allyl cysteine (SAC), S-allylmercapto-cysteine, arginine and other compounds at high temperature [56]. Likewise, the alliinase was denatured at high temperature which disable the conversion of alliin to allicin [57].

The PCA of BG samples and their chemical components, including those of antioxidants, sulfur-containing compounds and sugars, were illustrated in Figure 5. The result displayed 77.80% variability across the biplots, with PC1 contributing 51.05% and PC2 depicting 26.75%, respectively. Similar to the physiochemical properties, the BGs divided into two major groups, and those with lower iMC (BG5 and BG6) illustrated stronger influence in total flavonoid and phenolic content and antioxidant activity than the rest of the BG samples. Total free sugar was found to be highest in the BG1 due to the lower rate of Maillard reaction, resulting in a light brown colour. During heat processing, free sugar was released and used as the substrate for the reaction.

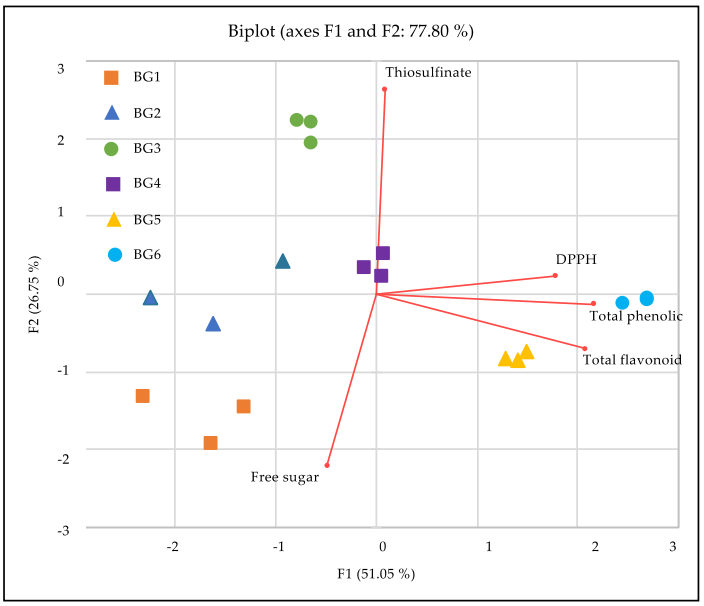

Figure 5. The PCA analysis with chemical compositions BG1, BG2, BG3, BG4, BG5 and BG6 were processed from raw material with different iMC (BG1, BG2, BG3, BG4, BG5 and BG6 with 72.60%, 66.78%, 59.99%, 56.44% 51.15% and 49.95%, respectively).

The FTIR spectrum between FG and BG after 60 days curing duration was illustrated in Supplementary Figure S1A. The broad band at 3265.94 cm^{-1} shows the N-H stretching. The peak at 1631.24 cm^{-1} results from C=O bonding. The peak at 1057.45 cm^{-1} shows the C-N bond. When comparing the spectra between the FG and BG, the result advised that the C-N bond which represented the amino acid in BG was higher than that of FG. The C=O bond displayed the carboxylic acid. After heat treatment, the pH of BG increased with the increasing carboxylic acid contents.

The metabolites of BG and FG were identified using LC-QTOF mass spectrometry was show in Supplementary Table S1, and the comparison of the chromatograms was displayed in Supplementary Figure S1B. The figure showed that heat processing altered the metabolite profiles of the garlic, and the obvious changes were with those of high affinity or retention strength to the C18 stationary phase in the first 10 min. In these regions, the chemical compounds were categorised into three major groups, namely amino acids, sulfur-containing compounds and antioxidant compounds. In comparison with the processed products, it was found in the result that lysine in FG was heat sensitive; thereby, the level was undetectable in the BGs samples. Leucine and Proline, however, can be detected in the BGs samples and the decrease in iMC of raw material seemed to have an effect on their contents. The main sulfur-containing compounds detected in the FG samples were allicin and cysteine-containing compounds. Allicin was only found in non-processed garlic as mentioned by Block [4]. Alliin is the odour-free sulfur-containing compound that is a product of enzymatic conversion with alliinase to allicin during tissue damage. Allicin is an unstable and responsible unique garlic smell during tissue damage. Heat processing could alter the allicin to a more stable compound, S-allylcysteine (SAC), which is an antioxidant compound. Consequently, the amount of SAC in BG was higher when compared with the FG. The antioxidant potential in BG was higher than those of fresh material and increased with lower initial moisture content. FG contains a carbohydrate–phenol complex, and heat treatment could break the bond between phenol and other compounds to release the free forms of phenols [58]. In other studies, S-allylcysteine, amino acids, were detected, which was in line with our reports [25,59]. The first two principle components of the PCA score plot were responsible for 86.4% (67.89% for PC1 and 18.52% for PC2) of the overall variance of the metabolite profile (Figure 6). It is clearly observed that the metabolites were separated into two obvious groups. The bi-plot described that FG was clearly separated from BG

