

# THE EFFECT OF HUMIDITY AND TEMPERATURE ON TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF BLACK GARLIC PRODUCED FROM PEELED CLOVES OF GARLICS

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**Abstract.** Recently, using peeled cloves of garlcs for produce black garlic has been investigated, but the effect of humidity and temperature on antioxidant activity and polyphenol content of black garlic produced from peeled cloves of garlic have not been studied yet. In this study, peeled cloves of garlcs were fermented in a variety of temperatures (70oC, 75oC, 80oC, and 85oC), humidity (70%, 80%, and 90%), and aging time (6 days, 15 days, and 20 days), then total polyphenol contents and antioxidant activities of samples were analyzed. The data showed that humidity did not affect polyphenol contents and antioxidant capacities of final products but affected antioxidants formation rate instead. Furthermore, temperature has noticeable impact on polyphenol content and antioxidant capacity. At lower temperatures (70oC and 75oC), polyphenol contents and antioxidant activities were gradually increased during the fermentation. On the contrary, polyphenol contents and antioxidant activities of black garlcs incubated at 80oC and 85oC rapidly increased at early stage, then they were stable or decrease during later stage. The optimal conditions (75oC, 90% relative humidity, and 15 days) to produce black garlic with high polyphenol concentration and antioxidant capacity is determined ( $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> DW and  $82.21 \pm 0.88\%$ , respectively).

**Keywords.** Polyphenol, antioxidant activity, black garlic, temperature, food processing, relative humidity, garlic cultivar.

## 1 INTRODUCTION

Black garlic is a functional food produced from raw garlic (*Allium sativum* L.) via fermentation under high temperature and relative humidity. Fermentation of black garlic is a set of non-enzymatic browning reactions, such as Maillard reaction, caramelization and oxidation of phenolic compounds. Non-enzymatic browning reactions produce a typical dark brown color and antioxidant compounds. Thermal treatment process in company with high relative humidity leads to activation of protease, amylase... These enzymes hydrolyze protein into amino acids and convert starch into monosaccharide, respectively. Reducing sugar and amino acid react together to produce melanoidins, which give black garlic its color and flavors. Moreover, fermentation causes the formation of polyphenols during processing. Kim et al. (2013) reported that black garlic possessed higher total polyphenol content (5-8 times) and antioxidant activity as compared with raw garlic [1]. Polyphenols are important compounds contributing to bioactivity of black garlic, thus total polyphenol content is considered as the key parameter to evaluate quality of black garlic. During fermentation, allicin converts to tetrahydro-β-carboline derivatives and S-allyl-cysteine, alkaloids, and flavonoid-like compounds, which serving as important bioactive compounds in black garlic [2]. In consequence, black garlic possesses a typical black color with sweet-sour flavor omitting unpleasant smell and appears as well-known supplementary food. Furthermore, black garlic is widely distributed in a variety of types, such as capsule, extract, and juice.

Fermentation also enhances the nutrition components and bioactivity of black garlic. Anti-cancer, antioxidant, anti-obesity, anti-inflammatory, hypolipidemic, and hypocholesterolemic effects are well documented in previous report [3]. Jeong et al. (2013) demonstrated that black garlic extract could ameliorate Aβ-induced neurotoxicity and cognitive impairment [4]. Recently, hepatoprotective effect of black garlic was reported [5]. In previous study, immune system's protective effect of black garlic fermented from Ly Son garlic, a Vietnamese garlic cultivar, also was revealed. Ho and Vu (2014) suggested that black garlic protected immune cell population of spleen, lymph nodes, thymus gland, and bone marrow from irradiation [7]. It is an interesting point that antioxidant activity of black garlic is several folds higher

than raw garlic. Superoxide dismutase like activity and hydrogen peroxide radicals scavenging of black garlic in vitro are 10-fold higher than raw garlic [8]. Lee et al. (2009) determined that black garlic decreased hepatic TBARS level, a product of peroxidation of lipid, more effectively than raw garlic in diabetic mice [9].

Mature time, quality, and bioactivity of black garlic are primarily depended on processing treatment [10]. Fermentation rate of black garlic mostly correlates with thermal treatment; in other word, higher temperature treatment leads to shorter mature time. Zhang et al. (2015) proved that mature time of black garlic incubated at 60oC was 69 days whereas mature time of black garlic incubated at 70oC decreased down to 30 days [11]. Moreover, thermal treatment also affected concentration of reducing sugar and 5-hydroxymethylfurfural (HMF), a strong antioxidant compound. Reducing sugar content of black garlic fermented in 60oC was 2-fold higher than black garlic fermented in 90oC, which affected the sweet taste of product. On the other hand, HMF content of black garlic fermented in 90oC was over 2-fold higher than black garlic fermented in 60oC [11]. Furthermore, Nguyen et al. (2017) demonstrated that different thermal treatment led to a variety of total polyphenol content and antioxidant of black garlic produced from Phan Rang raw garlic, a well-known Vietnamese garlic cultivar [12]. Among three different thermal treatments, total polyphenol content and antioxidant activity of black garlic fermented in 60oC with relative humidity 70% at day 30 was highest as compared with 70oC and 80oC, but the mature time of black garlic fermented in 60oC was longest.

