Preliminary study on the fermentation medium of the black garlic production

S B Anoraga¹, A R Sari¹, J Wikarta² and I Sabarisman¹

¹Agroindustrial Program, Department of Bioresources Technology dan Veterinary, Vocational College, Universitas Gadjah Mada, Yogyakarta, Indonesia
²Department of Agroindustrial Technology, Faculty of Agriculture Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract. The present work shows the potential of the natural material which commonly used javanese to be applied as fermentation medium on black garlic production. Black garlic processed by conventional fermentation using rice cooker for 10, 15 and 20 days. Three materials were used as medium, such as bamboo chambers, clay chambers and aluminium foil. The quality parameters were weight loss, moisture content, and TDS (brix %). The experimental design used in this study was a Completely Randomized Design (CRD) with a single factor experiment. The measurement result was obtained by using ANOVA statistical analysis and further testing with the Duncan method in the SPSS program. Black garlic was processed using natural material like bamboo chamber (besek) and clay chamber (kendil) for fermentation medium have shown lower weight loss and higher total soluble solid (%brix) than modern method using aluminium foil. Natural material affected total soluble solid (%brix) of black garlic significantly (p < 0.05). TSS (%brix) increased during fermentation process whereas pH decreased from pH 6.1 to 3.6.

Keywords: back garlic, conventional method, fermented processing, material, medium

1. Introduction

Some Indonesian traditional food are produced by fermentation process. There are various method of traditional fermentation that depend on the region, community traditions, culture, and heritage. Fermentation is conducted to maintain food quality and extend the shelf life. Usually, traditional fermentation is run naturally through spontaneous fermentation [1]. Several material are commonly used as traditional fermentation medium such as bamboo, banana leaf, corn husk, water apple leaf, teak leaves, hibiscus leaf, etc. All of these material can be taken from environmental surrounding of home industry. Each material has advantageous effect for the product characteristic, especially in aroma, texture, flavor, and health beneficial [2]. In addition, a number of studies have proven that the differences in packaging and fermentation media can affect the product quality [3]–[5].

As one of functional foods, black garlic is also produced by fermentation process. It occurs in the chamber with controlled temperature and humidity thus causing chemical and biochemical reactions [6]. Various method for black garlic fermentation have been reported [7], [8]. Generally, these methods using modern industrial processing for fermentation with temperature, humidity and time variation [9]–[12]. Therefore, this is an opportunity to combine existing fermentation methods with local wisdom in
Indonesia. So, it is necessary to conduct a preliminary study to find out how the characteristic changes in black garlic during traditional fermentation.

In Indonesia, clay chamber, bamboo chamber and banana leaf were used for fermentation process of tempeh, tape, tempeoyak, oncom, etc. These materials were also applied as fermentation medium for black garlic production. Aside of changing nutrients and sensory, fermentation process also increases the bioactivity of black garlic. Black garlic has many benefits for the body such as anticancer, anti-obesity, immunomodulatory, hypolipidemic, antioxidant, hepatoprotective, and neuroprotective effects [13]. This research is a preliminary study to develop the black garlic fermentation process based on Indonesian local wisdom. The aim of this study was to investigate the application of natural media on the black garlic fermentation process.

2. Material and Method

2.1. Materials
The material used in this study were fresh single garlic (Allium sativum Var. Solo garlic) namely bawang lanang taken from local market in Yogyakarta, fresh banana leaf, bamboo chamber namely besek, clay chamber namely kendil, tissue, and aluminium foil. The Equipments used in this research ware pH meter, thermometer, hygrometer, rice cooker, refractometer, analytic balance, oven, soxhlet, and other supporting equipments such as filter cloth, cup, tray, and measuring cup. This preliminary study was conducted in Mei 2020 at The Engineering Process Laboratory, Agroindustry Program, Vocational College, Universitas Gadjah Mada.

2.2. Procedure
Fresh garlics were sorted to obtain uniform sizes, then weighed 2 kg, and put in a fermentation chamber. There are 3 variations of fermentation chamber: bamboo chamber, clay chamber, and aluminium foil. Samples are put in the chamber and covered with banana leaf, while for aluminium foil was covered with tissue. Garlic is fermented for 10 days, 15 days and 20 days in the rice cooker to control the fermentation temperature.

