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EFFECT OF THERMAL TREATMENT ON THE ANTIOXIDANT ABILITY OF BLACK GARLIC PRODUCED FROM PHAN RANG'S NATURAL FRESH MATERIAL

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ABSTRACT

Black garlic, a product made from the natural fresh garlic, is produced by maturing in an environment of sufficient moisture for a long enough period. Black garlic contains phenolic, flavonoid and has much higher antioxidant activity than that of the natural one. This study is aiming at determining the influence of maturing temperature and incubation time on the transformation of the natural fresh garlic into the black one. Influence of moisture, pH, concentration of phenolic, flavonoid, antioxidant activity and the organoleptic properties of the black garlic were investigated at the temperatures of 60, 70, 80 °C during the incubation period of 10, 20, 30 and 40 days. It was shown that moisture and pH decrease while handling temperature increasing and prolonged incubation period. The content of amino acid, phenolic compounds and flavonoids reached their highest values of 5.752 mg/g, 8534 μg GAE/g dry matter and 492.41 μg GE/g dry matters, correlatively, at the temperature of 60 °C in 30 days. The antioxidant activity determined by DPPH technique as well as the reduction capacity got the highest value at the temperature of 60 °C in the incubation period of 30 days. In comparison with the raw fresh garlic, the black one incubated at 60 °C in 30 days has super high phenolic components, flavonoids as well as the antioxidant activity. The produced black garlic is of soft structure, no pungent smell, sweet and sour tastes, appropriate for regular use.

Keywords: black garlic, incubation, thermal treatment, antioxidant activity, reduction capacity.

1. INTRODUCTION

Garlic (Allium sativum L.) is one of the most common ingredients used in many cuisines. For long time ago, it has been known as a traditional medicine used for treating illnesses. In Vietnam, the garlic is grown in many specialized areas such as Ly Son Island of Quang Ngai, Phan Rang of Ninh Thuan provinces, etc. Garlic contains a lot of bioactive compounds which have been characterized as antioxidants (polyphenols), antibacterials and antibiotics (allicin and its derivatives). Despite of its advantages, the consumption of garlic is very limited due to its strong flavor and pungent smell. Currently, people are interesting in the process of producing

black garlic from the fresh one. In this process, the whole bulbs of garlic are matured in an oven by which temperature and humidity are well controlled. During the process of thermal treatment, the biochemical reactions occurring in garlic make it turn black, the structure of garlic becomes soft, and its strong taste finally reduces to slightly sour and sweet. As compared to fresh garlic, the black garlic has much higher level of bioactive properties such as antioxidant activity [1]. Recently, many production processes of black garlic have been proposed, most of them are able to produce garlic with black color; however, the contents of bioactive compounds are very different.

In Vietnam, there are some kinds of black garlic sold in the market. However, investigations on producing black garlic from Vietnamese fresh garlic are very few, as such of Vu Binh Duong et al. who studied on Ly Son garlic [2]. Hence, this study will focus on determining the influence of maturing temperature and retention time to the transformation of the Phan Rang garlic into the black one from which optimized conditions of this process can be obtained.

2. MATERIAL AND METHODOLOGY

2.1. Materials

Garlic was purchased from Thanh Hai commune, Ninh Hai district, Ninh Thuan province. The raw materials were selected, washed, cut from stems and roots and then stored in a well ventilated area.

2.2. Thermal treatment

The whole bulbs of natural garlic were incubated in an oven (Memmert SNE 200 - Germany) with the stable relative humidity of about 70% and at controlled temperature of 60, 70 and 80°C for 10, 20, 30 and 40 days. The garlic was then analyzed to determine the moisture content, pH, total phenolic and flavonoid content, antioxidant activity and organoleptic properties.

2.3. Methods of analysis

- Moisture: the moisture was determined by drying the samples to constant mass in accordance with Vietnam Standard 9706-2013.

- pH: the pH of garlic juice was obtained using a pH meter (pH CYBERSCAN 510 - Eutech- Singapore).

- The total phenolic content: Total amount of phenolic compounds were analyzed by the method of Folin - Ciocalteu, combined with standardized line chart garlic acid (GA) [3]. The results were expressed as gallic acid equivalents (GAE) per gram of dry matter (μg GAE/g).

- Contents of flavonoid compounds were determined according to the method described in [3]: 3 ml of aluminum chloride 2 % and 3 ml of the pressed garlic sample were mixed in a glass tube and stirred at room temperature for 1 hour. The solution was characterized by absorption spectroscopy at the wavelength of 420 nm. The results were expressed in quercetin equivalent (μg QE)/g dry matter.

