Black Garlic Powder as Antioxidant and Antimicrobial Agents in Emulsion Sausage

Nachayut Chanshotikul* and Bung-Orn Hemung

School of Applied Sciences, Faculty of Interdisciplinary Studies, Khon Kaen University, Nong Khai, Thailand
Email: nachayut@kku.ac.th (N.C.); bunghe@kku.ac.th (B.O.H.)
*Corresponding author

Manuscript received May 7, 2024; revised June 4, 2024; accepted July 11, 2024; published December 24, 2024

***Abstract*—Thai garlic has high nutraceuticals, but pungent smell may limit the perception and consumption. Therefore, it was used to produce black garlic by humid-fermentation in order to reduce unacceptable characteristic as well as increasing bioactive compounds. The obtained black garlic was used to extract for the bioactive compounds using water as the solvent. This extracted was encapsulated with maltodextrin using spray drying technique to obtain the Black Garlic Extract Powder (BGEP). The ratio of extract to maltodextrin was controlled at 1:10, while that of the inlet temperature was 160 °C. The BGEP exhibited the DPPH radical scavenging ability of 20.21%, while the microbial inhibition by clear zone was found for 7.66 mm. BGEP at 0, 0.5 and 1.0% was used to incorporated in pork sausage recipe prior evaluating the quality. The results showed that BGEP affected slightly the chemical composition. Changes in color of samples was noticed, particularly the lightness. However, it could not affect the sensory attributes based on the overall acceptability. Moreover, the remaining antioxidant activity in sausage was still observed and this value was increased with increasing BGEP content. The total microbial count was inhibited upon addition of BGEP although significantly different was not observed between 0.5 and 1.0%. These results suggested that BGEP could be a functional ingredient to improve antioxidant activity and extend the shelf-life of pork sausage.**

***Keywords*—Thai black garlic, black garlic powder, antioxidant, antimicrobial, sausage**

I. Introduction

Garlic (Allium sativum) is a plant that has been cultivated for over 6,000 years. It is a popular seasoning for a variety of dishes, especially those made with meat. Garlic helps to reduce the strong, distinctive smell of meat and develop a more desirable flavor during cooking. Garlic has many health benefits, including promoting healthy and strong skin, boosting the immune system, supporting tissue growth in the body, preventing cancer [1]. However, some people may not like to eat garlic because of its strong, pungent smell. Additionally, raw garlic can irritate the stomach and digestive system for some people. To make garlic more palatable, innovations have been developed to reduce its pungent smell. It has also been reported that fresh garlic contains high levels of γ-glutamylcysteines and this compound is responsible for the pungent smell of fresh garlic. Although garlic powder is available in capsules, some consumers are still resistant to this form of consumption, as it is associated with taking medicine. Research into other innovations to reduce the pungency of garlic is ongoing.

Black garlic is a fermented form of garlic that has been developed by several researchers. The process involves fermenting garlic at a controlled temperature and humidity for a period of at least one month [2]. This process reduces the pungent smell and spicy taste of raw garlic and turns the garlic's white flesh black. The fermentation process causes chemical changes in the garlic, particularly the Maillard reaction, which produces a variety of Amadori compounds [3]. These changes lead to changes in the levels of sugar and some amino acids in black garlic.

Analysis of black garlic has shown that it contains increased levels of allicin, an organosulfur compound that has been shown to have several health benefits [4]. These benefits include reducing cholesterol, lowering blood pressure, fighting allergies, antibacterial, improving blood sugar control, and reducing the risk of cancer [5]. The bioactivities were evaluated in either peeled and with clove form [6]. However, the direct consumption of black garlic may still have limitations in terms of acceptance [7]. Therefore, the development of black garlic in a form with full efficacy of bioactive compounds by extraction would be challenged. Extraction of black garlic has been studied using various solvents, including water [8]. In one study, water was used at a ratio of 1:10 (black garlic: water) at 80 °C for 1 h, followed by centrifugation to separate the extract. Another study used 80% ethanol as the solvent [9]. In some cases, the extraction process may use ethanol with a concentration ranging from 70–90 %. The stability of bioactive compounds in the black garlic extract has been documented [10]. Moreover, transformation the black garlic extract into powder that has the potential to be a food additive, may increase the channels for consuming black garlic and create added value for garlic through food technology innovation. However, producing black garlic powder after extract has rarely been documented and should be investigated.