2.3. Analysis
The parameters observed in this study were water content, weight loss, pH value, and TDS (brix%) content of black garlic. The characteristics of the black garlic results were compared with black garlic in the market which produced by UKM Umara Black Garlic in the Yogyakarta. Testing the moisture content using thermogavimetry method. The black garlic was weighed as much as 3 g with three replicates, then placed into a cup and dried using an oven at 105 °C until the weight was constant. pH value was measured with pH meter ATC. TDS (brix%) was measured using ATAGO refractometer. The measurement result was obtained by using ANOVA statistical analysis and further testing with the Duncan method in the SPSS program.

3. Result and Discussion

3.1. Fermentation condition
Fermentation process was conducted using rice cooker at temperature approximately 65°C [14]. Physically, the fermentation process can be controlled with the rate of increase in the fermentation temperature starting from the beginning to the end of the fermentation process. Changes in compounds in the fermentation process are characterized by changes in temperature [18]. Figure 1 show the empirical data of temperature and humidity during fermentation process.
Figure 1. Average temperature and RH condition during fermentation process

Based on Figure 1 shows that the temperature in the rice cooker increased around 70-80°C, while relative humidity was fluctuated. Humidity inside rice cooker increased in initial 10 days then dropped until the end of fermentation. In general, garlic fermented into black garlic is carried out at a temperature of 60-70 °C with a fermentation time of 30 days [17]. The increase in temperature in the fermentation media is followed by a decrease in humidity in the fermentation media at the end of the fermentation process. The decrease in humidity is caused by the dryness of banana leaves as a cover for fermentation media. Dry banana leaves are caused by an increase in temperature in the fermentation media during the fermentation process. Based on Figure 1 shows that the longer the fermentation time, the temperature in the fermentation media will increase. Thus, it required a replacement of medium fermentation cover for a few days to keep the humidity inside fermentation chamber.

The fermentation process functions to change complex macromolecular compounds such as protein, fat, and carbohydrates or other compounds into simpler compounds. The protein in black garlic is broken down into amino acids such as cysteine (the basic component of volatile and non-volatile compounds from garlic) [19]. Warming up in the fermentation process of garlic will cause many chemical reactions, such as enzymatic browning and Maillard reactions which cause the color to change from white and yellow to dark brown. During the heating process, compounds in garlic that are unstable and smell bad are converted to stable and odorless compounds. As a result, garlic fermented into black garlic which generally has a sweet and sour taste rather than flavor and spicy taste [17].

3.2. Weight loss and moisture content
Weight loss is a process of weight loss caused by the process of respiration and transpiration rates that produce several components including water, gas, and energy. Components resulting from the process of respiration will experience evaporation which can cause black garlic to shrink weight [23]. The weight loss and moisture content during fermentation process was shown in Figure 2 and Figure 3 respectively.
Based on Figure 2 and Figure 3, it shows that the sample’s weight in each fermentation method got increased in initial 5 days, and then decreased sharply. Black garlic covered using aluminium foil has the sharpest weight loss. Because the temperature inside the aluminum foil is higher than other fermentation chambers (Figure 1.), so moisture of black garlic comes out easily. Humidity that is too low will cause the amount of weight loss in black garlic [21]. However, the fermentation method variation did not affect black garlic weight loss significantly.

Reduction in weight of black garlic is related to reduced water content during the fermentation process. Water content is a very important characteristic of food because it can determine the freshness and durability of the material. The water content in black garlic decreases with increasing temperature in the fermentation process [20]. High temperatures will evaporate the water content in the material so that the texture of the ground black garlic becomes soft compared to the texture of fresh garlic [9]. Storing food at high temperatures causes physiological processes to increase resulting in respiration and
transpiration processes that cause the process of evaporation and carbon loss [22]. The longer heating causes the mass of water in the material to evaporate [9].