In industrial processing, whole bulbs of raw garlic are used as material for fermentation; therefore, products remain husks after fermentation. It will take a while for consumers to remove black garlic husks, which is nonedible by-product and decrease product aesthetic attribute. Moreover, black garlic need to be peeled in manufacturing processes of other types of foods, such as juice, capsule, extract, canned vegetable. Due to their soft texture, peeling process might lead to deformation of cloves of black garlics. Therefore, production of black garlic fermented from peeled cloves of raw garlic has been research to facilitate consumers and prevent from deformation of cloves. However, the effect of processing parameters such as temperature and relative humidity on total polyphenol content and antioxidant activity of black garlic produced from peeled cloves of garlic have not been studied yet. In this study, we investigated the effect of different relative humidity and thermal treatment on total polyphenol content and antioxidant activity of black garlic produced from peeled cloves of Ly Son garlic, a well-known Vietnamese garlic cultivar.

## 2 MATERIALS & METHODS

### 2.1 Materials

Garlic (*Allium sativum*) was cultivated at An Hai commune, Ly Son Island, Quang Ngai province in March 2017. The raw materials were selected and removed deformed bulbs, washed cut from stems and root, after which whole bulbs were separated into single cloves. Then cloves of garlics with same size (approximately 7.0 × 4.0 × 2.0 mm) were selected and peeled to remove garlic husks. All reagents used in this study were in analytical grade

### 2.2 Black garlic preparation

To determine the effect of relative humidity on quality of black garlic, peeled cloves was fermented in aging chamber (Shellab, USA) at 70°C in 90%, 80%, and 70% relative humidity for 20 days according to Choi et al. [2]. Samples were collected for 0, 1, 5, 10, 15, and 20 days, after which samples were used for analysis of total polyphenol content and antioxidant activity.

Due to the effect of thermal treatment on maturation time, black garlics were manufactured at different times depending on different temperature in same relative humidity (90% RH). When the thermal treatment was at 70°C, black garlics were incubated for 20 days; and at 75°C, black garlic were fermented for 15 days. On the other hand, black garlic were manufactured at 80°C and 85°C for 6 days. Samples were collected once per day and analyzed for total polyphenol content and antioxidant activity.

### 2.3 Determination of total polyphenol content

Total polyphenol content of black garlic was determined spectrophotometrically according to the method described by Kim et al. with some modifications [1]. Briefly, samples (3g) were mashed in 10 ml

70% methanol at 70°C for 10 min. The extraction step was repeated three times. All extracts were pooled and filtrated by Whatman grade 1 filter paper (Sigma, USA). Filtrate was adjusted to 100 ml by adding distilled water. Then, 1 ml of sample extract was mixed with 1 ml Folin-Ciocalteu reagent for 5 min, following by adding 1 ml 20% Na<sub>2</sub>CO<sub>3</sub> (w/v) for 5 min. The mixture was adjusted to 10 ml by adding distilled water. The mixture was incubated at room temperature in dark room for 90 min, then the absorbance at 750 nm was measured. Results were expressed as gram gallic acid equivalents per kilogram dry weight of garlic sample (g GAE·kg<sup>-1</sup> DW).

#### 2.4 Determination of antioxidant activity

The antioxidant activity of black garlic was measured via the 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay using the method of Bae et al. with some modifications [13]. Briefly, the 0.2 mmol·L<sup>-1</sup> DPPH solution was prepared in ethanol. Then 1 ml of DPPH solution were mixed with 1 ml of garlic sample with different concentrations. The mixture was stand at room temperature in dark room for 30 minutes, after which the absorbance of sample was measured at 517 nm using a spectrophotometer. Control was the DPPH solution without black garlic extract, the DPPH radical scavenging activity was calculated as: DPPH radical scavenging activity (%) =  $\frac{A_1 - A_0}{A_1} \times 100\%$  (1); in which, A<sub>0</sub> is absorbance of control at 517 nm, and A<sub>1</sub> is absorbance of sample at 517 nm.

#### 2.5 Statistical analysis

The data were presented as mean ± standard deviation, all experiments were triplicated. Statistical analysis was performed using Statgraphics Centurion XVI software (Statpoint Technologies Inc., Warrenton, Virginia, USA). Differences between means of different groups were analyzed two-way ANOVA followed by multiple range tests, the criterion of statistical significance was set as  $p < 0.05$ .

