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Ultrasound-Assisted Extraction of Polyphenols from Pomelo (*Citrus Grandis Limonia* Osbeck L.) Peel

Nghiên cứu trích ly polyphenol từ vỏ bưởi (*Citrus Grandis Limonia* Osbeck L.) có hỗ trợ sóng siêu âm

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Abstract

The pomelo peel occupies 50% of the fruit mass in pomelo juice processing. It contains large amounts of phenolic compounds, which may provide benefits to human health. These components should be isolated. In this study, the effects of ethanol concentrations, material-to-solvent ratios (g/mL), temperatures and sonication time on total phenolic content (TPC), naringin content and antioxidant capacity (using DPPH assay) of extract solution was evaluated. The results showed that all experimental factors significantly influenced the extraction of total polyphenol content, naringin content, and antioxidant capacity of the extract. The extraction condition was ethanol 80%, material-to-solvent ratio of 1:25 (w/v) at 60 °C, and sonication time of 7.5 min, gave the extract had total phenolic content of 9.05 ± 0.08 mg GAE/g DM, naringin content of 4.65 ± 0.08 mg NE/ g DM, and antioxidant capacity of 4.76 ± 0.03 mg AAE/g DM. The ultrasound treatment was a useful method for improving the extraction of phenolic acid compounds from pomelo peel.

Keywords: Antioxidant capacity, naringin, pomelo peel, polyphenols, ultrasound-assisted extraction.

Tóm tắt

Vỏ bưởi chiếm 50% khối lượng phụ phẩm của quá trình chế biến nước bưởi. Trong vỏ bưởi chứa nhiều phenolic acid, hợp chất có nhiều tác dụng tốt đối với sức khỏe con người. Việc chiết tách hợp chất này ra khỏi vỏ bưởi là điều cần thiết. Trong nghiên cứu này ảnh hưởng của nồng độ ethanol, tỷ lệ nguyên liệu dung môi và nhiệt độ trích ly đến hàm lượng polyphenol tổng số (TPC), hàm lượng naringin và hoạt tính chống oxy hóa (phương pháp DPPH) của dịch trích đã được đánh giá. Kết quả nghiên cứu cho thấy tất cả các yếu tố khảo sát đều có ảnh hưởng đến hàm lượng polyphenol tổng số, hàm lượng naringin và hoạt tính chống oxy hóa của dịch trích. Điều kiện trích ly với ethanol 80%, tỷ lệ nguyên liệu và dung môi là 1:25 (g/ml) tại nhiệt độ 60 °C và thời gian xử lý siêu âm là 7,5 phút cho dịch trích ly có hàm lượng polyphenol tổng số là 9,05 ± 0,08 mg GAE/g vck, hàm lượng naringin là 4,65 ± 0,08 mg NE/ g vck và hoạt tính chống oxy hóa là 4,76 ± 0,03 mg AAE/g vck. Ứng dụng siêu âm có hữu ích trong cải thiện hiệu quả trích ly các hợp chất axit phenolic từ vỏ bưởi.

Từ khóa: Hoạt tính chống oxy hóa, naringin, vỏ bưởi, polyphenol, trích ly có hỗ trợ siêu âm.

1. Introduction

Pomelo peel is the main waste product in pomelo juice production accounting for nearly 50% of the fruit mass [1]. This fact necessitates the recycling of these wastes. The pomelo peel includes two parts - the albedo (white color) and flavedo (green color) that have a high content of bioactive phenolic compounds, which provides health benefits such as anti-inflammatory [2], body weight control [3] and anti-cancer [4] properties. The beneficial effects of polyphenols are mainly attributed to their antioxidant properties since they can act as chain breakers or radical scavengers depending on their chemical structures. Ultrasound-assisted extraction (UAE) is a potential and alternative extraction technology considered as the cheaper technique with lesser instrumental requirements. The principle of UAE is based on acoustic cavitation that is able to improve solvent penetration into the plant body itself and damage the cell walls of the plant, which facilitates the release of the bioactive compounds [5]. The previous studies showed that ultrasound-assisted extraction (UAE) method is used isolating hesperidin from

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Penggan (*Citrus reticulata*) peel [6], phenolic acids and flavanone glycosides from Satsuma Mandarin (Citrus unshiu Marc) peel [7], extracting total phenolic compounds from Orange (*Citrus sinensis* L.) peel [8] and Grapefruit (*Citrus paradisi* L.) peel [9]. This study was carried out to find the suitable conditions for ultrasound treatment to assist the solvent extraction of polyphenols from pomelo peel. The objectives of this study were to establish a solvent extraction method (ethanol concentration, material and solvent ratio, extraction time) and ultrasonic treatment to improve polyphenol extraction from grapefruit peel.

