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Composition of spontaneous black garlic fermentation in a water bath

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Abstract

Garlic was fermented spontaneously in a water bath with a temperature of 72 °C and relative humidity close to 90%. The fermentation periods were 0 (fresh garlic), 7, 14, and 21 days. Several physicochemical properties: antioxidant capacity, total polyphenol, flavonoid content, pH, and browning intensity were determined. All of the chemical properties of black garlic increased significantly during the fermentation, except the pH value. The pH value was decreased conveniently during the time of fermentation. Browning intensity as physical properties also increased during the fermentation. A volatile compound in garlic during the fermentation process was analyzed by SPME-GCMS and was quite different compared with fresh garlic. A water bath could be considered as fermentation instrument of black garlic processes.

Keywords: black garlic; volatile compounds; SPME-GCMS.

Practica Application: Production of black garlic by spontaneous fermentation in a water bath and its composition.

1 Introduction

Garlic has been used as a seasoning food in Asian and medical herb due to its potential benefits i.e. antimicrobial, antioxidant, antitumor and immunomodulatory activities (Banerjee et al., 2013; Sultan et al., 2014). However, its consumption is still limited because the taste, flavor, and odor aren't preferred (Tanamai et al., 2017; Ngan et al., 2017), it has a tendency to cause stomach upset and it might be toxic at high doses (Bae et al., 2012). Black garlic is a garlic product that is produced to be preferred and it easily consumed. Black garlic produced by heating garlic at high temperature and high humidity (Ngan et al., 2017; Kang et al., 2008; Bae et al., 2014; Choi et al., 2014; Kimura et al., 2017), enzyme treatment and curing (Wang and Sun, 2017). Black garlic is also defined as a fermented product, made by spontaneous fermentation of whole garlic bulbs (Kim et al., 2012; Lee et al., 2011; Sato et al, 2006). The term spontaneous fermentation of black garlic supported by Qiu et al. (2018) has been succeeded isolating of 4 microbe genera: *Thermus, Corynebacterium*, S*treptococcus*, and *Brevundimonas* from black garlic procesed in the heating oven at 80 °C for 12 days on vacuum sealed bags. On the other hand, Setiyoningrum et al. (2018) made black garlic through (unspontaneous) fermentation, inoculated with *Saccharomyces kluyveri* Y97. In this research, black garlic was made focus by spontaneous fermentation in a water bath.

There were increasing properties in black garlic compared with fresh garlic such as S-allyl cysteine (SAC) that increased 5 to 6 times (Bae et al., 2012; Wang et al., 2012), extended shelf-life (Chu et al., 2007), higher phenolic content 5-8 times (Kim et al., 2012), lower off- flavor (Kimura et al., 2017) and lower fructan content (Yuan et al., 2016). Black garlic has a sweeter taste (Ngan et al., 2017) caused by increases in glucose, fructose, and sucrose content (Zhang et al., 2015), as well as sticky and jelly-like texture (Bae et al., 2014). The previous study on producing black garlic with heating was carried out by Kim et al. (2012); Bae et al. (2014); Zhang et al. (2015); Zhang et al. (2016); Ngan et al. (2017); Wang and Sun (2017); and Lu et al. (2018). The thermal process leads to non-enzymatic browning reactions such as the Maillard reaction, caramelization and chemical oxidation of phenol (Bae et al., 2014) associated with increased antioxidant properties Several tools used to produce black garlic with the heating method, are, among others, oven drying (Ngan et al., 2017; Bae et al., 2012), heat and humidity box (Wang & Sun, 2017), chamber, humidity control room (Yuan et al., 2018) and thermohygrostatic chamber (Choi et al., 2014; Lu et al., 2018). A water bath is a thermostatic instrument widely used for incubating samples at a constant temperature over a long period of time in a water media. A water bath has control of temperature and high humidity. The aims of this study were to process black garlic with a water bath and characterization of its properties.