### 3 RESULTS AND DISCUSSION

#### 3.1 Effect of relative humidity on total polyphenol content of black garlic

Polyphenol is a class of organic compounds which is characterized by one or multiple phenolic rings in chemical structure. Polyphenols exert not only strong antioxidant activity but also account for many health effects of black garlic. Therefore, total polyphenol content is the important parameter to evaluate quality of black garlic. Regardless of relative humidity, total polyphenol contents of black garlcs were continuously increased during the fermentation period. It was observed that the amount of polyphenol of black garlic increased 2-fold after incubation in 90% relative humidity for 5 days (1.75 ± 0.15 versus 0.74 ± 0.08 g GAE·kg<sup>-1</sup> DW). Furthermore, total polyphenol content of black garlic manufactured in 90% relative humidity at 10<sup>th</sup> day was higher than black garlic collected at 5<sup>th</sup> day (5.40 ± 0.29 and 1.75 ± 0.15 g GAE·kg<sup>-1</sup> DW, respectively). The similar results were shown in black garlcs produced in 70% and 80% relative humidity for same day. At 20<sup>th</sup> day, total polyphenol content of black garlic fermented in 90% relative humidity (8.17 ± 0.26 g GAE·kg<sup>-1</sup> DW) was over 11-fold higher than fresh raw garlic (0.74 ± 0.08 g GAE·kg<sup>-1</sup> DW). These results were consistent with data from previous studies [1, 2]. In that study, Choi et al. proved that total polyphenol content of black garlic fermented at 70°C in 90% relative humidity for 21 days was 4-fold higher than the raw garlic [2]. Furthermore, Kim et al. suggested that total polyphenol content of black garlic was increased 4.6-7.8 fold after stepwise thermal treatment [1]. Of note, the peeled cloves of garlcs were used to manufacture black garlic in this study whereas the whole bulbs of garlic were used in previous studies instead. In previous study, Toledano-Medina et al. demonstrated that total polyphenol content of black garlcs produced from peeled cloves was higher than those produced from whole bulbs [14]. On the other hand, it was observed that there was no significant difference of total polyphenol contents among three sub-sets of relative humidity at each time point. At 10<sup>th</sup> day, the amount of polyphenol of black garlic fermented in 70% relative humidity (5.21 ± 0.39 g GAE·kg<sup>-1</sup> DW) was identical with those fermented in 80% and 90% relative humidity (5.58 ± 0.25 and 5.40 ± 0.29 g GAE·kg<sup>-1</sup> DW, respectively). Moreover, total polyphenol contents of black garlcs produced in 70%, 80%, and 90% relative humidity at the end of fermentation were similar (8.10 ± 0.23, 8.19 ± 0.34, and 8.17 ± 0.26 g GAE·kg<sup>-1</sup> DW, accordingly). The data indicated that relative humidity could not affect the total polyphenol content in final products and/or polyphenol formation rate.

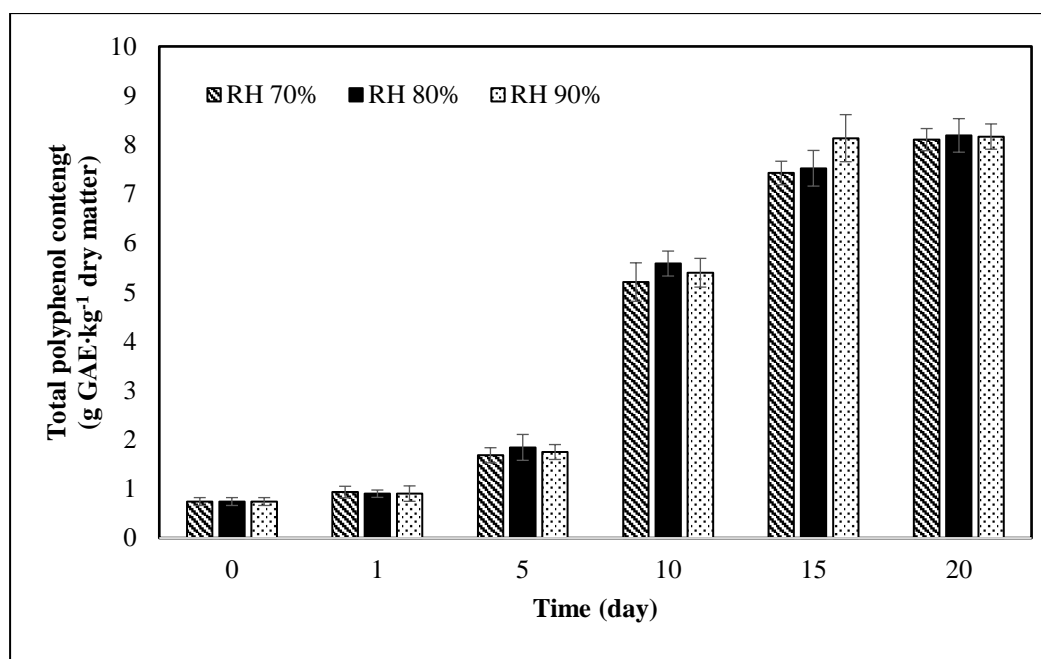


Figure 1. The effect of relative humidity (70%, 80%, and 90%) on total polyphenol content of black garlic produced from peeled cloves. Briefly, total polyphenol content of black garlics produced from peeled cloves was gradually increased during the fermentation. Regardless of relative humidity, total polyphenol content of black garlics was continuously increased during the fermentation period. After 20 days, total polyphenol content of black garlic manufactured in 90% relative humidity ( $8.17 \pm 0.26$  g GAE·kg<sup>-1</sup> DW) was similar with those produced in 70% and 80% relative humidity ( $8.10 \pm 0.23$  and  $8.19 \pm 0.34$  g GAE·kg<sup>-1</sup> DW, respectively). The data suggested that relative humidity could not affect the total polyphenol content in final product.