2. Experiment Design

2.1. Preparation of Pomelo Peel Powder

The pomelo peel powder preparation is comprised of the following steps: washing, cutting, drying, milling, sieving, and storing the citrus peel powder. Pomelo Năm Roi (*Citrus Grandis Limonia* Osbeck L.) peel composed of two parts- the albedo (white color) and flavedo (green color) were purchased from Le Trung Thien Co., Ltd. They were packed in a plastic bag and transported to the laboratory on the same day. Pomelo peels were carefully washed, manually cut by knife approximately 2 cm in length, and dried in the oven at 60 ºC until the moisture content of samples was approximately 10-13%. Then, the dried pomelo peel was milled and passed a 1mm sieve mesh, and stored in a hermetic bag or desiccator for further steps.

2.2. Chemicals

Ethanol, sodium carbonate (Na_2CO_3), diethylene glycol, sodium hydroxide (NaOH) were from Xilong (China). Standard naringin, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, Folin - Ciocalteau reagent, Gallic acid were from Merck (Germany).

2.3. Experimental Designs

2.3.1. Effect of ethanol concentrations on the extraction of polyphenols

The experiment was carried out to evaluate the effect of ethanol concentrations (0%, 20%, 40%, 60%, 80%, and 100% (mL/mL)) on the polyphenols and naringin content of the extract.

One gram of the peel powder was used for each extraction. The ratio of material and solvent was fixed at 1:20 (w/v). The mixture was made in a falcon tube and vortexed for one minute before extraction. Shaking and temperature stabilization (60 ºC) was employed by using a water bath with shaking at 200 rpm. After one hour of extraction, the mixture was cooled down by putting into ice water and then centrifuged at a temperature of 4 for 10 min at a speed of 5000 rpm. The supernatant was filtered using a filter paper (What -man No.1), and the filtrate was used for biochemical analyses (TPC, naringin, and antioxidant capacity).

2.3.2. Effect of material and solvent ratio on the extraction of polyphenols

The extraction was carried out as explained previously (in section 2.3.1). The ethanol concentration was taken according to the results of the first experiment. The material and solvent ratios (g/mL) were 1:10, 1: 15, 1: 20, 1: 25, and 1: 30. Parameters for analysis including TPC, naringin, and antioxidant capacity content were then obtained.

2.3.3. Effect of temperature on the extraction of polyphenols

The extraction temperatures were experimented at 25 ºC (room temperature), 40 ºC, 50 ºC, 60 ºC, and 70 ºC. The material and solvent ratio was taken according to the results of the experiment in section 2.3.2. Parameters for analysis including TPC, naringin, and antioxidant capacity content were then obtained.

2.3.4. Effect of sonication time on the extraction of polyphenols

After ethanol concentration, temperature, and material to solvent ratio have been found, ultrasound treatment was introduced into the extraction. Two gram of pomelo peel was extracted at a time. Before extraction, the mixtures were treated for 0; 2.5; 5; 7.5; 10 minutes by using UP100H Ultrasonic processor (Hielscher, Germany). The power was set at 20 W. Parameters for analysis including TPC, naringin, and antioxidant capacity content were then obtained.

2.4. Analytical Methods

2.4.1. Determination of total phenolic compound (TPC)

The total phenolic content was determined using spectrophotometric method, with Folin-Ciocalteu reagent and Gallic acid as a standard [10]. One mL of sample was mixed with 10 mL of Folin-Ciocalteu reagent 10% at room temperature. After 5 minutes, 10 mL of sodium carbonate (7%) was added. The mixture then was shaken and incubated at room temperature for 90 minutes. Blue color development was measured at 765 nm using US-VIS spectrophotometer. The content of total phenolic compounds was expressed as mg/g Gallic acid equivalent (GAE) of dry extract.