2 Materials and methods

2.1 Materials

Fresh garlic (*Allium sativum* L.) was obtained from the local market in Bogor, Indonesia. The reagents used in this research were methanol, aluminium (III) chloride (Ajax Finechem NSW, Australia), 1,1- diphenyl-2-picrylhydrazyl (Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), Follin-ciocalteu, ethanol potassium acetate and sodium carbonate (Merck,VWR International, Spain), ultrapure water (Generik Jakarta, Indonesia).

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2.2 Methods

Sample preparation and extraction of black garlic

The unpeeled fresh garlic was fermented spontaneously in a water bath with a temperature of 72°C and relative humidity closed to 90%. Sampling was made at different times for 7, 14, and 21 days. The extraction method in this research was described by Kim et al. (2012) with some modification. Measurement of pH and browning intensity Browning intensity and pH were determined in each sample during the fermentation process. Garlic browning intensity was determined by Yuan et al. (2018). The determination was obtained in duplicate.

Measurement of functional properties

The functional properties measured in this research were total polyphenol, flavonoid content and DPPH radical scavenging activity. Total polyphenol was determined using the Folin-Ciocalteu method by Chang et al. (2002) with some modification. In a 1.5 mL Eppendorf tube, 0.05 mL extract garlic was mixed with 0.8 mL distilled water, 0.05 mL of 10% (v/v) Folin-Ciocalteu, and 0.1 mL of 7% (v/v) sodium carbonate. The mixture was incubated at room temperature for 30 mins. The absorbance was read at 750 nm and total phenolics was calculated from a calibration curve, using gallic acid as standard. Total flavonoid was determined by the method of Chang et al. (2002) with minor modification (2002). To 0.05 mL extract garlic, 0.05 mL of 10% (w/v) $AlCl_3$ water solution, 0.05 mL of 1 M potassium acetate, 0.3 mL ethanol absolute, and 0.6 mL distilled water were added. After 30 mins incubation, the absorbance was read at 415 nm. The total flavonoid was calculated as quercetin from the calibration curve. For DPPH activity, 0.8 mL of DPPH methanolic solution was mixed with 0.2 mL of the garlic extract. The mixture was incubated at room temperature with darkroom condition for 30 mins. The absorbance was read at 517 nm and the radical scavenging activity was calculated as follows, radical scavenging activity (%) = (P-Q)/P x 100%, P: absorbance of blank, and Q: absorbance of the sample. This method follows Muanda et al. (2011) with minor modifications.

Volatile compound by SPME-GCMS

Chemical compounds as volatile form either in fresh garlic or black garlic were analyzed by SPME-GCMS as described by Molina-Calle et al. (2017) with a slight modification, two replicates. The absorption of volatile compounds in samples was performed at 60 °C for 40 mins by the SPME tool. Volatile compounds were trapped in the fiber of SPME was analyzed using an Agilent Technologies 7890A-5975 c inert XLEI/CI gas chromatograph (Agilent Technologies Co., USA) equipped with a mass spectra detector (MS) and fitted with an HP-5MS capillary column (length 30 m, 0.25 mm i.d, 0.25 µm film thickness). The 2, 4, 6 trimethylpyridine of 1% (Sigma Aldrich) was used as internal standard (20 µL/samples). The fiber of SPME was injected into GC-MS using split 1:5 and helium as a carrier gas. The injector temperature was 180 °C and the detector temperature was 280 °C. The oven temperature program was set as follows: initially at 40 °C, and held for 5 mins, increased 10 °C/min to 250 °C, and held for 5 mins. The cut solvent time was set at 2 mins. LRI (Linear Retention Index) was determined by comparing the retention time of all constituents of the samples with the retention time of homologous series of n-alkanes (C_6-$C_2$6) (Sigma – Aldrich Pte Lvtd, Singapore) on the same column and condition above.