### 3.2 Effect of relative humidity on antioxidant activity of black garlic

The increase of total polyphenol content of black garlic implied the elevation of antioxidant activity of black garlic during fermentation. To evaluate the antioxidant activity of black garlics, DPPH radical scavenging assay was performed. As shown in Figure 2, antioxidant activity of black garlic was gradually increased during the fermentation up to the 20<sup>th</sup> day. The DPPH radical scavenging activity of black garlic ( $75.50 \pm 1.06\%$ ) fermented at 70°C in 90% for 20 days was 30-fold higher than raw garlic ( $2.37 \pm 0.15\%$ ), which was identical with the data from previous study [12, 15]. Choi et al. demonstrated that antioxidant activity of black garlic fermented at same conditions increased over 16-fold after 21 days, as comparing with raw garlic. Of note, Nguyen et al. clarified that the antioxidant activity of Phan Rang black garlic, the other Vietnamese garlic cultivar, was reached the maximal value after 30 days of fermentation at 70°C in 70% relative humidity, and this value higher 19-fold than raw garlic [12]. The difference of increase of antioxidant activity could be explained that the black garlic produced peeled cloves possessed higher antioxidant than. On the other hand, there was not difference among antioxidant activities of peeled black garlics fermented in 70%, 80%, and 90% relative humidity after 20 days ( $72.50 \pm 1.21$ ,  $72.82 \pm 0.59$ , and  $75.50 \pm 1.06\%$ , respectively). From these data, we suggested that relative humidity did not affect the antioxidant activity of final products from peeled cloves of garlic. It was noted that relative humidity did not affect antioxidant activity of final products but accelerated reaction rate instead. It is well-known that relative humidity could affect moisture content of garlic which in turn enhances reactant solubility and mobility. Therefore, an increase relative humidity in certain level could accelerate reaction rate [15]. At 5<sup>th</sup> day, black garlic manufactured in 90% relative humidity showed the highest antioxidant activity among three sub-sets of relative humidity. Moreover, antioxidant activity of black garlic fermented in 90% relative humidity ( $70.03 \pm 0.60\%$ ) was higher than those in 70% and 80% relative humidity ( $32.63 \pm 0.29\%$  versus  $54.57 \pm 0.32\%$ , accordingly) at 10<sup>th</sup> day. Of note, antioxidant activity of black garlic produced in 90% relative humidity for 15 days ( $74.94 \pm 0.68\%$ ) was identical with black garlic incubated for 20 days ( $75.50 \pm 1.06\%$ ). Nevertheless, antioxidant activity of black garlic produced in 70% relative humidity for 15 days

was lower than with black garlic incubated for 20 days ( $55.40 \pm 0.36$  versus  $72.50 \pm 1.21\%$ , respectively). These results indicated that high relative humidity could increase the formation rate of antioxidant compounds in black garlic.

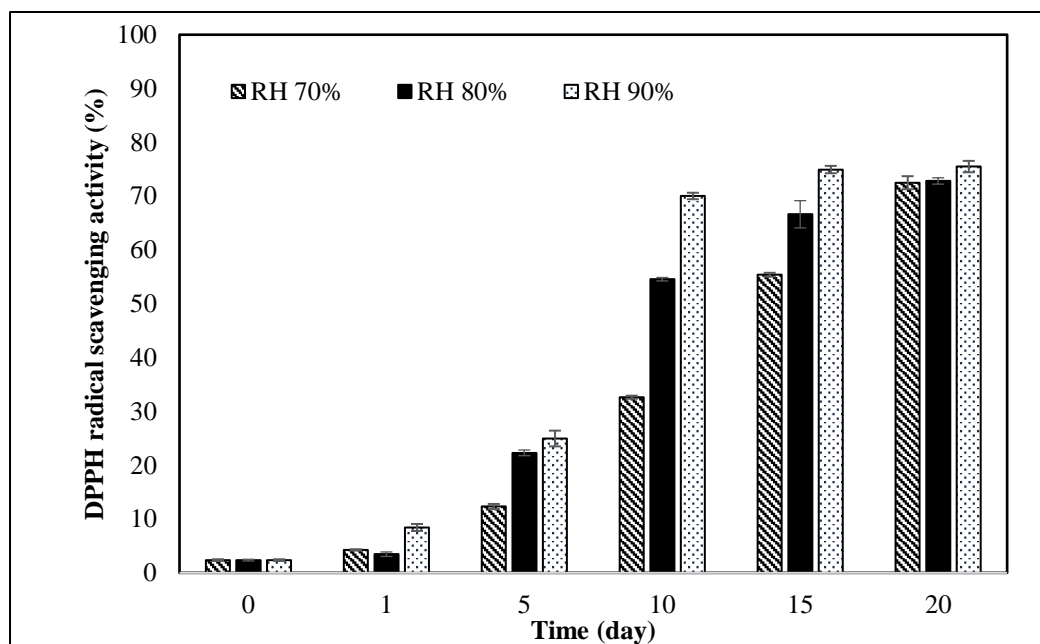


Figure 2. Effect of relative humidity (70%, 80%, and 90%) on antioxidant activity of black garlic. Briefly, antioxidant activity of black garlic produced from peeled cloves was gradually increased during the fermentation. Relative humidity did not affect maximal value of the antioxidant activity of black garlic. There was no significant difference of antioxidant activity of black garlicks among three sub-sets of humidity treatment at 20<sup>th</sup> day. However, relative humidity could increase the formation rate of antioxidant compounds. At the 10<sup>th</sup> day, antioxidant activity of black garlic manufactured in 90% relative humidity ( $70.03 \pm 0.60\%$ ) was higher than those in 70% and 80% relative humidity ( $32.63 \pm 0.29$  and  $54.57 \pm 0.32\%$ , respectively). Of note, antioxidant activity of black garlic produced in 90% relative humidity for 15 days was not different with those for 20 days whereas antioxidant activity of black garlic produced in 70% relative humidity for 15 days was less than those for 20 days. The results indicated that relative humidity could affect formation rate of antioxidant compounds.