- Antioxidant activity against free radical such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [4]: The garlic sample was diluted 2 times. Then, 2 ml of DPPH solution was added and stirred thoroughly. The mixture were kept in the dark at room temperature for 30 minutes and the absorbance was recorded at 515 nm. The antioxidant activity of the sample was performed using the standard curve of Trolox. Results are expressed as Trolox equivalent (TE) per gram of dry matter (μg TE/g).

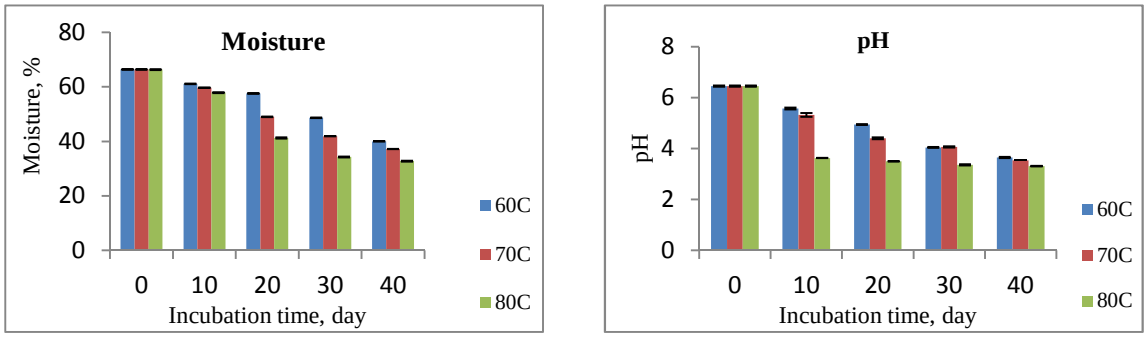
- Antioxidant activity and reduction capacity [4]: 1 mL of garlic was mixed respectively with 2 ml of phosphate buffer solution (pH 6.6, 0.2 M) and 1 mL of potassium ferrocyanide K_3 [Fe (CN) $_6$] solution. The mixture was incubated in a water bath at 50°C for 20 minutes and then 2.5 ml of trichloroacetic acid (TCA) solution was added at room temperature. After that, the solution was centrifuged at 650 rpm in 3 minutes and then 1 mL $FeCl_3$ solution of 0.1 % was added. The absorbance of the resulting solution was measured at 700 nm. The antioxidant activity of the sample was analyzed according to the standard curve of Trolox. Results are expressed as Trolox equivalent (TE) per gram of dry matter (μg TE/g).

- Data processing method: Each experiment was repeated three times; the results are presented as average of the repeats. The significant differences between samples were performed by ANOVA method (α = 5 %), using Statgraphic Centurion XV software.

3. RESULTS AND DISCUSSION

3.1. The effects of incubation time and temperature on moisture and pH of black garlic

The moisture of the raw garlic was 66.37 %. After 40 days under the thermal treatment, the moisture reduced to 40.03 %, 37.22 % and 32.78 % according to the incubation temperature of 60 ^{o}C, 70 ^{o}C and 80 ^{o}C, respectively. The pH of garlic decreased as well. The change of moisture in garlic also led to the change in the structure of black garlic. As results, the structures of black garlic were softest at 60 °C, dry and flexible at 70 °C, harder and brittle at 80 ^{o}C due to the loss of water.



(a) (b)

Figure 1. The effect of incubation time and temperature to the moisture (a) and the pH (b) of the produced black garlic.

As shown in the Figure 1, pH of the raw garlic was 6.45. At the 40 days of thermal treatment, the pH of black garlic samples were 3.65, 3.54 and 3.31 corresponding to the temperature of 60 °C, 70 °C and 80 °C, respectively. The reaction to form the organic acid was one of the reasons that caused the pH reduction of garlic. According to Liang T. et al. [5],

hexose and pentose were hydrolyzed into α and β-carbonyl or dicarbonyl at high temperatures, leading to the formation of carboxylic acids, which reduced the pH of garlic.

3.2. The effect of incubation time and temperature on the content of phenolics and flavonoids in black garlic

As shown in the Figure 2, the concentration of phenolic in raw garlic is 548.82 µg GAE/g dry matter. After 10 days of incubation, the concentration of phenolic in samples incubated at 80 °C garlic reached the highest value of 8165.25 µg GAE/g dry matter; however, until the 40th day, their concentration felt down to 4539.39 µg GAE/g dry matter.