Statistical analysis

Data were subjected by a one-way analysis of variance (ANOVA, p=0.05) using SPPS software (SPSS, Chicago, II, USA). Differences in samples were determined by the Duncan test.

3 Result and discussion

3.1 The pH and browning intensity

The decrease in pH value during the black garlic process shown in Table 1. Comparing fresh garlic (FG), the pH value of black garlic with all treatments did decline significantly ($p<0.05$). The same phenomenon has been described by Toledano-Medina et al. (2016) and Bae et al. (2014). The decrease in pH value on black garlic due to the increasing total acid content (Choi et al., 2014). Liang et al. (2015) reported that the concentration of acetic acid was gradually increased during the processing of garlic, degradation result of hexose during processing. Research by Choi et al. (2014) described that the total acidity of garlic has increased from 0.40mg/kg to 2.60mg/kg after 21 days of heating. During the fermentation process, acetic acid was formed and its concentration increased gradually (Table 2).

Browning intensity of black garlic increased during fermentation process significantly (Table 1.), due to the formation of several compounds resulting from Maillard browning. In the initial stages of the Maillard reaction, colorless intermediate products are produced due to the amine-sugar condensation process and Amadori rearrangement. At the intermediate stage, there are several reactions such as dehydration of sugar, fragmentation of sugar, and degradation of amino acids (degradation of Strecker) leading toan increased browning. In the final stage of the Maillard reaction, occurs the aldol condensation process, aldehyde-amine condensation, and formation of heterocyclic nitro compounds occur leading to a further increase of browning intensity (Choi et al., 2014; Billaud et al., 2004). The higher concentration of MRPs induced the higher browning intensity produced. Billaud et al. (2004) noticed that the formation of MRPs (Maillard Reaction the intensity Product) is initiated from the reaction between glucose and cysteine and is influenced by temperature and processing time.

Table 1. pH value and browning intensity of garlic during the fermentation process in a water bath.

Treatment	pH	Browning Intensity
FGB	6.06^a	0.013^a
BG7D	4.94^b	0.742^b
BG14D	4.31^c	0.973^c
BG21D	3.47^d	2.685^d

FGB: fresh garlic; BG7D: black garlic fermented for 7 days; BG14D: black garlic fermented for 14 days; BG21D: black garlic fermented for 21 days. Different superscript letters in the same column indicate significantly different means among samples according to Duncan test at P<0.05

Table 2. Changes of functional properties of garlic fermented spontaneously in a water bath.

Treatment	Total polyphenol (mg QE/kg dry basis)	Flavanoid content (mg GAE/kg dry basis)	Antioxidant activity (%inhibition of 0.2mM DPPH
FGB	89468.55[a]	2348.65[a]	21.67[a]
BG7D	101328.71[b]	3825.51[a]	26.03[b]
BG14D	132510.22[c]	16255.38[b]	89.1[c]
BG21D	157312.77[d]	27191.38[c]	90.54[c]

FGB: fresh garlic; BG7D: black garlic fermented for 7 days; BG14D: black garlic fermented for 14 days; BG21D: black garlic fermented for 21 days. Different superscript letters in the same column indicate significantly different means among samples according to Duncan test at P<0.05