samples leading with allicin and gamma-glutamyl-S-(1-propenyl) cysteine sulfoxide. The BG1–BG4 samples, which were produced from raw material of higher initial moisture content, had similar chemical profiles, with amino acid and antioxidant compounds playing significant influence, such as phellamurin and jasmonic acid. The lower iMC samples (BG5 and BG6) grouped together, which were led by amino acid and oligopeptide. From these findings, we can determine that the iMC of raw material contributes vastly to the chemical compositions that contribute to the physical properties, as well as the bioactive potentials, of BG.

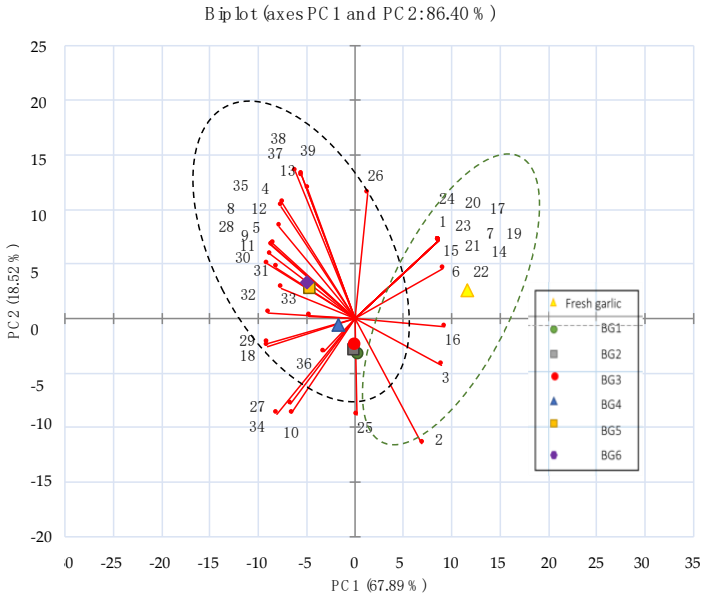

Figure 6. Principal component analysis analyses of the analysed metabolites of garlic samples. BG1, BG2, BG3, BG4, BG5 and BG6 were processed from fresh garlic with different iMC (BG1, BG2, BG3, BG4, BG5 and BG6 with 72.60%, 66.78%, 59.99%, 56.44% 51.15% and 49.95%, respectively). The numbers are correspondent with active compounds as reported in Table 1.

4. Conclusions

This study highlights that the initial moisture content of fresh garlic plays a crucial role to the physicochemical attributes of the finished product. High initial moisture content material gave a black garlic product that was lighter in both colour and texture, and of low acidity. On the other hand, the darker product of sweet-mellow taste, elastic texture and higher acidity that were favourable in the market can be achieved by more dehydrated materials (moisture content < 50%). Additionally, the low initial moisture content induced water solubility of the active compounds, thus improving antioxidant potentials in the finished product. The results from this study can be used to set out the criteria for raw material quality control and to minimise losses occurring due to the production of rejects.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7120535/s1, Figure S1: (A) The FTIR spectrum between fresh garlic and black garlic. (B) The QTOF chromatogram of fresh garlic and black garlic sample, Table S1: Compounds identified from garlics by Quadrupole Time of Flight-Liquid chromatography/mass spectrometry technique.

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