### 3.3 Effect of thermal treatment total polyphenol content of black garlic

Changes in total polyphenol content of black garlic produced from peeled cloves in different thermal treatment were shown in Figure 3. Regardless of thermal treatment, total polyphenol content of black garlicks was significantly increased after fermentation ( $p < 0.05$ ). As the temperature was set at 85°C, the amount of polyphenols in black garlic increased dramatically after one day, after which it was steadily increased. Of note, total polyphenol content of black garlic fermented at 85°C after one day was higher than black garlic fermented at 70°C ( $1.60 \pm 0.27$  versus  $0.90 \pm 0.16$  g GAE·kg<sup>-1</sup> DW, respectively). However, there was no significant difference in total polyphenol content of black garlic fermented at 85°C and 70°C after 4 days ( $1.67 \pm 0.29$  and  $1.41 \pm 0.15$  g GAE·kg<sup>-1</sup> DW, respectively). These data implied that reaction rate of black garlic at 85°C was higher than reaction rate at 70°C, especially in early time point. Of note, there was no significant difference of total polyphenol content of samples collected from day 1<sup>st</sup> to day 6<sup>th</sup> at 85°C. The same pattern was observed as comparing between black garlic fermented at 80°C and 70°C in same period. These data were comparable with the results from previous study, in which Zhang et al. indicated that total polyphenol contents of black garlicks incubated at 80°C and 90°C increased in early stage and then they decreased at later stage of fermentation [11]. These data suggested that the higher temperature (80°C and 85°C) did not only increase formation rate of polyphenols but also accelerate consumption rate of polyphenols. Therefore, these data demonstrated that the accumulation of polyphenols at high thermal treatment was hindered during fermentation.

On the contrary, at lower temperatures (70°C and 75°C), total polyphenol content of black garlics continuously increased during the fermentation, which implied that the accumulation rate of polyphenols surpassed the consumption rate of polyphenols at these temperatures throughout fermentation. Total polyphenol content of black garlic fermented at 70°C increased 11-fold after 15 days as comparing with raw garlic ( $8.13 \pm 0.48$  and  $0.74 \pm 0.08$  g GAE·kg<sup>-1</sup> DW, accordingly), then total polyphenol content was unchanged during later stage ( $8.17 \pm 0.26$  g GAE·kg<sup>-1</sup> DW). The same pattern was observed in black garlic fermented at 75°C. The amount of polyphenol of black garlic increased 11-fold after 12 days ( $7.98 \pm 0.36$  g GAE·kg<sup>-1</sup> DW), after which it was steady during later stage ( $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> DW). It could be explained that the formation rate of polyphenols was faster as higher temperature, thus the increase of temperature at certain level resulted in the elevation of polyphenols in black garlic. Among four thermal treatments, the maximum value of total polyphenol content of black garlics produced at 70°C was higher than those produced at 80°C and 85°C. Moreover, the maximum value of total polyphenol content of black garlic manufactured at 70°C was identical with black garlic manufactured at 75°C ( $8.21 \pm 0.36$  and  $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> DW, respectively), which suggested that these processing conditions (70°C and 75°C) were superior to obtain total polyphenol content.