3.2 Functional properties of black garlic

It seems logical to state that an increase in polyphenol and flavonoid content in black garlic is due to antioxidant properties in this product (Toledano-Medina et al., 2016). Total flavonoids and polyphenols of black garlic are shown in Table 2. There were a significantly increasing total flavonoid and total polyphenol of black garlic from 7 days to 21 days fermentation process in a water bath. The total flavonoid of BG14D (black garlic fermented for 14 days) and BG21D was significantly higher than fresh garlic. Meanwhile, the total polyphenols of BG7D, BG14D, and BG21D were significantly higher than fresh garlic. These results indicate that the time period of fermentation has an important role in the increased total flavonoids and polyphenols of black garlic in addition to temperature and RH condition. These results are consistent with those obtained by Kim et al. (2012), who reported that total flavonoids and polyphenol in garlic might be increased by the heating process. In this study, the total flavonoid of black garlic increased up to 11.5-fold compared with fresh garlic, whereas the total polyphenol of black garlic increased to just 1.7-fold compared with fresh garlic. These results were different from the study by Kim et al. (2013), the total flavonoid and polyphenol of black garlic were increased about 1.5-fold and 10-fold compared with fresh garlic. Moreover, total flavonoids and polyphenols were optimum at 21 days of fermentation. The study from Choi et al. (2014) reported that total flavonoid and polyphenol of black garlic with heating over 21 days (28 and 35 days) obtained less optimum results compared with 21 days of heating.

Several reasons explain why the total flavonoids and polyphenols increased after the fermentation process. First, the fermentation process breaks the bound form like glycosylated and esterified, thus leading to an increase in free forms. Second, it is caused by the decrease in enzymatic oxidation involving the antioxidant compounds. Third, it is caused by an increase in the levels of complex polyphenol from the later phase of the browning reaction (Kim et al., 2013).

In this research, antioxidant capacity increased along with fermentation period significantly ($p<0.05$). The result of this research is in line with research by Toledano-Medina et al. (2016) and Choi et al. (2014). BG21D had 20% of antioxidant capacity higher than Choi et al. (2014) in the same heating period. During the heating process, allin is an unstable compound in fresh garlic converted into a stable compound such as SAC (S-allyl cysteine) which has a high antioxidant capacity (Choi et al., 2014; Toledano-Medina et al., 2016; Amagase, 2006; Corzo-Martinez et al., 2007). The concentration of SAC in black garlic was 97 µg/g, while its increase reaches of 5 to 6-fold compared with fresh garlic (Bae et al., 2012). In addition, SAC, the research by Lee et al. (2009) reported that an increase of antioxidant capacitywas also related to an increase of polyphenols, which were derivatives from allin. These findings were in line with this research, whereas an increase of antioxidant capacity was associated with an increase in the total polyphenol (Table 2) Antioxidant capacity of BG21D increased to 4.5 folds compared with fresh garlic.

3.3 Volatile compounds by SPME-GCMS

Changes of volatile compounds during the manufacturing of black garlic were shown in Table 3. Fresh garlic has several signature-sulphur containing compound as well as mentioned by Arslaner (2020), who investigated yogurt enriched by garlic paste. Some of the sulfur volatiles in fresh garlic, allyl mercaptan di-2-propenyl tetrasulfide and di(1-propenyl) sulfide, were not detected in black garlic. During the fermentation process, an increase of several sulfur compounds such as dimethyl disulfide, 2-vinyl-1,3-dithiane, allyl sulfide, diallyl trisulfide, 1,2-dithiolane, methyl 2-propenyl disulfide, 1,3,5-trithiane, and cyclooctasulfur occurred. Findings in this research were quite similar to Molina-Calle et al. (2017) and Zhong-yi et al. (2012).

Other novel compounds also formed in black garlic and their concentration did increase during the fermentation period, such as allyl alcohol, acetic acid, butanediol, benzaldehyde, and isopropyl myristate. The formation of acetic acid and its increasing concentration affect the pH value of black garlic. Black garlic contains richer in methyl alcohol, acetone. Benzeneacetaldehyde (4.61 µg/g) which were only detected in BG21D. This result was in line with Molina-Calle et al. (2017) that identified the volatile compound of 5 weeks heated garlic. Fresh odor and floral flavor which is characterized by 2-butenal detected in FG and decreased in BG7D. Its concentration was not detected along with an increase in the fermentation period. This phenomenon was in line with Kim et al. (2011) and Molina-Calle et al. (2017).