2.4.2. Determination of naringin content

Naringin content was determined by Davis test with naringin $C_{27}H_{32}O_{14}$ as the standard analytical solution [11]. Ten milliliters of diethylene glycol were put into a test tube containing 0.1 mL of centrifuged sample. Then, 0.1 mL of sodium hydroxyl solution NaOH were added into the test tube, mixed well, and allowed to stand for 10 minutes. Yellow color

development was measured at 420 nm using US-VIS spectrophotometer. The naringin content was determined from the linear equation of a standard curve prepared with naringin standard. The content of naringin was expressed as mg/g naringin (NE) equivalent of dry extract.

2.4.3. Determination of antioxidant capacity

By radical scavenging ability, the antioxidant capacity of sample was measured using 2,2-diphenyl-1-picrylhydrazyl stable radicals (DPPH assay) [12] with some modifications. The stock solution DPPH was prepared by dissolving 24 mg DPPH into 100 mL ethanol to reach the absorbance 1.1 ± 0.02 unit at 517 nm. 150 µl centrifuged sample was taken into 2850 µl DPPH solution and placed for 30 min in the dark. The color development is measured at 517 nm using US-VIS spectrophotometer. The antioxidant capacity was determined from the linear equation of a standard curve prepared with ascorbic acid. The antioxidant capacity was expressed as mg/g ascorbic acid equivalent (AAE) of dry extract.

2.5. Statistical Analysis

All experiments were carried out in triplicates. Data and results were analyzed by using SPSS software and *p*-value (< 0.05) and ANOVA One-way analysis of variance (ANOVA) with Tukey's test was used to determine the significant differences ($p < 0.05$) between the means.

3. Results and Discussion

3.1. Effect of Concentration of Ethanol on Polyphenol Extraction

The ethanol concentration showed a significant effect ($p < 0.05$) on the total polyphenols (TPC) and naringin content, as well as on the antioxidant capacity (Table 1).

The highest values of TPC (6.51 mg GAE/g DM), naringin (3.16 mg NE/g DM), and antioxidant capacity (3.65 mg AAE /g DM) were achieved when it was extracted at 80% of ethanol concentration at 60 ℃ for one hour, which was considered the optimum concentration of solvent extracted from pomelo peel (Table 1). The previous research showed that 70-80% ethanol has the highest antioxidant content [13,14]. Therefore, 80% ethanol was used as the extraction solvent for the next experiments. The general principle of solvent extraction "like dissolves like" demonstrated that solvents only extract those phytochemicals which have similar polarity with the solvents [15].

3.2. Effect of Material-To-Solvent Ratio on Polyphenol Extraction

The ratio of material and solvent was shown to have a significant effect ($p < 0.05$) on TPC, naringin content and antioxidant capacity. According to Table 2 the yields of TPC, naringin and antioxidant capacity were increased proportionally with the increase of material and solvent ratio (g/mL) from 1:10 to 1:30. The extract solution of the ratio 1:25 and 1:30 had the TPC, naringin content, and antioxidant capacity higher than the other ratios. However, the values of TPC (mg GAE/g DM), naringin (mg NE/g DM), antioxidant capacity (mg AAE/g DM) analyzed at 1:25 ratio (7.14, 3.53, 4.61, respectively) and 1:30 ratio (7.28, 3.66, 4.99, respectively) were not significantly different. For that reason, the material-to-solvent ratio (1:25) was considered for the extraction process from pomelo peel, which was fixed on the next experiments.

Table 1. The effect of ethanol concentrations on TPC, naringin content, and antioxidant capacity of the pomelo peel extract.

