EFFECT OF EXTRACTION CONDITIONS ON POLYPHENOLS, FLAVONOIDS, S-ALLYL CYSTEINE CONTENT AND ANTIOXIDANT ACTIVITY OF BLACK GARLIC EXTRACTS

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ABSTRACT

Black garlic is obtained from fresh garlic (*Allium sativum* L.) that has been aged for a long time at a high temperature under high humidity. Black garlic exerts metabolic and cardiovascular beneficial effects. In this study, (*i*) water and various concentrations of ethanol (50 % and 100 %) in water were used as solvent in the extraction of black garlic with different black garlic:solvent ratios (1:5, 1:10 and 1:15 w/v); (*ii*) the process was investigated in the temperatures ranging of 40 to 80 ^{o}C during 30 to 120 minutes, as well as determination of bioactive compounds content (total polyphenols, total flavonoids and S-allyl cysteine content) of all obtained extracts. The antioxidant activity was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The obtained results indicated that the aqueous ethanol (50 %) extracts presented the highest contents of polyphenols and flavonoids (7.94 mg GAE/g and 3.37 mg QE/g, respectively) and DPPH radical scavenging activity at black garlic: solvent ratio of 1:10 (w/v); while the water extract showed the highest S-allyl cysteine content (76.0 mg/kg) at the 1:10 ratio. The suitable condition for extracting of total polyphenols and flavonoids from black garlic was 60 ^{o}C for 90 minutes. With respect to SAC extraction, the suitable temperature and time of 80 ^{o}C and 90-120 minutes, respectively were found.

Keywords: antioxidant activity, black garlic, bioactive compounds, extraction.

1. INTRODUCTION

Garlic (*Allium sativum* L.) has been used as a medicine to treat many different diseases for humans. Due to the presence of several bioactive compounds that are effective in proven therapeutics. Black garlic is a newly processed food prepared by subjecting whole raw garlic to thermal processing for about a month under controlled high temperature and high humidity. The browning process occurred during aging. Garlic cloves was turning dark, give them more sweet taste (high soluble solid content) and alters their consistency to chewy and jelly-like. The pH of black garlic also decreased from about 6.0 in fresh garlic to less than 3.8 in black garlic. This explains the longer preservation of black garlic [1]. An important change during aging process was the increase in polyphenol content [2] and thus increased antioxidant capacity [3]. On the

other hand, the variability of some unstable ingredients and the smell of fresh garlic became stable and odorless compounds during aging, predominantly of sulfur organic compounds such as S- Allyl cysteine (SAC), also increase the antioxidant activity [4]. SAC is known as a water soluble bioactive compound for its extremely high antioxidant capacity [5]. SAC is formed during hydrolysis of γ-glutamyl-S-allylcysteine by the enzyme γ-glutamyl transpeptidase (γ-GTP, EC 2.3.2.2). SAC content in fresh garlic is about 20-30 μg/g and increases about 6 times during aging [6]. However, the activity of γ-GTP can be affected by heat treatment [7] and therefore, high temperatures may limit SAC formation during aging.

The objective of the extraction method is to obtain the maximum content of compounds with the highest quality [8]. During extraction, all the beneficial compounds must be extracted without altering their chemical structure [9]. Plant phenolic compounds have been extracted by various solvent systems [10]. However, the nature of the solvents and the conditions (temperature and duration) of extracts strongly influence the extraction efficiency and antioxidant capacity due to the presence of different antioxidant compounds with differentiation polarity and chemical characteristic. This makes them soluble or insoluble in a particular solvent [11]. The antioxidant activity of phenolic compounds is significantly affected by the polarization of solvent extraction. Therefore, proper solvent and extraction conditions are important for extraction of plant-derived materials. Extraction solvent is usually selected according to the extraction purpose, the polarity of the components. In addition, there is currently no data available on the appropriate extraction conditions of bioactive compounds in black garlic and their antioxidant capacity. Therefore, the objective of this study was to identify and select proper extraction methods to obtain the highest levels of bioactive compounds from black garlic.

