# OPTIMIZING CONDITIONS FOR TOTAL PHENOLIC CONTENTS USING ULTRASOUND-ASSISTED EXTRACTION FROM CASHEW TESTA (*Anacardim occidentale* Linn.)

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**Abstract**: The optimal experiments on cashew testa (*Anacardim occidentale* Linn.) are conducted with three factors of extraction (including liquid-solid ratio, extraction time, and extraction temperature), and the responses is total phenolic content (TPC). The result shows the highest of TPC at 375.73 mg of gallic acid equivalent/g dry weight (mg GAE/g DW) when the solvent is methanol, liquid-solid ratio at 42.6 (mL/g), extraction time at 97.8 (min), and extraction temperature at 48.5°C. The quantitative analysis of five main components of cashew testa including catechin, epicatechin, epigallocatechin gallate (EGCG), epicatechin gallate and caffein collected from the seven regions of Vietnam is performed by HPLC. The results showed that epicatechin (44.539 mg/g DW) was the highest content, following by epicatechin gallate (13.501 mg/g DW).
**Keywords**: Box-Behnken, Cashew testa, Catechin, Epicatechin, Total phenolic, Untrasoud.

## 1. INTRODUCTION

Cashew (Anacardim occidentale Linn.) is a fruit and seed crop distributed in many countries in around the world, and cashew testa is the waste from cashew nuts processing. The previous studies about cashew testa show that it is a really good source for tannin and (+)- catechin, (-)-epicatechin, and those compounds are also the main major components of polyphenol in the cashew testa [1-3]. That finding is in agreement with the study of Trox et al. (2011), in which (+)- catechin và (-)-epicatechin were about 5.7 and 4.46 g/kg, respectively [1]. Besides, (+)- catechin and (-)-epicatechin exhibited several health benefits, such as scavenging free radicals and retarding extracellular transformation induced by ultraviolet (UV) radiation and pollution [4]. In another report, catechins could activate collagen synthesis and prevent the increase of metalloproteinase enzymes [5]. Free-radical scavenging activities of EGCG and epicatechin gallate on free-radical scavengers were better than many other standards of antioxidants, such as ascorbic acid (Vitamin A), tocopherol (Vitamin E), and trolox [6-7]. Hence, catechins became the compounds that have been increasingly used in the field of food, medicine, pharmaceutical, and cosmetics and are being actively studied in various approaches. Therefore, cashew testa is one of the rich sources of catechins and is worthy of attention and exploitation. In addition, the extraction with ethanol of cashew testa was shown to give the expression of a significant level of antioxidant activity, which was supposed by its phenolic composition [8-9]. On the other hand, the cashew testa was also used as food for rats and the cashew testa had no effect on liver, heart, lungs, kidneys, and intestinal weights [10].

In this study, the best conditions of the extraction time, extraction temperature and liquid-solid ratio to yield phenolic from cashew testa and analyzed the major components of polyphenol in the cashew testa collected from Binh Phuoc in Vietnam were figured out.

## 2. MATERIALS, EXPERIMENTAL AND ANALYSIS METHODS

### 2.1 Materials

Cashew testa was collected from Binh Phuoc province, Vietnam, at the time of harvest and processing. After being separated from the raw cashew nuts, the testa was brought to the laboratory, washed, and dried at a temperature of 50°C to achieve a moisture content of 6.52±0.01 (%). The samples were then finely ground and sieved to a particle size of 0.5-1 mm. After processing, the cashew testa samples were placed in polyethylene (PE) bags and stored in airtight plastic containers at room temperature (20-22°C), avoiding light and humidity, before conducting experiments and analyses.