3.3. pH value

pH is a value that indicates the degree of acidity of an object, substance, or solution [23]. The effect of different fermentation methods on pH value of black garlic was shown in Table 1. Fresh garlic has pH value approximately 6.18 and black garlic has pH value around 3.6-3.7. It shown that pH value decreased during the fermentation process. Increased storage times can lead to organic acid decomposition and volatilization resulting in a decrease of the total acid content [24]. The fermentation method variation did not affect pH value of black garlic significantly, even it was influenced by fermentation time [9].

| Fermentation time (day) | pH       |
|-------------------------|----------|
|                         | Besek    | Kendil   | Aluminium foil |
| 0                       | 6.18 ± 0.17 | 6.18 ± 0.17 | 6.18 ± 0.17 |
| 5                       | 4.545 ± 0.01 | 4.51 ± 0.07  | 3.83 ± 0.01  |
| 10                      | 4.015 ± 0.02 | 3.94 ± 0.01  | 3.425 ± 0.02 |
| 15                      | 3.77 ± 0.01  | 3.88 ± 0.01  | 3.475 ± 0.02 |
| 20                      | 3.745 ± 0.01 | 3.785 ± 0.01 | 3.615 ± 0.01 |

The decrease in pH is due to the respiration process during the fermentation process. The respiration process cause the utilization of acids organic so it can reduce the total acid in black garlic. In the process of respiration will transform pyruvic acid and other organic acids aerobically to CO₂, H₂O, and energy [22]. A pH decrease during fermentation process can encourage the hydrolysis process which caused increase in glucose and fructose levels [15]. So, it can be said if the decrease in pH is closely related to the increase of the brix value.

The effect of a decrease in pH will affect the enzyme activity so that it can give a distinctive taste of black garlic [9]. The enzyme activity is influenced by the higher fermentation temperature. High temperatures will cause the transformation of allin compounds into allicin as an inactivation of the allinase enzyme due to a descrease in water content. The allicin compound is very instrumental in the formation of flavor and sour taste in black garlic to be not as pungent as fresh garlic [16].

3.4. Brix (%)

The effect of different fermentation methods on %brix value of black garlic was shown in Table 2. Natural medium affected the %brix value of black garlic significantly. Thus, the sweet taste of black garlic may be influenced by increased sugar levels.

| Fermentation time (day) | %brix       |
|-------------------------|-------------|
|                         | Besek       | Kendil    | Aluminium foil |
| 0                       | 39.23 ± 1,17<sup>a</sup> | 39.23 ± 1,17<sup>a</sup> | 39.23 ± 1,17<sup>b</sup> |
| 5                       | 37.4 ± 1.6<sup>ab</sup>  | 36.65 ± 1.54<sup>a</sup> | 36.26 ± 2.61<sup>b</sup> |
| 10                      | 38.45 ± 3.79<sup>ab</sup> | 42.925 ± 3.03<sup>a</sup> | 32.1 ± 1.75<sup>b</sup> |
| 15                      | 41.975 ± 3.08<sup>ab</sup> | 43 ± 4.57<sup>a</sup> | 40.825 ± 5.98<sup>b</sup> |

Based on Table 2 shows that fresh garlic has %brix value approximately 39.23, and increased during fermentation processing. Fresh garlic with fermented media chamber (besek) and clay chamber (kendil)
experienced a decrease in total dissolved solids on the fourth day then increased until the end of storage. While fresh garlic on aluminium coating fermentation media decreased the total dissolved solids until the seventh day of storage, then increased at the end storage. The value of brix of garlic at the end of storage on the fermented media of chamber (besek), clay chamber (kendil), and aluminium coating was 41.975; 43; and 40,825.

The content of reducing sugars in the process of making black garlic is influenced by two factors, namely the hydrolysis reaction of polysaccharides into reducing sugars and the occurrence of the Maillard reaction. The existence of the hydrolysis reaction of polysaccharides into reducing sugars will cause reducing sugars to increase, whereas the Maillard reaction will cause reducing sugars to decrease [10]. Based on the results of table 2 shows the decrease in sugar levels at the beginning of the fermentation time is due to the faster Maillard reaction compared to the hydrolysis reaction of polysaccharides. Whereas at the end of the fermentation time, a polysaccharide hydrolysis reaction occurs which causes an increase in the total dissolved solids.