2. MATERIALS AND METHODS

2.1. Materials

Fresh garlic was harvested at Van Hai ward, Phan Rang-Thap Cham city, Ninh Thuan province. Black garlic was processed at Can Tho University by using the aging method of whole garlic bulbs.

2.2. Methods

2.2.1. Preparation of black garlic extract

Black garlic was ground and mixed with distilled water or ethanol solutions (50 % and 100 %) with black garlic and solvents at different ratios of 1:5, 1:10 and 1:15 (w/v). The mixtures were kept standing at room temperature for one hour. Then, the extracts of the black garlic were filtered through Whatman® 41 filter paper. After selecting the appropriate treatments from above experiment, black garlic was extracted at 40 - 80 ^{o}C for 30 - 120 minutes and filtered through Whatman® 41 filter paper. All extracts were kept at 4 ^{o}C before analysis.

2.2.2. Analysis methods

Total polyphenols content (TPC) (mg gallic acid equivalents (GAE) per g of dry weight (d.w)) was determined by Folin-Ciocalteu method [12]. Phenolic reacts with phosphomolybdic acid in the Folin-Ciocalteau reagent which appears to be blue color in the alkaline medium. The absorbance was recorded at 765 nm by a spectrophotometer. Gallic acid was used as a standard

for the calibration curve. The phenolic content was reported as gallic acid equivalents (mg) using the following linear equation (mg GAE/g d.w).

Total flavonoids content (TFC): total flavonoids content was determined by colorimetric method with $AlCl_3$ solution in an alkaline-photometric medium [13]. The absorbance of reaction solution was measured at 415 nm. Based on the quercetin calibration curve to determine total flavonoids content of samples. The results are expressed in mg of quercetin equivalent (QE) per g of dry weight (mg QE/g d.w).

Antioxidant capacity (%): radical scavenging activity was analyzed by testing of DPPH (2,2-diphenyl-1-picrylhydrazyl). DPPH radical scavenging activity was determined by Blois [14]. Antioxidants scavenge the DPPH by giving hydrogen, reducing the absorbance at maximum wavelength, and the color of reaction solution to fade; then, solution change from violet to light yellow.

Determination of S-allyl cysteine by high performance liquid chromatography (HPLC). Sample preparation: the extracts of black garlic were filtered through a 0.45 μm syringe filter (Sartorius AG, Goettingen, Germany) and the solution was used to analyze. S-allyl-L-cysteine (≥ 98 %, SAC was purchased from Sigma-Aldrich Co. LLC) and was used as a standard for the calibration curve.

HPLC analysis conditions: HPLC-UVD system (Shimadzu, Shimadzu Corporation, Japan) was used to analyze S-allyl cysteine content in extract of black garlic samples including: an LC-10AD pump, a SPD-10A UV/Vis detector, a CTO-10AC column thermostat and a manual sample injector. Separation of the analyte was carried out using an LiChroCART® column (250 × 4 mm, 5 μm, Merck Millipore, Merck Millipore Corporation, Germany) at room temperature, and SAC was detected at 210 nm. The mobile phase consists of 0.1 % H_3PO_4 solution and acetonitrile solution (Sigma-Aldrich Co. LLC) with isocratic elution. Flow rate of 0.5 mL/min and injection volume of 10 μL.

2.2.3. Statistical analysis

The experiment was repeated three times. The means and standard deviations were also caculated and plotted using Microsoft Excel software. The data were analyzed by two-way ANOVA followed by using Fisher's Least Significant Difference (LSD) test using Statgraphics Centurion XVI (version 16.0; Statpoint Technologies, Inc., USA). Differences were considered statistically significant at $P < 0.05$ for all tests.

