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THE EFFECT OF THERMAL TREATMENT ON ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF BLACK SHALLOT (*ALLIUM ASCALONICUM*)

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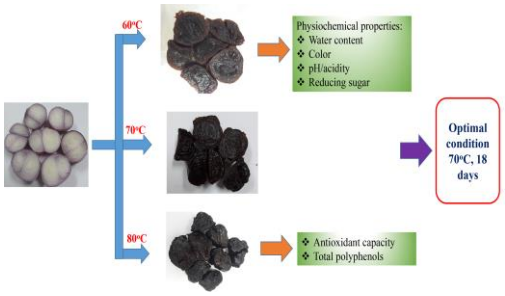
Graphical abstract



Abstract

Black shallot is a newly developed food product from shallot (*Allium ascalonicum*), which is characterized by dark brown color, sweet taste and jelly texture. However, the effect of thermal treatment on physicochemical properties, bioactive compounds, antioxidant activity has not been assessed yet. In this study, we determined the changes of some physicochemical properties (reducing sugar, pH and acidity), polyphenols, and its antioxidant activity (via DPPH radical scavenging assay) during aging process at three different temperatures (60, 70, 80°C) with sampling intervals 3 days for a 21-day period. All thermal treatment conditions gradually reduced water content and pH versus increased the acidity, reducing sugar, total polyphenol content, and antioxidant activity. The optimal conditions to produce the antioxidant and polyphenols - enriched black shallot were 70°C for 18 days (63.95 ± 1.24 % and 52.86 ± 2.02 mg of GAE/g DW, respectively). These findings not only gives the basis for the optimization of processing for improvement food quality with desired attributes but also suggests black shallot as the promising source to extract bioactive compounds for application in pharmaceutical and food industry.

Keywords: Antioxidant activity, black shallot, physicochemical properties, polyphenols, thermal treatment

1.0 INTRODUCTION

Shallot is one of the most well-known spices in Asian culinary and a good source of protein, fiber, minerals, and vitamins including vitamin A, B, and C. It also possess a variety of phenolic and flavonoid compounds including gallic acid, apigenin, eriodictyol, quercetin, iso-quercerin, rutin, kaempferol, catechin and tannic acid. The presence of these bioactive compounds in shallot gives an explanation

of its health benefits such as anti-cancer, anti-viral, anti-bacterial, anti-obesity, anti-microbial, anti-inflammatory, anti-oxidant, hepatoprotective, nephroprotective activities [1]. In addition, shallot also is one of the most traded commodity over the world which accompanied by onions account for 3.87 billion US dollars in 2020 [2]. In Vietnam, shallot is one crucial crops with some specialized farming areas including Ninh Thuan (Ninh Hai Town), Soc Trang (Vinh Chau Town), and Quang Ngai (Ly Son island) Provinces and contributes to local economy (with onions) about 3.26 million US dollars [2]. Recently, overproduction of local product and competition with low-price shallot imported from other countries have led to reduction of profitable returns to farmers as well as less competency and sustainability of local shallot farming. According to Saptana *et al*. (2021), the main points to improve the competiveness of shallot are its quality, differentiation from rival products, consumer acceptance, and sustainability [3]. Therefore, diversification of processing product from shallot is a potential approach to create adding value to shallot and augment competiveness of local horticultural product.

Fresh shallot has mild pungent flavor and is tender in texture with high moisture which limit its long-term storage and uses. Traditionally, some processing products have been developed in Vietnam market such as dried shallot, ground shallot powder, crispy fried shallot, pickled shallot, shallot jam. Recently, some modern solutions have been developed, namely drum drying shallot powder, shallot juice, shallot extract [4, 5]. In 2010, Wongmekiat *et al*. have developed and reported the nephroprotective effect of shallot extract, which could be used as an adjunct remedy for cyclosporine treatment [4]. Moreover, Setyadjit and Sukasih also have produced the fiber and antioxidant -enriched shallot powder using drum dryer with addition of cassava starch 10% and maltodextrin 5% [5]. Among them, the utilization of aging and thermal processing to transform fresh garlic with unpleasant and sharp flavour to the dark brown, gummy texture, sweet taste, and without the offensive flavour product, named as black shallot or black purple onion, is the promising and innovative approach.