Ethanol concent-ration (%)	Total Polyphenolic content (mg GAE/g dm)	Naringin content (mg NE/g dm)	Antioxidant capacity (DPPH) (mg AAE/g dm)
0	$4.84^{c} \pm 0.06$	$1.56^{e} \pm 0.07$	$2.73^{d} \pm 0.04$
20	$4.98^{c} \pm 0.06$	$2.43^{d} \pm 0.04$	$3.51^{b} \pm 0.01$
40	$5.53^{b} \pm 0.02$	$2.61^{c} \pm 0.07$	$3.53^{b} \pm 0.01$
60	$5.60^{b} \pm 0.02$	$2.82^{b} \pm 0.06$	$3.57^{b} \pm 0.02$
80	$6.51^{a} \pm 0.23$	$3.16^{a} \pm 0.05$	$3.65^{a} \pm 0.01$
100	$5.36^{b} \pm 0.04$	$2.33^{d} \pm 0.04$	$2.84^{c} \pm 0.03$

The results expressed as mean ± STDEV (n = 3). Dm: dry matter. The values get different letters in a column showing the significant difference at p < 0.05 using Student's t

Table 2. The effect of different material and solvent ratios on TPC, naringin content, and antioxidant capacity of the pomelo peel extract

Material: solvent ratio	Total Polyphenolic content (mg GAE/g dm)	Naringin content (mg NE/g dm)	Antioxidant capacity (DPPH) (mg AAE/g dm)
1:10	$4.57^{c} \pm 0.03$	$2.50^{d} \pm 0.04$	$1.93^{e} \pm 0.02$
1:15	$5.85^{b} \pm 0.07$	$2.70^{c} \pm 0.04$	$2.87^{d} \pm 0.01$
1:20	$6.00^{b} \pm 0.09$	$3.19^{b} \pm 0.08$	$3.79^{c} \pm 0.10$
1:25	$7.14^{a} \pm 0.09$	$3.53^{a} \pm 0.08$	$4.61^{b} \pm 0.05$
1:30	$7.28^{a} \pm 0.05$	$3.66^{a} \pm 0.05$	$4.99^{a} \pm 0.05$

The results expressed as mean ± STDEV (n = 3). dm: dry matter. The values get different letters in a column showing the significant difference at p < 0.05 using Student's t

Similar results were reported by other researchers as well. The polyphenol content increased with the solid-to-solvent ratio increase [16,17,18]. Mass transfer principle explains the antioxidant content difference between samples. The driving force during mass transfer is the concentration gradient between the solid and the bulk of the liquid, which is greater when a higher solvent-to-solid ratio is used.

3.3. Effect of Temperature on the Extraction of Polyphenols

The increase in temperature from 25 ºC to 60 °C increased the contents of polyphenols and naringin, also for the antioxidant capacity in extraction (Table 3). When the temperature was 70 ºC, they decreased slightly. This result was similar to other investigations that TPC content extracted from citrus peel decreased at high temperatures [6]. The increased temperature can accelerate the extraction of TPC. It increases both the diffusion coefficient and the solubility of phenolic compounds in the extraction solvent and decreases the viscosity of the solvent, thus it facilitates phenolic compounds passage through the solid substrate mass [19,20]. It was reported that at high temperatures, the phytochemical compounds were decomposed, which explains why extraction temperature rise to 70 ºC did not improve the TPC, naringin content and antioxidant capacity.

The highest TPC (6.99 mg GAE/g DM), naringin content (3.57 mg NE/g DM) and the antioxidant capacity (4.62 mg AAE/g DM) were recorded at the treatment of 60 ºC . Therefore, in this experiment, the temperature at 60 ºC was considered to be the optimum temperature for polyphenols extraction and used in the next experiment.

Table 3. The effect of different temperatures on TPC, naringin content, and antioxidant capacity of the pomelo peel extract

Temper-ature (°C)	Total Polyphenolic content (mg GAE/g dm)	Naringin content (mg NE/g dm)	Antioxidant capacity (DPPH) (mg AAE/g dm)
25	$4.83^{d} \pm 0.04$	$2.17^{e} \pm 0.03$	$3.75^{d} \pm 0.02$
40	$6.06^{b} \pm 0.10$	$2.72^{d} \pm 0.01$	$4.26^{c} \pm 0.02$
50	$7.17^{a} \pm 0.06$	$3.01^{b} \pm 0.04$	$4.50^{b} \pm 0.07$
60	$6.99^{a} \pm 0.06$	$3.57^{a} \pm 0.03$	$4.62^{a} \pm 0.02$
70	$5.82^{c} \pm 0.09$	$2.89^{c} \pm 0.05$	$3.84^{d} \pm 0.04$