The increase in total dissolved solids is influenced by an increased in carbohydrates due to the enzymatic heating process so that it is able to convert carbohydrates into sugars [10]. The longer the heating, the sugars as a component of carbohydrates is more soluble so that the total dissolved solids increase. Carbohydrates contained in black garlic can improve the sweet taste due to sucrose.

4. Conclusion
Black garlic was processed using natural material like bamboo chamber (besek) and clay chamber (kendil) for fermentation medium have shown lower weight loss and higher total soluble solid (%brix) than modern method using aluminium foil. Natural material affected total soluble solid (%brix) of black garlic significantly (p < 0.05). TSS (%brix) increased during fermentation process whereas pH decreased from pH 6.1 to 3.6.

References
[1] I S Surono 2016 Ethnic fermented foods and beverages of Indonesia. In J. P. Tamang Ethnic fermented foods and alcoholic beverages of Asia Delhi: Springer p. 341-382.
[2] N Şanlier, B B Gökcen and A C Sezgin 2019 Health benefits of fermented foods,” Crit. Rev. Food Sci. Nutr., vol. 59, no. 3, pp. 506–527.
[3] R D Setyawardhani 2008 Pengaruh Jenis Kemasan Dan Volume Ketan terhadap fermentasi serta perubahan mutu tape ketan hitam selama penyimpanan (Effect of packaging and volume on fermentation and quality change of tape black ketan during storage) Thesis, Institut Pertanian Bogor. [In Indonesian]
[4] R Retno 2013 Pengaruh pembungkus yang berbeda terhadap kadar etanol dan organoleptik tape uwi (Dioscorea alata L) (Effect of different packaging on ethanol and organoleptic levels of uwi tape) Thesis, Thesis, Universitas Muhammadiyah Surakarta. [In Indonesian]
[5] I Adi Wagesu, N Semadi Antara, and G Ganda Putra 2017 Pengaruh ph awal media dan lama fermentasi terhadap produksi kalsium sitrat dari limbah brem dengan menggunakan Aspergillus niger ATCC 16404 (Effect of initial ph media and fermentation time on the production of calcium citrate from brem waste by using Aspergillus niger ATCC 16404) J. Rekayasa Dan Manaj. Agroindustri, vol. 4, no. 4, pp. 70–79. [In Indonesian] [6] R De Cássia Mirela Resende Nassur, E. V. De Barros Vilas Boas, and F. V. Resende, “Black garlic: Transformation effects, characterization and consumer purchase intention,” Comun. Sci., vol. 8, no. 3, pp. 444–451, 2017.
[6] R De Cássia Mirela Resende Nassur, E V De Barros Vilas Boas, and F V Resende 2017 Black garlic: Transformation effects, characterization and consumer purchase intention Comun. Sci., vol. 8, no. 3, pp. 444–451.
[7] E. B. M. Daliri et al. 2019 Effects of different processing methods on the antioxidant and immune stimulating abilities of garlic Food Sci. Nutr., vol. 7, no. 4, pp. 1222–1229.
[8] F Xiong, C H Dai, F R Hou, P P Zhu, R H He, and H Le Ma 2018 Study on the ageing method
and antioxidant activity of black garlic residues *Czech J. Food Sci.*, vol. **36**, no. 1, pp. 88–97.

[9] R. Zhafira 2018 Effect of Aging Time on Physical, Chemical, and Antioxidant Activity of Single Clove Black Garlic Product *J. Pangan dan Agroindustri*, vol. **6**, no. 1, pp. 34–42.