Black shallot is processing product obtained via aging process of shallot in high temperature and regulated relative humidity. The ageing process of shallot, similar with other aged products of *Allium* species, initiated numerous complex chemical reactions, including enzymatic browning reactions, for example, oxidation of phenols, and non-enzymatic browning reactions, the Maillard reaction and caramelization [6, 7]. Moreover, thermal treatment results in and accelerates the Maillard reaction, a non-enzymatic browning process initiated by condensation of reducing sugar with amino groups of amino acids, to generate dark brown, high molecular weight compounds, melanoidins, which in turn convert fresh shallot to a dark brown product, black shallot [8]. Aging process improved not only sensory attributes, such as sweet taste and omitting of pungent flavor but also some health beneficial effect of black shallot, namely antioxidant, anti-inflammatory, and anti-cancer effects. Some bioactive compounds of black shallot such as quercetin, branched chain amino acids, isoleucine, leucine, valine, and organosulfur compounds, including isoalliin, also are elevated as compared to fresh garlic [9]. Recently, some researchers have developed some novel processing food product from black shallot, such as ultrasonic assisted extract of black shallot [10]. In spite of rising demand of black shallot and the growing number of research on application of black shallot, manufacture of black shallot is still limit in pilot-level due to variation of product quality among batches. In this study, we investigated the effect thermal treatment, one of the most impact factor on product quality during aging, on physicochemical properties and antioxidant activity of black shallot during fermentation and determined the optimal condition to produce black shallot with desired attributes.

2.0 METHODOLOGY

2.1 Chemicals and Reagents

3,5-Dinitrosalicylic acid (DNS), 2,2-Diphenyl1-picrylhydrazyl (DPPH), gallic acid, and quercetin were supplied by Sigma-Aldrich Company (USA). Other chemicals and reagents using in the study, unless otherwise noted, were at analytical grades and also provided by Sigma-Aldrich Company (USA).

2.2 Production of Black Shallot

Shallots (*Allium ascalonicum*) were collected from Vinh Chau Town, Soc Trang Province, Vietnam in March 2018. Black shallots were prepared using Tran *et al*. protocol [11]. Briefly, shallots were selected by some criteria (without decaying or pest, even purple color and diameter) and peeled to remove the outer skin, followed by incubation of shallot in aging chamber (Shellab, USA) with three different temperatures (60, 70, 80°C) and relative humidity 70% for a 21-day period. The samples were collected with 3-days interval to determine physicochemical characteristics (color, water content, acidity, pH, and reducing sugar content), total polyphenol content, and antioxidant activity.

2.3 Determination of Physicochemical Attributes of Black Shallot

Water content of black shallot during aging process was measured using Vietnam's Sector Standard 10TCN 842:2006 for determination of water content and dry matter in fruit and vegetable products [12].

The pH values of samples were measured using a pre-calibrated pH meter (HI 8424, Hanna, USA) in compliance to Vietnam's National Standard TCVN 7806:2007 (ISO 1842:1991) [13]. Acidity was determined via titration methods with 0.1 M NaOH solution in compliance to Vietnam National Standard TCVN

5483:2007 (ISO 750:1998) from the Directorate for Standards, Metrology and Quality of Vietnam, the results were expressed as milligram per kilogram dry weight (mg/kg DW) [14].

Color values of the sample was estimated using a CR-410 Chroma Meter (Konica Minolta, Nederland). The instrument was calibrated with a white tile and color was presented as CIE L*a*b* system, in which L* implies the brightness (from black to white), a* indicates the redness (from green to red), b* shows the yellowness (from blue to yellow). The total color difference (ΔE) was calculated using following formula:

$$\Delta E = \sqrt{(L(t) - L_0)^2 + (a(t) - a_0)^2 + (b(t) - b_0)^2}$$

in which L_0, a_0, b_0, are color values of control (fresh garlic), and $L_{(t)}$, $a_{(t)}$, $b_{(t)}$, are color values of samples (black shallot at chosen time point) [15].

Reducing sugar content was estimated via DNS methods using the standard curve of glucose as reference material. The absorbance of sample was determined at 540 nm, and the data were presented as milligram per kilogram dry weight (mg/kg DW) [16].