Therefore, the aim of this study was to produce black garlic from Thai variety and extract the bioactive compounds prior spray drying into powder. In addition, the remaining activity after incorporation into sausage was evaluated for the feasibility in meat industries.

II. Materials and Methods

A. Materials

The garlic used in the experiment was purchased from Makro Department Store, Muang District, Nong Khai, Thailand. The garlic bulbs were peeled and separated into cloves for further processing into black garlic. The peeled garlic was then fermented in a humidity-controlled pot at a temperature of 68–72 °C for 26–28 days. This method was modified from the previous report [2]. The garlic was then peeled for further chemical composition analysis.

The skin and visible fat were trimmed out from chicken breast. Thereafter, the flesh one was ground before dividing into a portion of 200 g. These samples were packed in vacuum condition prior to store at −20 °C until used.

B. Proximate Analysis of Black Garlic

Proximate analysis of black garlic was done according to the AOAC (2000) [11]. Moisture content was based on oven drying at 105 ºC for over 12 h. Petroleam extract from soxlet extraction was used as crude fat. Total nitrogen from Kjeldahl method was used to calculate crude protein using conversion factor of 6.25. Total fiber was based on acid digestion. Mean and average were based on 3 replications.

C. Black Garlic Extraction and Encapsulation

The black garlic powder (5 g was mixed with De-Ionized (DI) water, (100 mL) in a Duran bottle. Thereafter, the bottle was extracted by a microwave heating (SAMSUNG, model ge107y, Malaysia) at 180 W for 90 min. The extracted was then centrifuged at 6,000×g for 20 min prior to filtration through a filter paper (Whattman® number 4). The filtrate was mixed with maltodextrin solution at a ratio of 1:10 and controlled the solid content in the mixture to be 30%. Then, the mixture was homogenized at 5,000 rpm for 40 sec for being ready for spray drying (B-290, Buchi, Switzerland). The inlet temperature was controlled at 160 °C. Drying condition was set the air flow rate at 439 L/h, the pressure to bev0.23 bar, and the flow rate to 357 L/h. The powder obtained is stored in an opaque plastic bag in a desiccator until use. This powder is called Black Garlic Extract Powder (BGEP).

D. Characterization of BGEP

1) DPPH radical scavenging ability. Sample (1 g) was dissolved in hexane (10 mL) before analyzing for the DPPH radical scavenging ability. Sample solution (0.5 mL) was mixed with ethyl acetate (1.5 mL) and DPPH solution (1.5 mL) before incubating in the dark condition for 30 min. Then, the sample was read for absorbance at 515 nm (A sample). The control was prepared by taking off samples from the reaction cocktail and used as A control. The DPPH radical scavenging ability was calculated according to the equation 1:

$$\text{DPPH radical scavenging ability (\%)} = \left(\frac{A\ control - A\ sample}{A\ control}\right) x100 \quad (1)$$

2) Antimicrobial activity. Antimicrobial activity was tested using disc diffusion against the spoilage microorganisms isolated from spoilage sausage. The microbial load on the agar plate was controlled over log 5 CFU/g. Powder was dissolved in DI water by controlling the concentration at 10%. Thereafter, the filter paper disc was dipped in solution prior to put into the spread plate and being incubated at 37 °C for 48 h. The clear zone around disc was measured to report the clear zone.