### 2.2 Experiment methods

#### 2.2.1 Ultrasound-assisted extraction (UAE)

Cashew testa (1 g) was added to a beaker and filled with MeOH. And then, the beaker was placed in an ultrasonic extractor (Sonica 3200 EPs) at the right conditions of extraction time, liquid-solid ratio, and extraction temperatures. After extracting, the extracts were stored at 5ºC for the next analysis [11]

#### 2.2.2 Optimal experimental design

Box-Behnken design (BBD) was applied for the experimental design using three variables including liquid-solid ratio (A) (mL/g), extraction time (B) (min) and extraction temperature (C) (ºC) with three levels. The resulted design has a total of 17 experiments, in which consisted of a 12-factorial design, and 5 center points (Table 1). The main interactional effects of the factors in the experimental region of A, B, C on TPC yields (Y) were applied in these experiments [12]. The independent variables were coded at three levels (-1, 0, +1) and the influence of extraction includes A (40-60), B (80-100 min), C (40-60ºC). The impacts of experimental variables on the response were described by second-order polynomial equation:

$$Y = \beta_0 + \sum_{j=1}^{3} \beta_j X_j + \sum_{j=1}^{3} \beta_{jj} X^2_j + \sum\sum_{i<j} \beta_{ij} X_i X_j \quad (1.1)$$

Where Y represents the response function, $\beta_o$ is the intercept; $\beta_j$ is the linear coefficients; $\beta_{jj}$ is the quadratic coefficient; $\beta_{ij}$ is the interaction coefficient; $X_i$ and $X_j$ are independent variables [13]. Model response surface of three factors liquid-solid ratio, extraction time, extraction temperature. The result is shown in Table 2.

### 2.3. Analysis methods

#### 2.3.1 Determination of total phenolic content

The TPC in the cashew testa extracts was quantified by the Folin-Ciocalteu (FC) method, followed the previous research by Singleton and Rossi [14] with small changes. 0.5 mL extract solution was added into the test tubes containing 2.5 mL of FC 10%, shaken for hommogenous, and kept for 3-8 min in the dark. Then the mixture was added 2 mL $Na_2CO_3$ 7.5%, shaken, and incubated in the dark for 60 min. The absorbance was taken by a UV–vis spectrophotometer (Thermo GENESYS 20 UV-Vis) at 765 nm and garlic acid was used as a standard compound.

#### 2.3.2. Determination of main compounds in cashew testa by HPLC

The cashew testa sample was extracted under the same conditions (optimal condition) with A (43 mL/g), B (98 min) and C (48ºC), solvent MeOH. After filtering, the samples were stored at low temperature for analysis.

Five compounds (catechin, epicatechin, epicatechin gallate, EGCG, caffein) were separated and analyzed by liquid chromatography system with a UV detector (UHPLC-UV Ultimate 3000 of Thermo, USA), Hypersil C18 BDS column (250 mm × 4.6 mm ×5 µm) [15]. The solvents of mobile phase were MeOH (A) and $H_2O$ containing 0.1% $H_3PO_4$ (B). Progress decompose by gradient mode as follows: from 0 to 0.5 min, 95% B; 0.5 to 8.0 min 95-83% B; 8.0 to 10.0 min 83-70% B; 10.0 to 15.0 min 70-55% B; 15.0 to 20.0 min 55-5% B; from 20.0 to 22.0 min 5-95% B; from 22.0 to 23.0 min 95% B. Flow rate 1.0 mL/min with time run sample 23 min. Using UV probe with wavelength 265 nm, column temperature 30ºC, sample injection by system automatically with a sample volume of 5 µL. Calculate the result based on the standard curve equations of the standards at the same condition.

#### 2.3.3 Statistical analysis

Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA) and the experiments were performed in triplicate. The data analysis tool in Microsoft Excel 2013® was used to analyze the experimental results of the response surface designs. Design expert software (Version 11) was applied to model analysis and selection.

## 3. RESULT AND DISCUSSION

### 3.1. Fitting the Box-Behnken model for TPC yield

Three single factors that included liquid-solid ratio (A), extraction time (B) and extraction temperature (C) (°C) were selected and used for experiments where Y defined as the response value. There were two regression equations of the correlation between the response variables and the experiment factors, which analyzed multiple regression on the experimental data and conducted using Design-Expert Software. The model of Y response was function of A, B, and C and noted in equation 1.2.