2.4 Determination of Antioxidant Activity

Antioxidant activity was estimated via determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity of samples using Choi *et al*. procedure with some modifications [17]. Samples (2 g) were homogenized in 15 ml of hydro-ethanolic solution (water: ethanol with ratio 1:3 v/v) using mortar and pestle. The mixture was vortexed for 2 min, followed by extraction with assistance of ultrasound [10]. The extract was obtained via centrifugation at 4°C. The procedure was repeated twice. A volume of extract (0.2 mL) was mixed with 0.5 mL of 0.2 mM DPPH solution, the mixture was added up to 3 mL using distilled water. The mixture was incubated in dark for 30 min and the absorbance of mixture was recorded at 517 nm. DPPH radical scavenging activity was calculated as the following formula: DPPH radical scavenging activity (%) = *Ao-A1/Ao* ×100%; in which, A_0 is the absorbance of control at 517 nm, and A_1 is the absorbance of the sample at 517 nm.

2.5 Determination of Total Polyphenol Content

Total polyphenol content was measured using the method described by Yamuangmorn *et al*. with some modifications [18]. Samples (5 g) were homogenized in 25 mL of 70% methanol using mortar and pestle. The homogenate was extracted with assistance of ultrasound, then the extract was obtained via centrifugation, and the procedure was repeated twice, then the volume of extract was adjusted into 100 mL. A volume of sample (2.4 mL) were mixed with 0.15 mL Folin-Ciocalteu reagent, followed by adding of 0.45 mL of 1M Na_2CO_3 [17]. The reaction was performed in dark room for 30 min, the absorbance of mixture was measured at 750 nm. Total polyphenol contents of samples were plotted from standard curve using gallic acid as reference material, the results were expressed as gallic acid equivalent mg/g dry weight (GAE mg/g DW).

2.6 Statistical Analysis

All analyses were performed in triplicate and data were expressed in form of mean ± standard deviation. Statgraphics Centurion XV software (USA) was used to conduct the statistical analysis. ANOVA variance analysis and Least Significant Difference (LSD) test were used to assess whether the differences in groups statistically significant (p<0.05).

3.0 RESULTS AND DISCUSSION

3.1 The Effect of Thermal Treatment on Color Indices of Black Shallot

Color is a crucial factor affecting the consumer perception of food quality and correlate with some other attributes such as moisture and pigment level of products [19]. Furthermore, during aging process and thermal treatment, Maillard reaction occurs and produces melanoidins; therefore, color also is an indicator of maturation of black shallot and can be used as a means to monitor Maillard reaction between aging batches. After thermal treatments at 60°C, 70°C, 80°C for 21 days, fresh shallots changed their colors and produce dark brown products. However, the products incubated at 60°C could not develop a homogenous brown color, whereas black shallots treated at 70°C and 80°C were developed an uniform brown color (Figure 1).

Table 1 The changes of color indices of black shallot during aging process at 60°C, 70°C, 80°C

Incubation time (day)	0	3	6	9	12	15	18	21
				60°C				
L	43.55 ± 0.18[a]	25.77 ± 0.01[b]	19.07 ± 0.04[c]	15.58 ± 0.07[d]	13.15 ± 0.02[e]	11.71 ± 0.01[f]	9.46 ± 0.01[g]	7.92 ± 0.01[h]
a*	12.37 ± 0.41[a]	4.89 ± 0.03[b]	3.13 ± 0.04[d]	4.30 ± 0.03[c]	4.26 ± 0.05[c]	3.16 ± 0.02[d]	3.26 ± 0.08[d]	-0.17 ± 0.12[e]