When the incubation temperatures were set at 60 °C and 70 °C, the levels of phenolic compounds increased and reached the highest value of 8545.76 and 8168.40 µg GAE/g dry matter after 30 days of incubation, respectively. At the 40th day of incubation, the amount of phenolic compounds in black garlic samples decreased.

In short, the total phenolic contents in black garlic after the incubation process increase 8-15 times more than the ones in fresh garlic. The garlic incubating process increases the amount of phenolic acids, which contribute to increasing the total phenolic contents in black garlic. Phenolic compounds in plants often associated with other components such as fiber, protein or sugar to form the complex structure. The content of phenolic compounds could increase due to the broken links in the complex structure (ester linkages, linked glycoside) under the effect of temperature leads to the release of free compound [3].

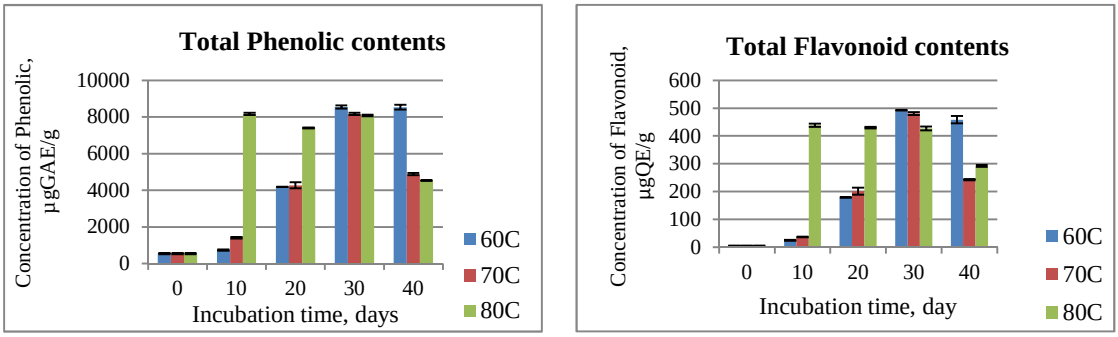


Figure 2. The effect of incubation time and temperature on the contents of phenolic and the flavonoid in the produced black garlic.

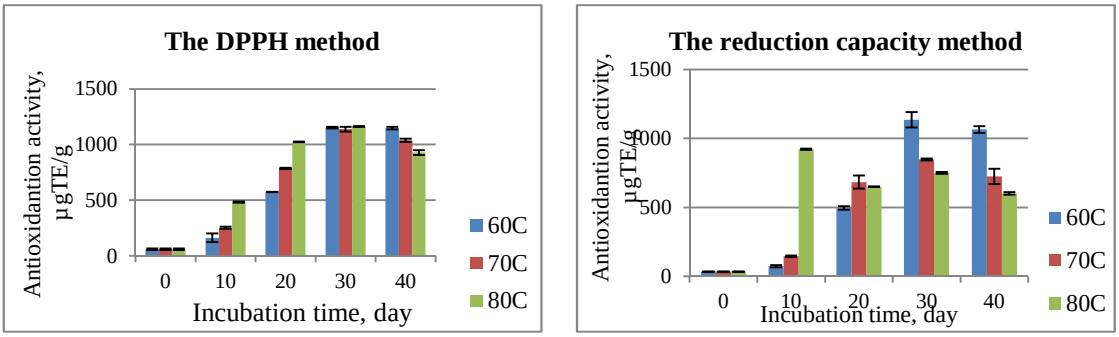
The total flavonoid content in raw garlic is 5.73 µg QE/g dry matters. At 60 °C and 70 °C, the content of flavonoid increased to 25.15 and 37.17 µg QE /g dry matter, respectively after 10 days of incubation and more; and these contents could reach maximum levels of 492.41 and 480.74 µg QE/g dry matter at the 30th day and then reduced slightly at the 40th day. When the incubation temperature was set at 80 °C, the content of flavonoids increased rapidly to 438.33 µg QE/g dry matter after 10 days and then decreased slightly.

3.3. The effect of incubation time and temperature on antioxidant activity

The antioxidant activity of black garlic were determined by DPPH method and reduction capacity method, the results were presented in Figure 3.

Based on the data obtained by the DPPH method, the figure showed that the antioxidant activity of raw garlic sample was 59.61 µg TE/g dry matters. During the thermal treatment, the antioxidant activity of the samples increased and reached the maximum values at the 30th day

(1152.16; 1138.15 and 1162.21 µg TE/g dry matter according to the heating temperature of 60 °C, 70 °C and 80 °C, respectively). After 40 days of incubation, the antioxidant activities of these samples reduced down to 1147.46; 1038.36 and 928.51 µgTE/g dry matters, respectively.