3. RESULTS AND DISCUSSION

3.1. Effect of black garlic: solvent ratios and types of solvent on bioactive compounds content and antioxidant capacity of black garlic extracts

Total polyphenols content of black garlic extracts according to the ratio of black garlic/solvent and different extraction solvents showed significant differences ($p < 0.05$) (Fig. 1a). The highest total polyphenols content (7.94 mg GAE/g d.w) was obtained with 50 % ethanol solution, followed by 100 % ethanol solution and the lowest that was distilled water (4.97 mg GAE/g d.w) at ratio of extraction 1:10. Total polyphenols content was obtained by the extraction ethanol solution (50 % and 100 %) was higher than which in distilled water. This might be due to the presence of impurities (organic acid, glucide, soluble protein). Impurities

effected on the identification of phenolic compounds. On the other hand, for all three types of solvents, the 1/5 extraction rate showed the lowest total polyphenols content was obtained; 1:10 and 1:15 extraction ratios showed no significant difference ($p > 0.05$).

Total flavonoids content was obtained in the extracts according to black garlic:solvent ratios and the different solvent types were shown in Figure 1b. There was no significant difference ($p > 0.05$) between the content of flavonoids obtained in 100 % ethanol solution and distilled water. This means that the plant contains more flavonoids heteroside than that aglyone. The highest level of total flavonoids content (3.37 mg QE/g d.w) in 50 % ethanol solution was obtained at extracted ratio of 1:10.

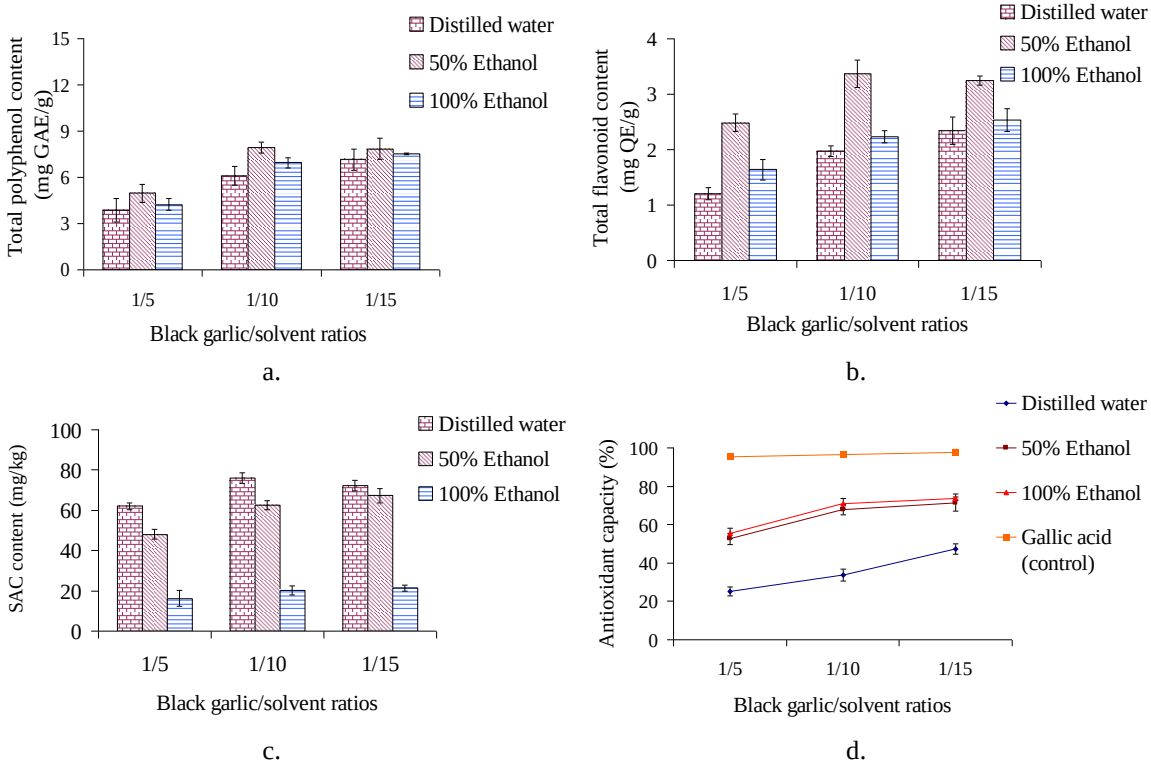

Figure 1. Total polyphenols content (a), total flavonoids content (b), S-allyl cysteine (c) and antioxidant activity (d) in black garlic extracts which were extracted by using different black garlic:solvent ratios and solvent types.