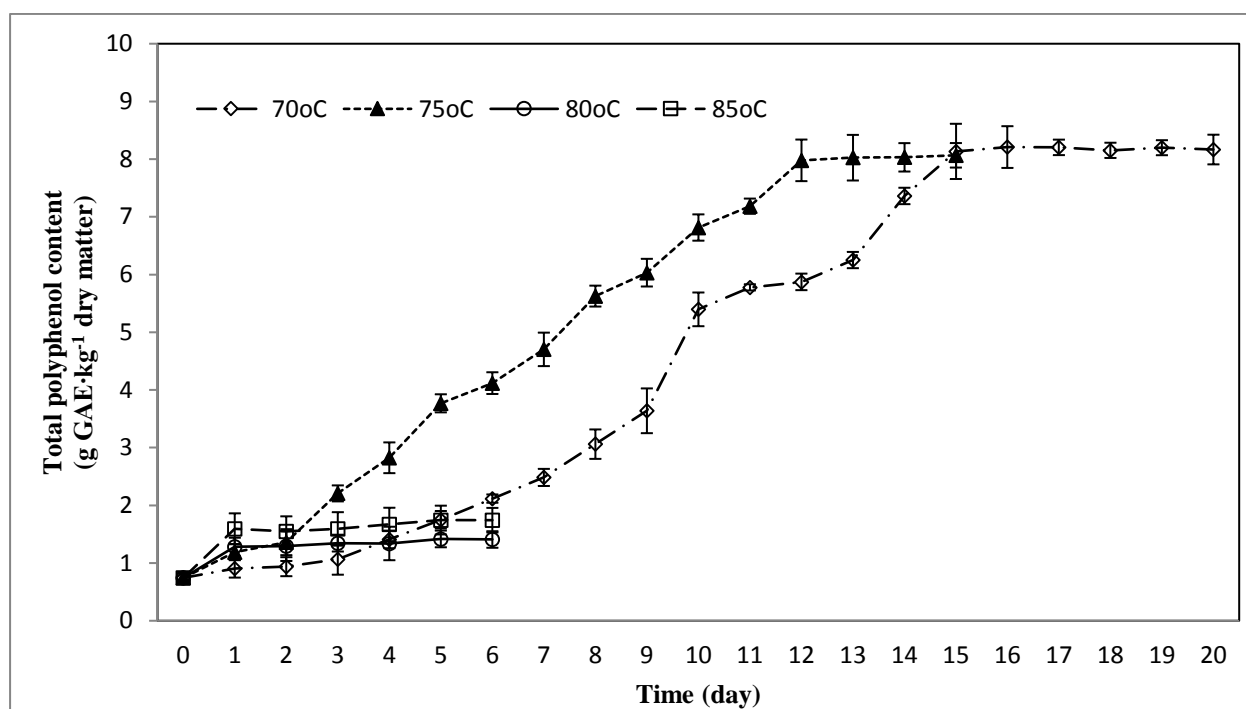


Figure 3. Effect of thermal treatment on total polyphenol content of black garlic. Total polyphenol content of black garlic produced at 70°C and 75°C was gradually increased during the fermentation. Of note, total polyphenol content of black garlic fermented at 85°C increased two-fold after one day as comparing with raw garlic ( $1.60 \pm 0.27$  versus  $0.74 \pm 0.08$  g GAE·kg<sup>-1</sup> DW, respectively), then it was stable during later stage ( $1.74 \pm 0.21$  g GAE·kg<sup>-1</sup> DW). The same pattern was observed in those produced at 80°C. These data suggested that the higher temperature (80°C and 85°C) did not only increase formation rate of polyphenols but also accelerate consumption rate of polyphenols.

Therefore, the accumulation of polyphenols at high thermal treatment was hindered during fermentation. Among these temperature, garlics incubated at 70°C and 75°C showed higher total polyphenol content ( $8.21 \pm 0.36$  and  $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> DW, respectively) than those incubated at 80°C and 85°C ( $1.42 \pm 0.14$  and  $1.75 \pm 0.25$  g GAE·kg<sup>-1</sup> DW, respectively), which suggested that these temperatures (70°C and 75°C) were superior to obtain total polyphenol content.

### 3.4 Effect of thermal treatment on antioxidant activity of black garlic

The effect of thermal treatment on antioxidant capacity of black garlic was investigated by DPPH radical scavenging assay. The antioxidant activity of black garlic from four subsets of thermal treatment

was shown in Figure 4. When the temperatures were set at 80°C, the antioxidant activities reached maximal values after 1 day ( $85.55 \pm 0.85\%$ ), and then antioxidant activity was continuously reduced down to  $45.63 \pm 0.74\%$  at the sixth day. The same trend was observed in black garlic fermented at 85°C. It was shown that antioxidant activity of black garlic incubated at 85°C was notably increased ( $87.46 \pm 1.01\%$ ) after 2 days, after which it continuously decreased during the later period ( $68.40 \pm 3.29\%$ ). On the contrary, antioxidant activities of black garlics manufactured at 70°C and 75°C showed gradually accumulation during fermentation. At the 15<sup>th</sup> day of fermentation, it was observed that black garlic manufactured at 75°C also processed higher antioxidant capacity than black garlic manufactured at 70°C ( $82.21 \pm 0.88\%$  versus  $74.94 \pm 0.68\%$ , respectively). These results were consistent with other previous reports. In previous study, Bae et al. demonstrated that DPPH scavenging activities and reducing capacities of black garlics produced at higher temperature were higher than those produced at lower temperature [13]. While Woo et al. investigated the effect of garlic aromatic compounds on electron donor ability to DPPH radicals at a variety of temperatures (100 - 120°C), they also observed the electron donor ability to DPPH radicals, which implied the DPPH scavenging activity, highly correlated with temperature [16]. These data suggested that the high thermal treatment could increase of antioxidant capacity of black garlic.