(a) (b)

Figure 3. The effect of incubation time and temperature on the antioxidant activity of the produced black garlic determined by DPPH method (a)and reduction capacity method (b).

By using the reduction capacity method, the initial antioxidant capacity of raw garlic was 34.60 µgTE/g dry matters. After 10 days of thermal treatment at 80 ^{o}C, the black garlic gained its maximum antioxidant capacity of 922.74 µg TE/g dry matter. Meanwhile, the samples treated at 60 °C and 70 ^{o}C reached maximum antioxidant capacities at the 30th day with the values of 1136.01 and 848.95 µg TE/g dry matter, respectively. After 40 days of incubation, the antioxidant activity of these samples were reduced to 1065.62, 724.47 and 601.65 µg TE/g dry matter corresponding to 60 °C, 70 °C and 80 °C.

Antioxidant activities of black garlic determined by both methods show the same trend in variation. Actually, there is a relationship between phenolic and flavonoid contents of black garlic with antioxidant capacity. However, the results obtained by DPPH method are higher than the ones by reduction capacity method. This may be due to difference mechanism of chemical reactions occurring in these methods. According to Huang et al. [4], the mechanism in DPPH method is related to the hydrogen transfer while the one in reduction capacity method is known as the electron transfer in which the compounds being able to capture the free radicals such as the thiol hydrogen and carotenoids don't show their antioxidant activity. This may be the reason why results are difference in two methods.

4. CONCLUSION

As results, the content of antioxidant (phenolic compounds and flavonoids) and its activity reached the highest level as the samples were matured at 60 °C after 30 days. This optimal condition provides the best organoleptic properties of the black garlic such as soft structure, no pungent smell, sweet and sour tastes. Furthermore, antioxidant activity determined by DPPH technique was 1152.16 µgTE/g dry matters (i.e. 19 times higher than the one in the raw fresh garlic), the content of phenolic and flavonoid compounds reached 8545.76 µgGAE/g dry matters and 492.41 µgQE/g dry matter (i.e. nearly 16 and 86 times increased), respectively. In conclusion, the incubation at 60 °C during 30 days is optimum condition to obtain the highest antioxidant activity in black garlic produced from the Phan Rang original garlic material.

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TÓM TẮT

HIỆU QUẢ CỦA VIỆC XỬ LÍ NHIỆT ĐẾN KHẢ NĂNG KHÁNG OXY HOÁ CỦA TỎI ĐEN SẢN XUẤT TỪ TỎI PHAN RANG

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Tỏi đen là một sản phẩm từ tỏi tươi, được sản xuất bằng cách ủ nhiệt trong môi trường có đủ độ ẩm trong thời gian dài. Tỏi đen có chứa các hợp chất phenolic, flavonoid và có hoạt tính kháng oxy hóa cao hơn nhiều so với tỏi tươi. Mục đích của nghiên cứu này là xác định ảnh hưởng của nhiệt độ ủ và thời gian ủ nhiệt đến sự chuyển hóa từ tỏi tươi thành tỏi đen. Độ ẩm, pH, hàm lượng các hợp chất phenolic, flavonoid, hoạt tính kháng oxy hóa và các tính chất cảm quan của tỏi đen được khảo sát ở nhiệt độ 60, 70, 80 ^{o}C trong thời gian ủ 10, 20, 30 và 40 ngày.

Các nghiên cứu đã chỉ ra rằng độ ẩm và pH của tỏi bị giảm khi tang nhiệt độ xử lí và kéo dài thời gian ủ. Hàm lượng acid amin, các hợp chất phenolic, flavonoid đạt giá trị cao nhất tương ứng là 5,752 mg/g, 8534 µg GAE/g chất khô và 492,41 µg GE/g chất khô ở nhiệt độ 60 ^{o}C với thời gian ủ 30 ngày. Hoạt tính chống oxy hóa xác định theo phương pháp DPPH và năng lực khử cũng cho giá trị cao nhất khi ủ ở nhiệt độ 60 ^{o}C trong thời gian 30 ngày. So sánh với tỏi nguyên liệu, tỏi đen được sản xuất khi ủ ở 60 ^{o}C trong 30 ngày có các thành phần hợp chất phenolic, flavonoid và hoạt tính kháng oxy hóa cao vượt trội. Tỏi đen sản xuất ra mềm dẻo, không còn mùi hăng, có vị chua ngọt, thích hợp cho việc sử dụng.

Từ khóa: Tỏi đen, ủ, xử lí nhiệt, khả năng kháng oxy hóa, năng lực khử.