E. Preparation of Pork Sausages

The model of pork sausage was carried out according to previous study [12] with slight modification as the formulation similar in Table 1. Effect of BGEP at 0.5 and 1.0% was evaluated in comparison with control (without BGEP). The minced pork was chopped with a quarter amount of ice for 30 s using hand-type food mixer (Bowl Rest™ mixer, Hamilton Beach, N.C., U.S.A). Salt, sodium nitrite, and ice were added before chopping for 1 min. Minced back fat and BGEP was finally added and continuing chopping for 1 min to obtain the batter with temperature below 16 °C. The batter (40 g) was loaded into centrifuge tubes (50 mL) before removing trapped air by centrifugation (200×g for 30 s). All tubes were heated at 80 °C for 30 min before cooling in ice-water for 15 min. All cooked sausages were kept in a cold room over night before determining the characteristics.

Table 1. The pork sausage recipe supplemented with BGEP

Ingredients	BGEP levels (%)		
	0	0.5	1.0
Pork mince (g)	300	300	300
Pork back fat (g)	100	100	100
Ice (g)	95	95	95
NaCl (g)	7.5	7.5	7.5
Sodium nitrite (g)	0.0015	0.0015	0.0015
Sodium polyphosphate (g)	2.5	2.5	2.5
BGEP (g)	0	2.5	5.0
Total (g)	505.00	507.50	510.00

F. Analysis of Sausage

1) Proximate analysis

Proximate analysis of sausage was performed according to the AOAC (2000) according to section B.

2) Antioxidant by DPPH activity.

DPPH activity of sausage samples were performed according to the details appeared in section D.

3) Color

Color values of sausage were measured using the Hunter color meter (Color reader, CR-10, Minolta, Japan). Color values were reported as Hunter L^*, a^*, and b^* for lightness, redness, and yellowness, respectively.

4) Microbial analysis.

Total plate count of sausage sample storage at 4 °C for a week was used to determine aerobic microorganisms in sausage sample using spread plate technique. The sausage sample was homogenized aseptically and taken (10 g) to mix with sterilized water (90 mL). Serial dilution was made before taking sample for 0.1 mL to spread on plate count agar. All plates were incubated at 37 °C for 24 h. The colony appeared on plate was recorded as colony forming unit/ g of sample (CFU/g).

5) Sensory analysis

Acceptance of the 3 sausage formulas was evaluated using a hedonic test with a score of 1–9. More than 15 trained panelists (aged 18–45 years) were involved in the test. The samples were grilled in an oven at 220 °C for 15 min. They were then sliced into 1 cm thickness and served to the judges. Each judge was asked to evaluate the acceptance of each formula in terms of color, aroma, flavor, texture, and overall preference.

G. Statistical analysis

A significant difference at confidential level ($P < 0.05$) was considered for mean values, which was based on the ANOVA analysis using the PASW statistics 16 (SPSS Inc., Chicago IL, USA).

III. Results and Discussion

A. Proximate Analysis of Black Garlic

From the experiment of analyzing the chemical composition of black garlic, it was found that the chemical composition of black garlic after drying had the highest crude protein content (Crude protein) at 20.01±0.19% (Table 2). The next highest component was moisture, while the ash and crude fiber contents were similar. The component that was almost not found in this study was crude fat, which was found to be very low. The black garlic that had been fermented at a temperature of 40–90 °C for 11 days had a moisture content of 58.48% [13], which is higher than the value reported in this study. This is because the black garlic in this experiment was dried to remove moisture, which resulted in a lower value than previous studies. Additionally, the high moisture content of the sample also resulted in a low percentage of protein and fat in the sample in the reference document, which was found to have 0.98% and 0.56% fat and protein, respectively.

Table 2. The chemical composition of black garlic

Compositions	Amount (%)
Moisture	8.01±0.20
Crude fat	0.11±0.01
Crude protein	20.01±0.19
Total ash	5.37±0.43
Crude fiber	5.40±0.02

Mean ± SE was averaged from 3 replications.