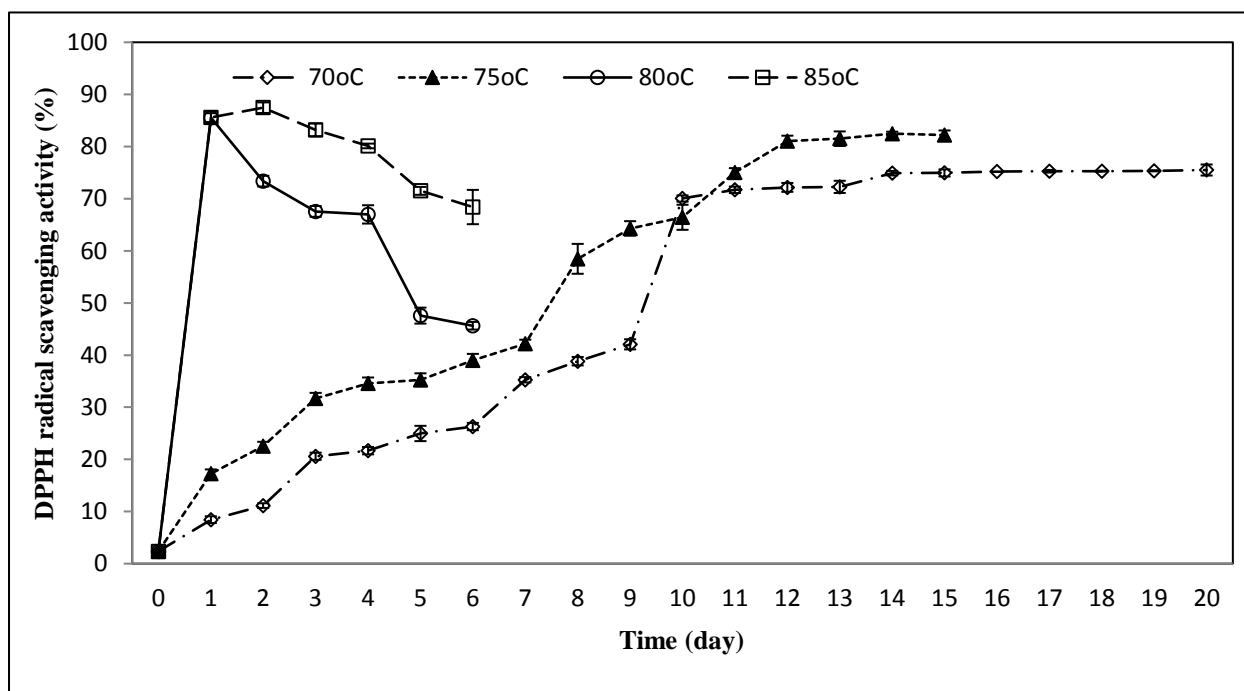


Figure 4: Effect of thermal treatment on antioxidant activity of black garlic. When temperatures were set at 80°C and 80°C, the antioxidant activities promptly increased in early stage of fermentation, and then antioxidant activity was continuously decreased during later stage. On the contrary, antioxidant activity of black garlics manufactured at 70°C and 75°C gradually accumulated during fermentation. Among all thermal treatments, black garlic produced at 80°C and 85°C possessed the highest antioxidant capacities than those produced at 75°C and 70°C. Furthermore, antioxidant activity of black garlic fermented at 75°C was higher than those fermented at 70°C. Of note, black garlic incubated at high temperatures (80°C and 85°C) could easily acquire bitter flavor and bone-dry texture. On the other hand, the polyphenol concentration of black garlics produced at 75°C was the highest value. These data suggested the optimal temperature for obtaining high polyphenol concentration and antioxidant capacity was 75°C in 90% relative humidity for 15 days ( $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> DW and  $82.21 \pm 0.88\%$ , respectively).

Among all thermal treatments, black garlic produced at 80°C and 85°C possessed the highest antioxidant capacities ( $85.55 \pm 0.85\%$  and  $87.46 \pm 1.01\%$ , respectively) than those produced at 75°C and 70°C. Of note, the time to obtain the highest antioxidant activities of garlic fermented at 80°C and 85°C so short that black garlics produced in this temperature were immature [11]. Although high temperatures (80°C

and 85°C) could increase the antioxidant capacities of black garlic, Zhang et al. observed that black garlic incubated in these temperatures could easily acquire bitter flavor, bone-dry texture, and burning smell [11]. Furthermore, antioxidant activity of black garlic fermented at 75°C was higher than black garlic fermented at 70°C ( $82.48 \pm 0.36\%$  and  $75.50 \pm 1.06\%$ , respectively). On the other hand, the polyphenol concentration of black garlics produced at 75°C was the highest value. These data suggested the optimal temperature for obtaining high polyphenols and antioxidant capacity was 75°C for 15 days ( $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> DW and  $82.21 \pm 0.88\%$ , respectively).

#### 4 CONCLUSIONS

When black garlic was produced in different relative humidity and different temperatures, a variety of antioxidant activity and total polyphenol content during fermentation was observed. Briefly, relative humidity did not affect antioxidant capacity of the final product but increased the antioxidant compounds formation rate instead. Of note, thermal treatment had significant impact on antioxidant activity and total polyphenol content of black garlic. Among thermal treatments, lower temperatures such as 70°C and 75°C were superior to obtain total polyphenol content whereas antioxidant activity of black garlic fermented at 75°C was higher than those fermented at 70°C. In conclusion, the incubation at 75°C in 90% relative humidity during 15 days is optimal to acquire the high polyphenol amount and antioxidant capacity of black garlic manufactured from peeled cloves of Ly Son garlic ( $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> DW and  $82.21 \pm 0.88\%$ , respectively). However, quality of black garlic is very complex with many attributes that need to be concerned besides DPPH radical scavenging capacity and total polyphenol content, such as color, flavor, S-allyl-cysteine content, reducing sugar content, allicin content, total acid content. More research works should be performed to clarify the changes of other physicochemical and antioxidant properties of black garlic during fermentation in these conditions.

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#### REFERENCES

- [1] J. S. Kim, O. J. Kang, and O. C. Gweon, Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps, *Journal of Functional Foods*, vol. 5, no. 1, pp. 80-86, 2013.
- [2] I. S. Choi, H. S. Cha, and Y. S. Lee, Physicochemical and antioxidant properties of black garlic, *Molecules (Basel, Switzerland)*, vol. 19, no. 10, pp. 16811-16823, 2014.
- [3] 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 production, bioactivity, and application, *Journal of Food and Drug Analysis*, vol. 25, no. 1, pp. 62-70, 2017.
- [4] J. H. Jeong, H. R. Jeong, Y. N. Jo, H. J. Kim, J. H. Shin, and H. J. Heo, Ameliorating Effects of Aged Garlic Extracts against A $\beta$ -Induced Neurotoxicity and Cognitive Impairment, *BMC Complementary and Alternative Medicine*, vol. 13, no. 1, pp. 268, 2013
- [5] J. H. Shin, C. W. Lee, S. J. Oh, J. Yun, M. R. Kang, S. B. Han, H. Park, J. C. Jung, Y. H. Chung, and J. S. Kang, Hepatoprotective Effect of Aged Black Garlic Extract in Rodent, *Toxicological Research*, vol. 30, no. 1, pp. 49-54, 2014.
- [6] S. Kodai, S. Takemura, S. Kubo, H. Azuma, and Y. Minamiyama, Therapeutic administration of an ingredient of aged-garlic extracts, S-allyl cysteine resolves liver fibrosis established by carbon tetrachloride in rats, *Journal of Clinical Biochemistry and Nutrition*, vol. 56, no. 3, pp. 179-185, 2015
- [7] A. S. Ho and B. D. Vu, Evaluating the immune cells protective effect of Lyson black garlic extract in mice exposed to radiation, *Journal of Military Pharmaco-medicine*, vol. 9, pp. 31-38, 2014.