[10] Nelwida and B Berliana 2019 Kandungan Nutrisi Black garlic Hasil Pemanasan dengan Waktu Berbeda (Black Garlic Nutrition Content Heating with Different Time) *Jurnal Ilmiah Ilmu-IlmuPeternakan* vol. **22**, no. 1, pp. 53–64. [In Indonesian]

[11] N P N Ilham 2018 Pengaruh suhu fermentasi pada beberapa varietas bawang putih terhadap mutu black garlic (The effect of fermentation temperature on some white variety on variety of black garlic quality) Thesis, Universitas Mataram. [In Indonesian]

[12] N Winona 2018 Pengaruh Lama Fermentasi Terhadap Beberapa Komponen Mutu Solo Black Garlic dari Bawang Putih (Allium Sativum, L) Varietas Lumbu Hijau (The Effect of Fermentation Time on Several Quality Components of Solo Black Garlic from Garlic (Allium Sativum, L) lumbu hijau Varieties) Thesis, Univ. Mataram. [In Indonesian]

[13] Gia Buu Tran, Tan Viet Pham and Ngoc Nam Trinh 2019 Black Garlic and Its Therapeutic Benefits *IntechOpen*, 85042.

[14] R Ahamed, Z Islam, M M Rashid, and M Meftahul 2014 Modeling and performance analysis of electric rice cooker 3rd International Conference on Mathematical Applications in Engineering 2014 (ICMAE’14), 23rd-25th Sept. 2014.

[15] O J Kang 2016 Physicochemical characteristics of black garlic after different thermal processing steps *Prev. Nutr. Food Sci.* vol. **21** no. 4.

[16] Imam, Padu Nawazul, Nazaruuddin, and Wiharyani Werdinginship. 2018. Pengaruh Suhu Fermentasi Pada Beberapa Varietas Bawang Putih Terhadap Mutu Black Garlic. *Artikel Ilmiah*. Universitas Mataram. Mataram. [In Indonesian]

[17] Corzo-Martines, M., Corzo, N., dan Villamiel, M. 2007. Biological Properties of Onions and Garlic. *Trends Food Sci Technol.* **18**(12): 609-625.

[18] Rasadi, Yuke. 2015. Karakteristik Fisik dan Kimia Biji Kakao (Theobroma cacao L.) Hasil Fermentasi Variasi Wadah Kotak Kayu, Krat Plastik dan Daun Pisang di Pusat Penelitian Kopi dan Kakao Indonesia. *Skripsi*. Universitas Jember. Jember. [In Indonesian]

[19] Delfita, Rina dan Aidhya Irhash Putra. 2015. Pembuatan Bawang Putih Tanpa Aroma (Allium sativum L.) Menggunakan Fermentasi dengan Jamur Tempe dan Uji Aktivitas Antioksidannya. *Jurnal Seminar Nasional Pendidikan dan Sains Biologi*. ISBN:978-602-74224-0-7. [In Indonesian]

[20] Zhang, X., Li, N., Lu, Liu., Pengli and Qiao, X. 2016. Effect of Temperature On The Quality of Black Garlic. *Journal Science Food Agricultural*, **96**: 2366-2372.

[21] Purnomo, Edi, Sri Widodo Agung Suedy, dan Sri Haryanti. 2017. Pengaruh Cara dan Waktu Penyimpanan terhadap Susut Bobot, Kadar Glukosa dan Kadar Karotenoid Umbi Kentang Konsumsi (Solanum tuberosum L. Var Granola). *Jurnal Anatomi dan Fisiologi*. 2 (2): 2257-6751. [In Indonesian]

[22] Muchtadi, T.R dan Sugiyono. 1992. Ilmu Pengetahuan Bahan Pangan. Departemen Pendidikan dan Kebudayaan. Direktorat Jenderal Pendidikan Tinggi. Pusat Antar Universitas. Institut Pertanian Bogor. Bogor. [In Indonesian]

[23] Wills, RBH. 1981. *An Introduction To The Physiology And Handling Of fruits And Vegetables*. Australia: NSW Ltd.

[24] Ding, Yanfang, Yongli Jiang, Yun Deng, Yanyun Zhao. 2020. Effect of Packaging Materials and Storage Temperature on Water Status, Mechanical and Thermal Properties of Black Garlic. *Journal Food Packaging and self Life*. 24: 10057.