S-allyl cysteine, is a water-soluble bioactive compound, was formed during the hydrolysis of γ-glutamyl-S-allylcysteine by enzyme γ-glutamyl transpeptidase. The research results in Figure 1c showed that SAC content of the black garlic extracted by distilled water (76.0 mg/kg) was significantly higher than which of the ethanol solutions (50 % and 100 %) (62.6 mg/kg and 20.3 mg/kg, respectively) at extraction rate of 1:10. Indeed, ethanol solvent was not suitable for extracting of SAC. The ratio of extraction is 1:5 showed the lowest SAC content; however no significant difference ($p > 0.05$) was found at other ratios (1:10 and 1:15) in all three types of solvent.

Antioxidant capacity of distilled water extracts (25.2 - 47.4 %) was lower than which of ethanol solution (50 % and 100 %) (52.6 - 71.4 % and 55.5 - 73.7 %, respectively) (Fig. 1d). The 1:5 extraction ratio showed that antioxidant activity was the lowest. This may be related to levels of bioactive compounds that present in garlic extracts. However, this relationship was not clear in this experiment. S-allyl cysteine content was analyzed by HPLC (Fig. 2).

a. b.

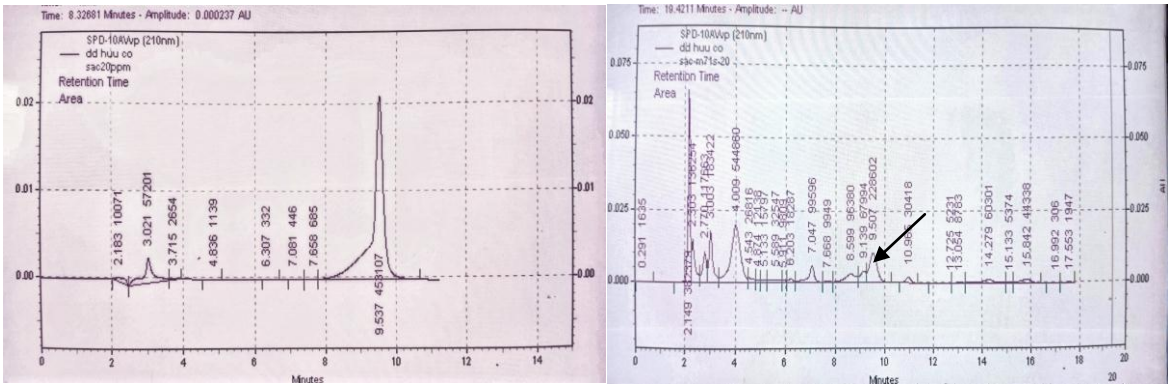

Figure 2. HPLC chromatograms of pure SAC solution (a) and SAC in black garlic extracts.

According to the principle of mass transfer operation in which dynamics for mass transfer is considered a concentration gradient between the solid and solvents. Large amounts of solvent could be produce a higher concentration gradient, so that, leading to increased diffusion rates, which accelerate extraction of solid by solvent [15]. In addition, the exposure of biomolecules and solvents was increased when solvent ratio increased so that improving the extraction efficiency. However, the levels of bioactive compounds will not increase further when equilibrium (solubility) occurs [16].