Incubation time (day)	0	3	6	9	12	15	18	21
				60°C				
b*	-1.83 ± 0.04^{a}	6.88 ± 0.03^{e}	4.87 ± 0.06^{c}	9.12 ± 0.06^{g}	6.59 ± 0.04^{d}	7.44 ± 0.05^{f}	7.47 ± 0.05^{f}	3.20 ± 0.07^{b}
ΔE		21.16 ± 0.02^{a}	27.01 ± 0.03^{b}	31.1 ± 0.04^{c}	32.57 ± 0.01^{d}	34.41 ± 0.01^{e}	36.49 ± 0.05^{f}	38.10 ± 0.02^{g}
				70°C				
L	43.55 ± 0.18^{a}	22.49 ± 0.01^{b}	17.65 ± 0.03^{c}	15.84 ± 0.07^{d}	14.76 ± 0.02^{e}	11.68 ± 0.01^{f}	8.08 ± 0.02^{g}	6.46 ± 0.02^{h}
a*	12.37 ± 0.41^{a}	2.67 ± 0.03^{d}	6.30 ± 0.10^{b}	1.96 ± 1.00^{e}	4.55 ± 0.06^{c}	2.26 ± 0.06^{de}	-0.43 ± 0.10^{f}	-0.01 ± 0.11^{f}
b*	-1.83 ± 0.04^{a}	6.02 ± 0.02^{c}	8.93 ± 0.02^{e}	5.26 ± 1.73^{c}	7.85 ± 0.04^{d}	5.72 ± 0.02^{c}	2.56 ± 0.06^{b}	2.81 ± 0.07^{b}
ΔE		24.47 ± 0.02^{a}	28.69 ± 0.03^{b}	30.48 ± 0.04^{c}	31.36 ± 0.01^{d}	34.27 ± 0.01^{e}	37.96 ± 0.05^{f}	39.37 ± 0.02^{g}
				80°C				
L	43.55 ± 0.18^{a}	22.30 ± 0.18^{b}	16.76 ± 0.01^{c}	15.09 ± 0.03^{d}	12.20 ± 0.04^{e}	9.13 ± 0.1^{f}	8.63 ± 0.06^{g}	5.33 ± 0.55^{h}
a*	12.37 ± 0.41^{e}	3.20 ± 0.03^{c}	4.66 ± 0.01^{d}	0.53 ± 0.04^{b}	0.33 ± 0.05^{b}	-0.23 ± 0.10^{a}	0.30 ± 0.05^{b}	0.32 ± 0.17^{b}
b*	-1.83 ± 0.04^{a}	6.76 ± 0.12^{f}	8.55 ± 0.04^{g}	2.60 ± 0.02^{b}	2.78 ± 0.04^{c}	2.70 ± 0.06^{bc}	3.39 ± 0.01^{d}	3.95 ± 0.10^{e}
ΔE		24.69 ± 0.21^{a}	29.75 ± 0.02^{b}	31.13 ± 0.04^{c}	33.9 ± 0.03^{d}	36.93 ± 0.06^{e}	37.31 ± 0.05^{f}	40.49 ± 0.49^{g}

The data were expressed as Mean ± SD. a, b, c, d, e, f, g in rows indicate the significant differences among groups using LSD tests (p<0.05)

As shown in Table 1, L values of black shallots from all thermal treatment (60°C, 70°C, and 80°C) gradually and significantly decreased (7.92 ± 0.01, 6.46 ± 0.02, 5.33 ± 0.55 at day 21, respectively) as compared to fresh shallot (43.55 ± 0.18). There was a decline of a* index (redness) of all black shallot after aging at three different conditions, whereas b* index of black shallot increased after aging (Table 1). We also observed a sharp rise of total color difference values of black shallots treated with three temperatures, which was correlated with the color changes of products. The typesetting process has changed the structure of paragraph. Change to

'Moreover, shallots possess an abundant amount of anthocyanins, a major class of red to blue flavonoid pigments. During the aging and thermal treatment process, anthocyanins are hydrolyzed, which in turn makes the color changes of black shallot. Additionally, Oancea and Draghici suggested that thermal treatment over 45°C could result in degradation of anthocyanin extracted from red onion [20]. Therefore, a* index (redness) of black shallots in this study was dramatically decreased (from 12.37 in fresh shallot to a range of -0.17 to 0.32). Heat is a key factor of Maillard reaction and can speed up chemical reaction rate. Therefore, the higher temperature results the faster aging process and dramatically accumulate browning products [21]. Of note, the total color difference of end-product treated with 80°C was highest (40.49 ± 0.49), followed by those treated with 70°C and 60°C (39.37 ± 0.02 versus 38.10 ± 0.02, respectively). Taken together, the best conditions to produce characterized dark brown color for black shallot are 70°C and 80°C.

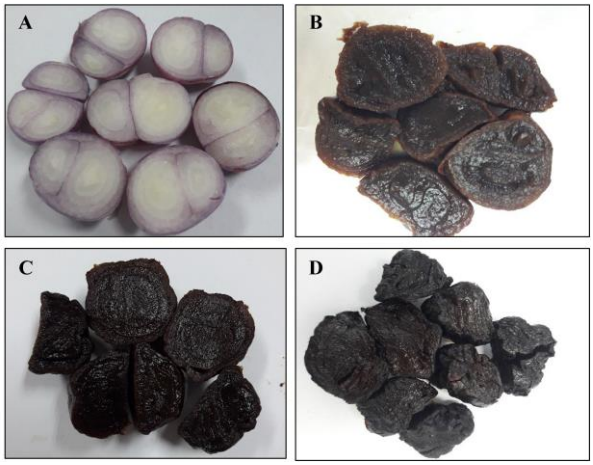


Figure 1 The end-products of aging process at three different temperatures. A. fresh shallot; B. black shallot aged at 60°C, C. black shallot aged at 70°C, D. black shallot aged at 80°C