B. Characteristic of BGEP

1) Physical appearance

Thai black garlic was extracted with water using the same method as for black garlic extraction. It was found that the extract had low viscosity and could be directly mixed with maltodextrin to form a viscous slurry that could be dried into a powder using spray drying. This process is considered to be encapsulation of the extract with maltodextrin, which allows the liquid extract to be converted into a powder for ease of use and storage [14]. However, the biological activity of the extract, including its antioxidant activity and ability to inhibit spoilage microorganisms in sausage, should be evaluated to determine the impact of encapsulation on maintaining such activity. The study found that the extract was successfully converted from liquid to powder using the encapsulation technique. The powder had a fine texture and was similar in color to flour, with a grayish-white color (Fig. 1).

Fig. 1. Physical appearance of BGEP.

2) Antioxidant and antimicrobial activities

Encapsulated black garlic extract powder obtained from this experiment, when dissolved in water at a concentration of 10%, had an antioxidant capacity of 20.21±0.67%. The antioxidant capacity detected was similar to the values obtained from previous research. The previous report stated that encapsulation of the extract still maintains its antioxidant activity [15]. In addition, the ability to inhibit spoilage microorganisms obtained from spoiled sausage was confirmed by the results of the experiment in Table 3, which showed that a clear zone was found around the disc in a wide area. This information confirms that water-extracted black garlic extract encapsulated with maltodextrin still has the ability to inhibit spoilage microorganisms from sausage. The results of this experiment also reflect that the encapsulated extract powder has the potential to extend the shelf life of sausage.

Table 3. antioxidant and antimicrobial activities of BGEP

Activity	Value
DPPH radical scavenging activity (%)	20.21±0.67
Antimicrobial activity as clear zone (mm)	7.66±0.20

Mean ± SE was averaged from 3 replications.

C. Characteristic of Sausage Supplemented with BGEP

1) Proximate analysis

The chemical composition of sausage formulated with BGEP showed that the moisture content decreased when the BGEP was 1.0% (Table 4). This may be due to the presence of maltodextrin in the sample, which is consistent with the increasing fiber content. The amount of fat extracted from the sausage sample decreased when the amount of extract was increased. This may be since the fat part is replaced by the non-fat part in the extract. It was found that the formula with the most extract had the least amount of fat. When considering the amount of protein in emulsion sausage, it was found that the amount did not differ significantly statistically. The analysis results showed that the addition of encapsulated powder in the sausage production formula slightly reduced the amount of fat but did not affect other quantities.

Table 4. The chemical composition of sausage with BGEP

Compositions	BEGP levels (%)		
	0	0.5	1.0
Moisture	$60.80±0.53^a$	$60.02±0.57^a$	$59.08±0.36^b$
Crude fat	$19.24±0.69^a$	$18.97±0.80^b$	$17.05±0.27^c$
Crude protein	$16.61±0.76^a$	$16.09±0.32^a$	$16.81±0.60^a$
Crude fiber	$0.07±0.01^b$	$0.08±0.03^b$	$0.14±0.07^a$

Mean ± SE was averaged from 2 replications.

a-c Different letters within the same row indicate statistically different at p<0.05

2) Color value

The color of the sausage may not appear to be very different when evaluated by the naked eye. When it was measured with a colorimeter, the produced sausage was relatively lower value of whiteness, as the lightness value was less than 50. While yellow and red color were found in the color components, which is indicated by the finding that a and b values are positive. It was found that increasing the concentration of BGEP reduced the brightness of the product, while the values of yellowness and redness increased the most at the highest level of extract addition as well (Table 5). The results of this study show that the addition of BGEP powder may have some impact on the quality factor in terms of the color of the product. However, this must be evaluated by sensory evaluation to ensure that the level of color quality change is detected by consumers.