3.2. Effect of extraction temperature and time on bioactive compounds in black garlic extracts

Based on the data obtained from the study, the regression equations were established (equations 1, 2 and 3). The relationship between temperature (X: 40 ^{o}C - 80 ^{o}C) and time (Y: 30-120 mins) of extraction on TPC, TFC and SAC content (equations 1, 2 and 3) by using 50 % ethanol solution was found with a high coefficient of determination ($R^2 \geq 0.85$). Response surface models shows the effects of temperature and time extracted on TPC, TFC and SAC content which can help predict the results of the extraction process shown in Figure 3.

$$TPC\ (mg\ GAE/g) = -23.949 + 0.7\ X + 0.325\ Y - 0.0051\ X^2 - 0.0016\ Y^2 - 0.00106\ XY \quad (1)$$

$$R^2 = 0.85$$

$$TFC\ (mg\ QE/g) = -6.382 + 0.162\ X + 0.151\ Y - 0.001\ X^2 - 0.0007\ Y^2 - 0.0009\ XY \quad (2)$$

$$R^2 = 0.85$$

$$SAC = -99.151 + 2.759\ X + 1.268\ Y - 0.015\ X^2 - 0.0049\ Y^2 + 0.0002\ XY \quad (3)$$

$$R^2 = 0.97$$

The extraction temperature influences the solubility, mass transfer rate (diffusion coefficient) and stability of phenolic compound [8]. In a limited, high temperature increases the

extraction efficiency by enhancing the diffusion intensity and solubility of the substance to be extracted in the solvent. Exceeding that limit, high extraction temperatures will reduce total polyphenols content and total flavonoids content [17]. Heating increases the solubility and diffusion of compounds, reduces viscosity, increases mass transfer and transportation of solvent into the cell [18]. In addition, Mohamad *et al*. [19] reported that high temperatures can reduce cellular barriers due to weakening of the walls and the cell membranes, which facilitates solvent contact with the compounds, enhancing the ability to extract. Extraction time effects on the ability to extract bioactive compounds. Short extraction time, bioactive compounds were not extracted completely. In contrast, the extraction time is too long, some bioactive compounds will be oxidized leading to the quality and quantity of bioactive compounds will decrease. Because most bioactive substances that are sensitive to high temperatures, keeping them in the long run will lead to decomposition.