3.2 The Effect of Thermal Treatment on Water Content of Black Shallot

The water contents of black shallots decreased over incubation time regardless thermal treatment conditions, and the higher temperature produced black shallots possessing the less water contents (Figure 2). As shown in Figure 2, fresh shallot had a high water content (81.77 ± 2.76%) but the water contents were lower in black shallots after aging process at 60°C, 70°C, 80°C (71.24 ± 1.21%, 65.60 ± 1.82%, and 58.24 ± 2.69%, accordingly). Interestingly, total color difference of black shallot at 80°C is the highest values, followed by those of 70°C and 60°C after 21 days. According to Eichner and Karel, high water content in food could reduce Maillard reaction rate; therefore, there is an inverse correlation between the change in color and water content of product [22]. Moreover, the higher temperature also sped up the loss of water in products; for instance, the water content of black shallot produced from 60°C (74.56 ± 1.14%) was higher than that of 80°C after 15 days (62.20 ± 2.17%, p<0.05). These results were similar with Zhang *et al*. report, in which the higher thermal treatment produced the less moisture products [23]. One of explanation for this phenomenon is that thermal treatment produces a concentration gradient between the outer layer and inner core due to water evaporation, and the higher temperature results acceleration of driving force for moisture diffusion among layers [24]. In addition, water content also plays an important role in food and agricultural products texture, at least in its functions as a plasticizer [25]. Among three thermal treatments, products of 60°C possessed highest water content, and infused, soft texture, while black shallots incubated at 70°C had more elastic texture than that of 60°C along with less water content. Product at 80°C had a hard, rigid texture as well as the least water content among three treatments. Therefore, water content of product is an important parameter to monitor product attributes during aging process of black shallot.

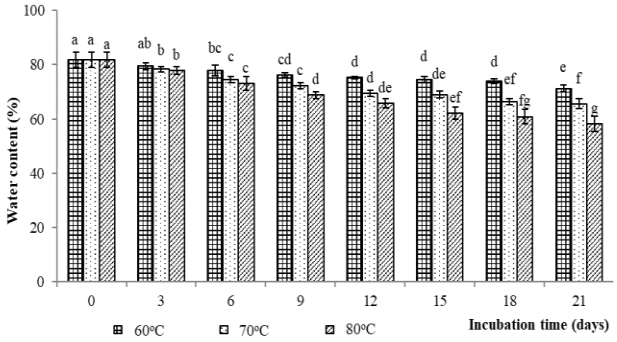


Figure 2 The changes in water content of black shallot during aging process at three temperatures (60°C, 70°C, 80°C). a, b, c, d, e, f, g in same group of treatment denote the significant differences among samples at different time-points using LSD tests (p<0.05)

3.3 The Effect of Thermal Treatment on pH and Acidity of Black Shallot

After 21 days of incubation at 60°C, 70°C and 80°C pH values of black shallots fell into 4.28 ± 0.17, 4.21 ± 0.11 and 4.17± 0.19, respectively; while the pH of fresh shallot was 6.24 ± 0.08 (p<0.05). During incubation at the same temperatures, pH of black shallot also steadily dropped (Figure 3). These results were identical with Kang *et al*. research, in which pH values of black garlic ranged from 4.01 to 5.27 after thermal treatment and was less than pH of fresh garlic (6.29) [6]. The pH value has the strong connection with browning reaction. As the consequence, the high pH values in initial stage of aging process enhances the color changes of all samples (total color differences of black shallot treated at 60°C, 70°C, and 80°C as 21.16 ± 0.02, 24.47 ± 0.02, and 24.69 ± 0.21, correspondingly) at day 3; whereas low pH at the later stage may hinder the browning reaction rate [26].