Table 5. The color value of sausage with BGEP

Color values	BEGP levels (%)		
	0	0.5	1.0
*L**	65.92±0.43^{a}	64.81±0.27^{b}	64.93±0.45^{b}
*a**	2.87±0.13^{b}	2.83±0.17^{b}	2.96±0.13^{a}
*b**	16.17±0.45^{c}	16.88±0.35^{b}	17.04±0.25^{a}

Mean ± SE was averaged from 5 replications.

$^{a-c}$ Different letters within the same row indicate statistically different at p<0.05.

3) DPPH activity and microbial count

The antioxidant activity of BGEP is still present and has been confirmed that the activity is not destroyed during sausage production. The antioxidant activity of sausages produced increases significantly with the increasing amount of BGEP (Table 6). This was because BGEP contains antioxidants, adding it to sausages gives sausages antioxidant activity. The results of this study may help to improve sausages as a healthy food product.

The total plate count also confirmed the ability of BGEP to inhibit spoilage microorganism in the sausage as seen in Table 5. However, increasing BGEP from 0.5 to 1.0% did not significantly different. Therefore, application of this power at only 0.5 could extend the shelf-life of sausage.

Table 6. The DPPH radical scavenging activity and microbial count of sausage with BGEP

Data	BEGP levels (%)		
	0	0.5	1.0
DPPH radical scavenging activity (%)	10.00±2.83^{c}	14.40±2.26^{b}	32.40±2.26^{a}
Total plate count (CFU/g)	5.8 ±0.4^{b}	4.3 ±0.5^{a}	3.9 ±0.2^{a}

Mean ± SE was averaged from 2 replications.

$^{a-c}$ Different letters within the same row indicate statistically different at p<0.05.

4) Sensory evaluation

The overall acceptance of the product was relatively low compared to other products on the market. This is evident from the average score of only 4–5 out of a total of 9. This was because all seasonings were taken off in order to evaluate the effect BGEP (Table 7). The judges compared the product to commercially available sausages and found that there were significant differences in taste. This was evidenced by a lower value of acceptability toward the taste of all sausage samples. However, the overall acceptability was not affected by the addition of BGEP. This suggested that BGEP still has the potential to be ingredient in sausage production.

Table 7. The sensory attributes of sausage with BGEP

Attributes	BEGP levels (%)		
	0	0.5	1.0
Appearance	5.00±1.84	5.30±1.70	5.30±2.10
Color	5.30±1.60	5.77±1.66	5.57±1.68
Texture	4.70±1.93	5.13±1.96	4.97±1.97
Taste	4.63±1.81	5.10±1.60	4.73±1.93
Overall acceptance	4.80±1.94	5.47±1.68	4.97±1.92

Mean ± SE was averaged from 2 replications.

^{ns} Not significantly different at p<0.05.

IV. Conclusion

Thai garlic could be used to produce black garlic that capable of bioactivities especially, antioxidant and antimicrobial activities. Extraction of those compounds could be possible using aqueous solvent. Spray drying converted the liquid extract into a stable powder form using maltodextrin as the encapsulating agent. This powder could be used as an additive in order to extend the shelf-life of sausage by retarding the lipid rancidity as well as microbial spoilage.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Nachayut Chanshotikul designed, conducted the experiments, and analyzed data regarding the black garlic powder characterization; Bung-Orn Hemung evaluated the qualities of sausage supplemented with black garlic powder, and organized the manuscript. All authors had approved the final version.

Acknowledgment

The authors wish to thank the Faculty of Interdisciplinary Studies for all facilities. Financial support from Khon Kaen University was also gratefully acknowledged. Special thanks go to Mr. Panuphong Sonkosa for the black garlic extraction.

References

[1] Y. M. Jung, S. H. Lee, D. S. Lee, M. J. You, I. K. Chung, W. H. Cheon, Y. S. Kwon, Y. J. Lee, and S. K. Ku, "Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects," *Nutrition Research*, vol. 31, pp. 387–396, May 2011.