- [8] E. Sato, M. Kohno, H. Hamano, and Y. Niwano, Increased anti-oxidative potency of garlic by spontaneous short-term fermentation, *Plant Foods Human Nutrition*, vol. 61, no. 4, pp. 157-160, 2006.
- [9] 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, no. 2, pp. 156-161, 2009.
- [10] Y.S. Queiroz, E. Y. Ishimoto, D. H. M. Bastos, G. R. Sampaio, and E. A. F. S. Torres, Garlic (*Allium sativum* L.) and ready-to-eat garlic products: *In vitro* antioxidant activity, *Food Chemistry*, vol. 115, no. 1, pp. 371-374, 2009.
- [11] 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, no.7, pp. 2366-2372, 2015.
- [12] T. N. Nguyen, N. H. Phan, T. T. T. Le, and D. T. Phan, Effect of thermal treatment on the quality of black garlic produced from Phan Rang's natural fresh material, *Vietnam Journal of Science and Technology*, vol. 55, no. 1, pp. 29-34, 2017.
- [13] S. E. Bae, S.Y. Cho, Y. D. Won, S. H. Lee, and H. J. Park, Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment, *LWT - Food Science and Technology*, vol. 55, no. 1, pp. 397-402, 2014.
- [14] M. A. Toledano-Medina, J. Pérez-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, 2016.
- [15] C. W. Won, H. B. Wijayanti, and B. R. Bhandari, Maillard Reaction in Limited Moisture and Low Water Activity Environment, in *Water Stress in Biological, Chemical, Pharmaceutical and Food Systems*, G. F. Gutiérrez-López, L. Alamilla-Beltrán, M.P. Buera, J. Welti-Chanes, E. Parada-Arias, and G.V. Barbosa-Cánovas, Eds. Springer, 2015.
- [16] K. S. Woo, H. S. Yoon, Y. R. Lee, J. S. Lee, D.J. Kim, J. T. Hong, and H. S. Jeong, Characteristics and antioxidative activity of volatile compounds in heated garlic (*Allium sativum*), *Food Science and Biotechnology*, vol. 16, pp. 822-827, 2007.

## ẢNH HƯỞNG CỦA ĐỘ ẨM VÀ NHIỆT ĐỘ LÊN HÀM LƯỢNG POLYPHENOL TỔNG SỐ VÀ HOẠT TÍNH KHÁNG OXY HÓA CỦA TỎI ĐEN SẢN XUẤT TỪ TỎI TƯƠI ĐÃ BÓC VỎ

**Tóm tắt.** Những năm gần đây, việc sản xuất tỏi đen từ tép tỏi đã bóc vỏ đang được quan tâm nghiên cứu; tuy nhiên, tác động của độ ẩm và nhiệt độ lên hoạt tính kháng oxy hóa và hàm lượng polyphenol của tỏi đen sản xuất từ tép tỏi đã bóc vỏ vẫn chưa được khảo sát. Trong nghiên cứu này, tỏi đen được lên men trong các nhiệt độ (70oC, 75oC, 80oC và 85oC) ở các điều kiện ẩm (70%, 80% và 90%) và thời gian khác nhau (6 ngày, 15 ngày và 20 ngày), sau đó chúng tôi tiến hành xác định hàm lượng polyphenol tổng số và hoạt tính kháng oxy hóa của sản phẩm. Kết quả cho thấy độ ẩm không ảnh hưởng đến hàm lượng polyphenol tổng số và khả năng kháng oxy hóa của sản phẩm cuối nhưng tác động đến vận tốc hình thành các hợp chất kháng oxy hóa. Thêm vào đó, nhiệt độ tác động rõ rệt lên hoạt tính kháng oxy hóa và hàm lượng polyphenol. Ở nhiệt độ thấp (70oC và 75oC), hàm lượng polyphenol và hoạt tính kháng oxy hóa tăng dần trong quá trình lên men. Ngược lại, hàm lượng polyphenol và hoạt tính kháng oxy hóa của tỏi đen lên men ở nhiệt độ 80oC và 85oC tăng lên nhanh chóng trong giai đoạn đầu, sau đó ổn định hoặc giảm ở giai đoạn sau. Điều kiện lên men ở nhiệt độ 75oC với 90% độ ẩm sau 15 ngày tạo ra tỏi đen với hàm lượng polyphenol và hoạt tính oxy hóa cao nhất ( $8.07 \pm 0.21$  g GAE·kg<sup>-1</sup> chất khô và  $82.21 \pm 0.88\%$ ).

**Từ khóa.** Polyphenol, hoạt tính kháng oxy hóa, tỏi đen, nhiệt độ, chế biến thực phẩm, độ ẩm tương đối, giống tỏi.

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