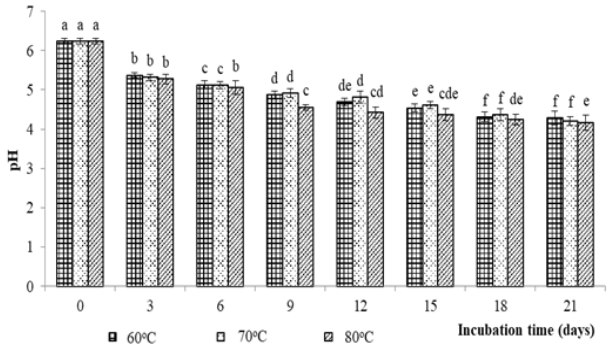


Figure 3 The changes in pH of black shallot during aging process at three temperatures (60°C, 70°C, 80°C). a, b, c, d, e in same group of treatment denote the significant differences among samples at different time-points using LSD tests (p<0.05)

During aging process, we observed a dramatic increase in acidity of black shallot regardless of thermal treatment conditions (p<0.05). Moreover, the higher temperature produced the more acidic product; for instance, acidity of black shallot incubated at 80°C was higher than that of 60°C at day 6 (Figure 4). The results were in agreement with pH values of black shallot in this study and the data from other research. In previous study, Moreno-Ortega *et al*. reported that there was a significant increase of tartaric acid, malic acid as well as total acids as compared to fresh shallot after 21 days of incubation at 65-70°C, 90% relative humidity [27]. Moreover, Choi *et al*. also observed a rise of total acids of black garlic after 21 days of aging process (over 4-folds higher than fresh garlic) [17].

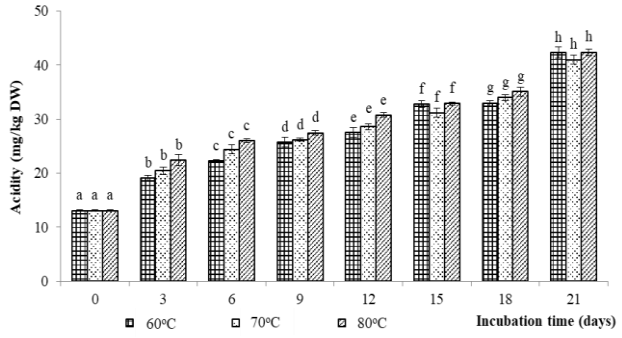


Figure 4 The changes in titratable acidity of black shallot during aging process at three temperatures (60°C, 70°C, 80°C). a, b, c, d, e, f, g, h in same group of treatment denote the significant differences among samples at different time-points using LSD tests (p<0.05).

3.4 The Effect of Thermal Treatment on Reducing Sugar Content of Black Shallot

Under thermal treatment, polysaccharides degraded into smaller molecules, such as oligosaccharides and monosaccharides, the major source of reducing sugar during aging process [28]. Furthermore, high temperature and high organic acid content results in instability of fructans, a class of fructose polymers mainly found in shallot, garlic and other *Allium* species [28]. As the consequence, reducing sugar content of black shallot are higher than that of fresh shallot. Reducing sugar content of black shallots incubated at 60°C and 70°C (700.43 ± 9.06 and 736.15 ± 9.34 mg/kg DW, respectively) significantly increased after 21 days as compared to fresh shallot (75.32 ± 2.41 mg/kg DW, p <0.05). Reducing sugar content of black shallot from 80°C steadily grew until reaching the peak after 18 days (828.17 ± 16.22 mg/kg DW), followed by a sharp decline at the end of aging process (656.04 ± 23.14 mg/kg DW, p<0.05) (Figure 5). Of note, reducing sugar content of black shallot aged at 80°C after 18 days in this study also were greater than those of 60°C after 21 days, and the products of 70°C possessed higher amount reducing sugar than those of 60°C (at day 21). The data are identical with Zhang *et al.* study. In previous study, the authors showed that reducing sugar of black garlic of 60°C (69 days) was lower those of 70°C (30 days) and 80°C (15 days) [23]. In addition, Moreno-Ortega *et al*. (2020) also reported a noticeable increase in fructose and glucose content in three varieties of onion during aging process [27]. Of note, reducing sugar can interact with amino acid in Maillard reaction under thermal treatment; therefore, the higher temperature can accelerate Maillard reaction rate as well as reducing sugar consumption rate. That may lead to a decline in reducing sugar content in black shallot at 80°C at later stage. Reducing sugar content may contribute to sweetness of products; therefore, the high reducing sugar content of products at 70°C implies that the quality and consumer acceptance of this product are better than the others [23].