The results expressed as mean ± STDEV (n = 3). dm: dry matter. The values get different letters in a column showing the significant difference at $p < 0.05$ using Student's t

3.4. Effect of Sonication Time on the Extraction of Polyphenols

The ultrasound-treated samples had higher polyphenol content and antioxidant capacity than the control sample. Increasing the sonication time from 0 to 7.5 minutes significantly affected the polyphenols yield extraction and antioxidant capacity in a positive way (Table 4). The TPC, naringin and antioxidant capacity reached the highest values at the ultrasound time of 7.5 minutes, at 9.05 (mg GAE/g DM), 4.65 (mg NE/g DM) and 4.76 (mg AAE/g DM) respectively. However, when sonication time was increased up to 10 minutes, there was a slight reduction in the polyphenol content. On the other hand, the naringin content was not affected by the sonication time. The naringin contents at 0, 2.5, 5, 7.5, and 10 minutes were 3.28, 3.55, 4.40, 4.65, and 3.90 (mg NE/g DM) respectively, which are not significantly different. It also indicated that polyphenols and antioxidant capacity had an increasing trend with an increase in sonication time and decrease slightly with a further increase in sonication time to 10 mins.

Table 4. The effect of different temperatures on TPC, naringin content, and antioxidant capacity of the pomelo peel extract

Sonic-ation time (min)	Total Polyphenolic content (mg GAE/g dm)	Naringin content (mg NE/g dm)	Antioxidant capacity (DPPH) (mg AAE/g dm)
0	$6.93^{e} \pm 0.09$	$3.28^{e} \pm 0.05$	$4.42^{d} \pm 0.02$
2.5	$7.39^{d} \pm 0.12$	$3.55^{d} \pm 0.05$	$4.57^{c} \pm 0.02$
5.0	$8.48^{b} \pm 0.07$	$4.40^{b} \pm 0.07$	$4.69^{b} \pm 0.02$
7.5	$9.05^{a} \pm 0.08$	$4.65^{a} \pm 0.08$	$4.77^{a} \pm 0.03$
10	$7.80^{c} \pm 0.10$	$3.90^{c} \pm 0.10$	$4.61^{c} \pm 0.02$

The results expressed as mean ± STDEV (n = 3). dm: dry matter. The values get different letters in a column showing the significant difference at $p < 0.05$ using Student's t

Furthermore, there was a significant difference between the times of each ultrasound-assisted extraction treatment (0; 2.5; 5; 7.5; 10 mins). The highest values of mass yield and content of TPC, naringin, and antioxidant capacity were obtained at a sonication time of 7.5 min (9.05 mg GAE/g DM, 4.65 mg NE/g DM, and 4.76 mg AAE/g DM, respectively) (Table 4). From this result of the experiment, the sonication time of 7.5 minutes was considered the optimal time for ultrasound-assisted extraction of polyphenols from pomelo peel.

The effects of ultrasound can be explained by cavitation. The phenomenon produced bubbles in the solvent. The rupture of the bubbles will crack the plant cell wall, which promotes the inter-penetration of the

solvent into the plant cells to dissolve phytochemical compounds. For this reason, the increase in sonication time led to the plant cells being completely cracked, increasing the extraction efficiency within a certain sonication duration [21]. However, samples treated with sonication for a long time can reduce the number of antioxidant components in the extract, which has been reported in a previous study [22].

4. Conclusion

The results of this study demonstrated that the extraction of polyphenol content, naringin content and the antioxidant capacity from pomelo (*Citrus grandis* (L.) Osbeck) peel were affected by the concentration of ethanol, material:solvent ratio, extraction temperature, and sonication time. The suitable extraction condition was found at material:ethanol 80% ratio of 1:25, temperature at 60 ºC and sonication time for 7.5 mins. Under these conditions, the highest TPC, naringin, and antioxidant capacity of extract were 9.053 mg GAE/g DM, 4.65 mg NE/g DM, and 4.76 mg AAE/g DM, respectively; when compared with the sample without ultrasound treatment (6.93 mg GAE/g DM, 3.28 mg NE/g DM, and 4.42 mg AAE/g DM).

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