The formation of furfural started at 14 days of fermentation (BG14D) and its concentration increased above 41 times higher at the end of the fermentation process. Furfural was formed as a consequence of the Maillard reaction during the fermentation or heating process. This compound was a result of the degradation of pentose sugar Molina-Calle et al. (2017). The presence of furfural leads to sweet taste and has an aromatic odor that is reminiscent of almonds (McKillip et al., 2005).

Table 3. Volatile compound during the manufacturing of black garlic.

No	Compound	LRIexp	Concentration (μg/g)*			
			FGB	BG7D	BG14D	BG21D
1	Methyl alcohol	545	1.85	16.06	4.90	35.32
2	Acetaldehyde	554	5.26	nd	nd	nd
3	Acetone	567	0.60	5.26	1.49	8.57
4	Allyl alcohol	583	nd	73.39	17.57	90.79
5	Acetic acid	635	nd	nd	0.77	17.84
6	Butanedial	648	nd	1.64	1.81	8.49
7	Allyl mercaptane	604	6.00	nd	nd	nd
8	2-Butenal	648	0.90	0.64	nd	nd
9	Allyl methyl sulfide	695	3.85	3.77	2.39	8.47
10	Dimethyl disulfide	735	3.27	1.93	1.23	5.64
11	Furfural	840	nd	1.30	0.80	43.19
12	Allyl sulfide	860	nd	nd	10.88	53.93
13	3,3-thiobis-1-propene	860	20.49	30.81	nd	nd
14	1,3-dimethyl-Benzene (m-xylene)	871	nd	nd	nd	2.14
15	Di(1-propenyl)sulfide	891	0.46	nd	nd	nd
16	Methyl 2-propenyl disulfide	918	112.52	8.99	4.06	23.62
17	1,3-Diathiane	931	112.52	0.35	nd	nd
18	Methyl 1-propenyl disulfide	941	5.86	0.63	0.61	0.56
19	Benzaldehyde	963	nd	5.25	2.58	60.11
20	Dimethyl trisulfide	971	0.15	9.36	6.23	nd
21	1,3,5-trimethyl-benzene	1023	nd	1.58	0.57	3.73
22	Isobornyl acetate	1029	nd	nd	nd	1.85
23	Benzeneacetaldehyde	1047	nd	nd	nd	4.61
24	1,2-Dithiolane	1053	0.21	nd	nd	nd
25	Diallyl disulfide	1094	591.67	210.46	238.38	62.84
26	2-Vinyl-1,3-dithiane	1102	nd	10.37	2.03	17.32
27	Di-2-propenyl tetrasulfide	1107	60.28	nd	nd	nd
28	Methyl 2-propenyl trisulfide	1145	3.31	252.58	92.91	521.53
29	Diallyl trisulfide	1302	nd	nd	322.89	961.76
30	Di-2-propenyl trisulfide	1308	10.44	1565.20	nd	nd
31	1,2-Dithiolane	1381	nd	40.30	29.12	320.67
32	Methyl 2-propenyl disulfide	1394	nd	138.41	47.98	119.33
33	Diallyl tetrasulphide	1560	5.84	667.94	130.11	175.84
34	1,3,5-Trithiane	1669	nd	nd	15.59	58.90
35	Isopropyl myristate	1826	nd	nd	0.34	3.49
36	Cyclooctasulfur	2072	nd	1.92	1.08	1.19

*nd: not detected. FGB: fresh garlic; BG7D: black garlic fermented for 7 days; BG14D: black garlic fermented for 14 days; BG21D: black garlic fermented for 21 days.

4 Conclusion

Black garlic was manufactured by fermentation in water bath at temperature of 72 °C and relative humidity close to 90%. Its characterization quite differs from its fresh form. When the pH value decreased, its browning intensity, antioxidant capacity, total flavonoid, and total polyphenol increased. Volatile compounds in black garlic were quite different from fresh garlic. Furfural as a Maillard browning product was formed in garlic after the 7 days of the spontaneous fermentation process.

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