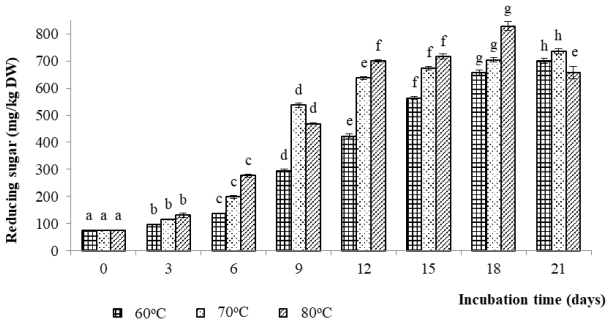


Figure 5 The changes in reducing sugar content of black shallot during aging process at three temperatures (60°C, 70°C, 80°C). a, b, c, d, e, f, g, h in same group of treatment denote the significant differences among samples at different time-points using LSD tests (p<0.05).

3.5 The Effect of Thermal Treatment on Total Polyphenol Content of Black Shallot

In the beginning of aging process, total polyphenol content of raw material (fresh shallot) was 16.60 ± 0.68 mg GAE/g DW (with the water content about 81.77 ± 2.76 %), while total phenol content of black shallot after aging process was dramatic increased regardless in term of temperatures with approximately 3 folds greater the fresh ones (Figure 6). In previous study, Vu *et al*. reported that fresh shallots collected from southern regions of Vietnam possessed total polyphenol contents ranging from 239.59 to 627.13 mg/100g fresh weight, which is consistent with the value of total phenol of fresh shallots in this study on wet basis [29]. Plant-based polyphenols are mainly associated with carbohydrates to form their glycosides which are cleaved into free form of phenolic compounds under thermal treatment, leading to increase polyphenol content in aged products [30, 31]. According to Kim *et al*., reduction of enzymatic oxidation and the increase in the levels complex polyphenols resulted of later phase of browning reaction also can contribute to polyphenol accumulation in products after thermal treatment [30]. As the consequence, all of black shallot after aging process increase their

polyphenol contents (Figure 6). Furthermore, Najman *et al*. (2020) also reported the similar results, in which total polyphenols of black garlic increased over two folds as compared to fresh garlic [32]. Note that, Monero-Rojas *et al*. (2018) indicated that quercetin, an important and abundant polyphenolic flavonoid of shallot and onion, increased over 1.6 times as compared to that of fresh shallot [9].

The higher temperature accelerated the polyphenol accumulation in black shallot; for instance, the polyphenol content of black shallot incubated 60°C reached its peak at day 18, whereas that of 80°C reached the peak of polyphenol content after 15 days. The data from Herlina *et al*. study also suggested that the higher temperature and longer incubation improved total polyphenol content in aged products [33]. That is in agreement with our results in total polyphenol content of black shallot treated with three different temperature in early stage of incubation. On the other hand, total polyphenol of black shallot incubated at 80°C in later stage (after day 15) significantly declined until the end of 21 days (50.71 ± 1.65 and 46.60 ± 1.20 mg GAE/g DW, respectively). This phenomenon implies that the release rate of phenolic compounds is slower the degradation rate due to the high thermal treatment and low pH in later stage of aging process. In term of combinational effect of three temperatures and different incubation times, black shallot produced at 70°C for 18 days possessed the highest polyphenol content (52.86 ± 2.02 mg GAE/g DW), followed those produced at 80°C for 15 days and 60°C for 18 days. Plant polyphenols is a crucial source of dietary antioxidants, which not only exerts protective effect against oxidative stress but also provides several health beneficial effects, including anti-cancer, anti-diabetic, anti-inflammatory effects as well as preventive effect from cardiovascular diseases, osteoporosis, and neurodegenerative diseases [34]. Therefore, this study suggests black onion as the good source for extraction of polyphenol as well as indicates the best condition to obtain the great polyphenol amount is incubation at 70°C for 18 days.