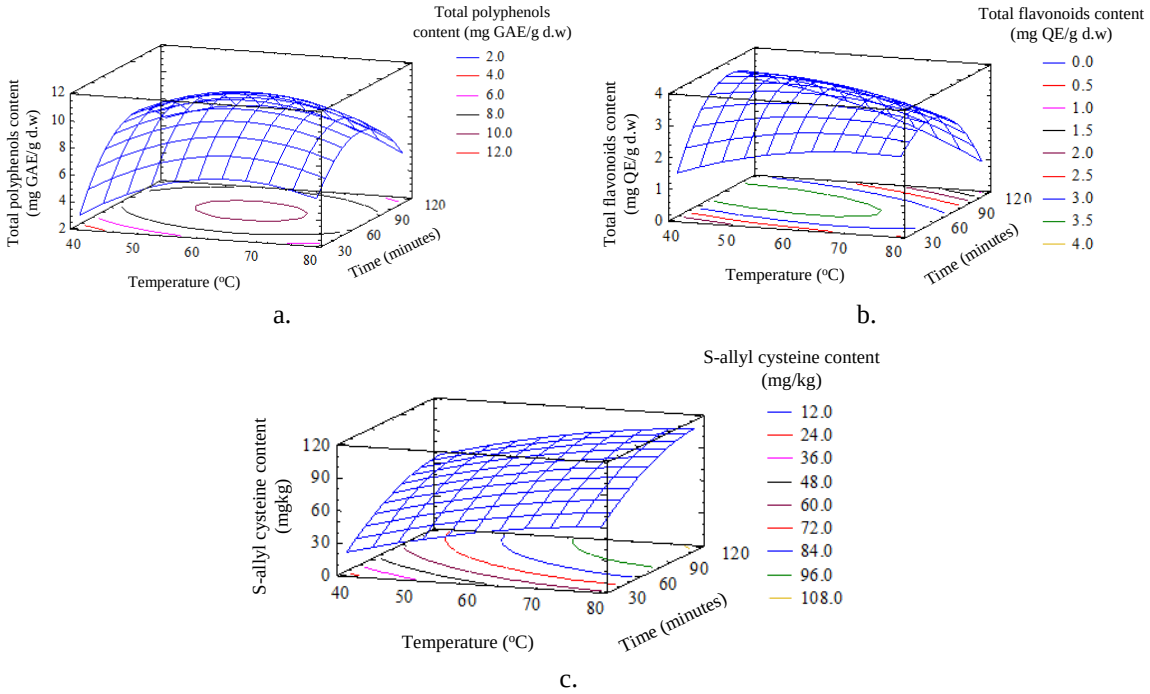

Figure 3. Effect of extraction temperature and time on TPC, TFC and SAC content in extracts of the black garlic.

4. CONCLUSIONS

Research results showed that black garlic/solvent ratios, types of solvent, extraction temperature and time effected on bioactive compounds content and antioxidant capacity in black garlic extract. The highest total polyphenols content and total flavonoids content (10.93 mg GAE/g and 4.20 mg QE/g, respectively) and DPPH radical scavenging activity presented in extract by using 50 % ethanol at ratio of black garlic:solvent of 1:10 and the suitable extraction temperature and time (60 °C and 90 minutes, respectively). In addition, S-allyl cysteine content in the extract reached the highest value (98 - 106 mg/kg) when using water extraction (black garlic:water of 1:10) at the temperature of 80 °C and the optimum extraction time between 90-120 minutes.

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TÓM TẮT

ẢNH HƯỞNG CỦA ĐIỀU KIỆN TRÍCH LI ĐẾN HÀM LƯỢNG POLYPHENOL, FLAVONOID, S-ALLYL CYSTEINE VÀ HOẠT TÍNH CHỐNG OXY HÓA CỦA DỊCH CHIẾT TỎI ĐEN

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Tỏi đen được chế biến từ tỏi tươi (*Allium sativum* L.) thông qua quá trình ủ chín trong một khoảng thời gian dài ở nhiệt độ và độ ẩm cao thích hợp. Tỏi đen có nhiều tác dụng có lợi đối với sự chuyển hóa trong cơ thể và bệnh tim mạch. Trong nghiên cứu này, (*i*) nước và dung dịch ethanol (50 % và 100 %) được sử dụng làm dung môi trích li tỏi đen với tỉ lệ nguyên liệu tỏi đen/dung môi khác nhau là 1/5, 1/10 và 1/15 (w/v) và (*ii*) Nhiệt độ và thời gian cho quá trình trích li trong khoảng 40 đến 80 ^{o}C trong thời gian từ 30 đến 120 phút. Tất cả các dịch trích đều được xác định hàm lượng các chất có hoạt tính sinh học (polyphenol tổng số, flavonoid tổng số và S-allyl cysteine). Hoạt tính chống oxy hóa được đánh giá bằng cách sử dụng phương pháp loại bỏ gốc tự do DPPH (2,2-diphenyl-1-picrylhydrazyl). Kết quả nghiên cứu cho thấy dịch trích li bằng dung dịch ethanol (50 %) có hàm lượng polyphenol và flavonoid tổng số (7,94 mg GAE/g và 3,37 mg QE/g, tương ứng) và khả năng loại bỏ gốc tự do DPPH là cao nhất với tỉ lệ tỏi đen:dung môi sử dụng là 1:10 (w/v). Trong khi đó, dịch trích trong nước cho hàm lượng S-allyl cysteine là cao nhất (76,0 mg/kg) ở tỉ lệ 1:10. Điều kiện thích hợp cho quá trình trích li polyphenol, flavonoid tổng số từ tỏi đen là 60 ^{o}C trong 90 phút và đối với SAC là 80 ^{o}C trong khoảng 90-120 phút.

Từ khóa: các hợp chất có hoạt tính sinh học, hoạt tính chống oxy hóa, tỏi đen, trích li.