[2] X. Zhang, N. Li, X. Lu, P. Liu, and X. Qiao, "Effects of temperature on the quality of black garlic," *Journal of the Science of Food and Agriculture*, vol. 96, pp. 2366–2372, May 2016.

[3] H. Yuan, L. Sun, M. Chen, and J. Wang, "The comparison of the contents of sugar, Amadori, and Heyns compounds in fresh and black garlic," *Journal of Food Science*, vol. 81, pp. 1662–1668, July 2016.

[4] U. Purev, M. J. Chung, and D.H. Oh, "Individual differences on immunostimulatory activity of raw and black garlic extract in human primary immune cells," *Immunopharmacology and Immunotoxicology*, vol. 34, pp. 651–660, Aug. 2012.

[5] E.-S. Jung, S.-H. Park, E.-K. Choi, B.-H. Ryu, B.-H. Park, D.-S. Kim, Y.-G. Kim, and S.-W. Chae, "Reduction of blood lipid parameters by a 12-wk supplementation of aged black garlic: A randomized controlled trial," *Nutrition*, vol. 30, 1034–1039, Sep. 2014.

[6] M. A. Toledano-Medina, J. Perez-Aparicio, R. Moreno-Rojas, and T. Merinas-Amo, "Evolution of some physicochemical and antioxidant properties of black garlic whole bulbs and peeled cloves," *Food Chemistry*, vol. 199, pp. 135–139, May 2016.

[7] S. Kimura, Y.-C. Tung, M.-H. Pan, N.-W. Su, Y.-J. Lai, and K.-C. Cheng, "Black garlic: A critical review of its application, bioactivity, and application," *Journal of Food and Drug Analysis*, vol. 25, pp. 62–70, Jan. 2017.

[8] Y.-M. Lee, O.-C. Gweon, Y.-J. Seo, J. Im, M.-J. Kang, M.-J. Kim, and J.-I. Kim, "Antioxidant effect of garlic and aged black garlic in animal model of type 2 diabetes mellitus," *Nutrition Research and Practice*, vol. 3, pp. 156–161, Jun. 2009.

[9] E. Sato, M. Kohno, H. Hamano, and Y. Niwano, "Increased antioxidative potency of garlic by spontaneous short-term fermentation," *Plant Foods for Human Nutrition*, vol. 61, pp. 157–160, Dec. 2006.

[10] S. H. Kim, E. Y. Jung, D. H. Kang, U. J. Chang, Y. H. Hong, and H. J. Suh, "Physical stability, antioxidative properties, and photoprotective effects of a functionalized formulation containing black garlic extract," *Journal of Photochemistry and Photobiology B: Biology*, vol. 117, pp. 104–110, Dec. 2012.

[11] AOAC, *Official Methods of Analysis*, 17th ed. AOAC International. 2000.

[12] N. Chanshotikul and B. Hemung, "Encapsulation of gac powder extract and its application in low-nitrite chicken sausage,"

International Journal of Food Engineering, vol. 5, no. 2, pp. 146–151, June 2019.

[13] J.-H. Shin, D.-J. Choi, S.-J. Lee, and J.-Y. Cha, "Changes of physicochemical components and antioxidant activity of garlic during its processing," *Journal of Life Science*, vol. 18, pp. 1123–1131, Aug. 2008.

[14] A. Y. Cinar, S. Altuntas, H. Demircan, A. N. Dundar, G. Taner, and R. A. Oral, "Encapsulation black garlic: Commparison with black garlic extract in terms of physicochemical properties, biological activities, production efficiency and storage stability," *Food Biosciences*, vol. 50, Part A, Dec. 2022.

[15] X. Lu, N, Li, X. Qiao, Z. Qiu, and P. Liu, "Composition analysis and antioxidant properties of black garlic extract," *Journal of Food and Drug Analysis*, vol. 25, no. 2, pp. 340–349, Apr. 2017.