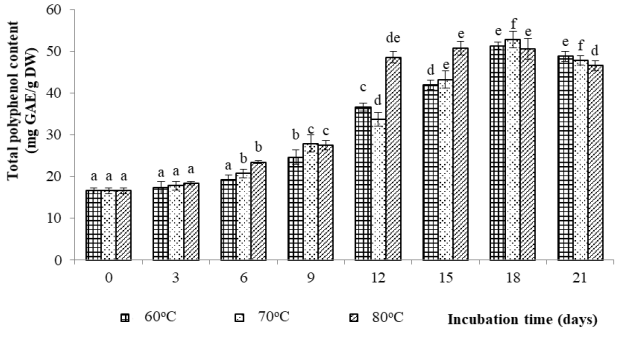


Figure 6 The changes in total polyphenol content of black shallot during aging process under three thermal treatment (60°C, 70°C, and 80°C). a, b, c, d, e, f, g in same group of treatment indicate the significant differences among samples at different time-points using LSD tests (p<0.05)

3.6 The Effect of Thermal Treatment on Antioxidant Activity of Black Shallot

The changes of antioxidant activity of black shallot were presented in Figure 7. There was a dramatic elevation of antioxidant activity of black shallots after aging process; for instance, the antioxidant activity of black shallot treated at 70°C after 21 days was 10 folds greater than that of fresh shallot (57.55 ± 0.92% versus 5.27 ± 0.94%, respectively). The results were in agreement with Tran *et al*. report, in which the black shallot extract exhibited a stronger antioxidant effect as compared to the extract from fresh shallot [11].

Furthermore, the higher temperature at early stage enhanced the antioxidant accumulation rate; for instance, the antioxidant activity of black shallot incubated at 80°C (16.19 ± 0.27%) was higher value that of 60°C (12.92 ± 0.78%) at day 3. In contrast, the highest temperature is not the best condition to obtain the great value of antioxidant activity; for example, the products from 70°C after 18 days possessed the highest antioxidant activity (63.95 ± 1.24%), followed by those of 80°C and 60°C (60.66 ± 0.26% and 58.92 ± 0.27%, respectively). Under three thermal treatment, we observed a steadily increase of antioxidant activity of black shallots with the peaks occurred at 18 day, followed by a reduction of antioxidant activity of black shallot at the end of aging process. The phenomenon was described by Choi *et al*. [17]. In previous study, the antioxidant activity black garlic incubated at 70°C in 90% RH increased along with aging process until day 21, and it steadily dropped at the later stage [17]. As mentioned in Figure 6, the total polyphenol content of black shallot accumulated during the aging process, which may be associated with the increase of antioxidant activity of black shallot. Note that, some products of Maillard reaction also can exhibit antioxidant activity [35] and heating could alter the Maillard reaction rate, which in turn affecting the antioxidant activity of aged products. In summary, the optimal aging condition to obtain the highest value of antioxidant activity and total polyphenols is incubation at 70°C for 18 days.

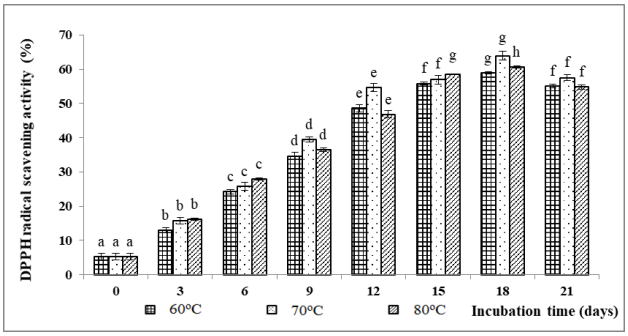


Figure 7 The changes in antioxidant activity of black shallot during aging process under three thermal treatment (60°C, 70°C, and 80°C). a, b, c, d, e, f, g, h in same group of treatment indicate the significant differences among samples at different time-points using LSD tests (p<0.05)

4.0 CONCLUSION

Thermal treatment is an important factor affecting not only the physicochemical properties but also bioactive compound content (polyphenols) and antioxidant activity of black shallot. Under thermal treatment, aging process noticeably decreased water content and pH value of shallot; on the contrary, the acidity, reducing sugar and total polyphenol contents, as well as antioxidant activity of black shallot dramatically elevated. The products incubated at 60°C is unable to develop the homogenous brown which may affect consumer perception; whereas those aged at 80°C possess lower water content (associated to its hard, and rigid texture) and reducing sugar content implying the less sweet taste of product. Among three temperatures, 70°C is the optimal aging condition to produce the highest antioxidant activity and polyphenol content black shallot after 18 days (63.95 ± 1.24 % and 52.86 ± 2.02 mg of GAE/g DW, respectively). These findings for production of information to select and regulate the aging process for production of the high quality of black shallot with desired attributes and suggest the potential utilization of black shallot as an innovative functional food and/or a source for isolation of antioxidants, bioactive compounds.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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