$Y_2=19.32+0.08A+0.36B–0.14C–0.36A^2–0.37B^2–0.31C^2$ (1.2)

**Table 1**: BBD with three-factor matrix of cashew testa and results

| Run | Factors of extractions | | | Responses surfaces |
|---|---|---|---|---|
| | A (g/mL) | B (min) | C (°C) | Y (mg GAE/g DW) |
| 1 | 40 | 90 | 50 | 375.03±9.86 |
| 2 | 40 | 90 | 50 | 372.05±8.74 |
| 3 | 50 | 90 | 40 | 353.8±0.51 |
| 4 | 50 | 90 | 60 | 344.14±0.64 |
| 5 | 50 | 80 | 50 | 334.67±5.06 |
| 6 | 30 | 90 | 60 | 342.59±2.55 |
| 7 | 40 | 90 | 50 | 371.07±4.35 |
| 8 | 30 | 80 | 50 | 328.84±3.79 |
| 9 | 30 | 90 | 40 | 351.68±4.46 |
| 10 | 40 | 80 | 60 | 327.61±8.79 |
| 11 | 40 | 90 | 50 | 373.74±3.71 |
| 12 | 40 | 90 | 50 | 374.3±3.40 |
| 13 | 30 | 100 | 50 | 353.46±2.29 |
| 14 | 40 | 100 | 60 | 355.84±3.64 |
| 15 | 40 | 80 | 40 | 343.21±1.33 |
| 16 | 40 | 100 | 40 | 364.3±2.46 |
| 17 | 50 | 100 | 50 | 366.73±7.21 |

Table 2 showed the summarization of the fitted model. The model performed good significant with $P<0.05$ (TPC <0.0001). Furthermore, the lack of fits of the models for TPC was 0.0696, that mean $P>0.05$ and had no significant. As this result of lack of fit, the model was good level of significant. Hence, all coefficients of determination were higher than 0.9 ($R^2$: 0.9879), which means, three factors (A, B, C) could explain 98.79% of the TPC response's result [16]. Those results pointed out that the models were the good predictability of the responses.

Based on the mentioned equations, TPC depended on all variable factors of the extraction process. In there, TPC response performed well significantly by the quadratic effects of A, B, and C factors, the TPC was impacted significantly by all three factors. Besides the good effect of three factors on the TPC yield, two-variable interaction including AB, AC, and BC had no good effects. Whereas the pairs of two-variable interaction, that is A-B, all two-variable interactions of three factors were not significant on TPC. According to the results of building the model that could describe the relationship between the independent variables and response value, and they were worth expectation.

**Table 2:** Result of experimental design

| **Source** | **TPC** | | | |
|---|---|---|---|---|
| | Coeffection | F-value | P-value | b |

| | | | | |
|---|---|---|---|---|
| Model | 19.32 | 63.27 | < 0.0001 | ** |
| A | 0.0760 | 9.00 | 0.0199 | * |
| B | 0.3559 | 197.43 | < 0.0001 | ** |
| C | -0.1439 | 32.28 | 0.0007 | ** |
| AB | 0.0474 | 1.75 | 0.2273 | |
| AC | -0.0037 | 0.0104 | 0.9216 | |
| BC | 0.0508 | 2.01 | 0.1995 | |
| $A^2$ | -0.3565 | 104.26 | < 0.0001 | ** |
| $B^2$ | -0.3682 | 111.19 | < 0.0001 | ** |
| $C^2$ | -0.3071 | 77.37 | < 0.0001 | ** |
| Lack of Fit (F) | 0.0696 | | | |
| $R^2$ | 0.9879 | | | |
| $R^2_{adj}$ | 0.9722 | | | |
| $P_{value}$ | < 0.0001 | | | |

A: Liquid-solid ratio (mL/g); B: Extraction time (min); C: Extraction temperature (°C); **: indicate highly significant ($p < 0.01$), *: indicate significant ($p < 0.05$).

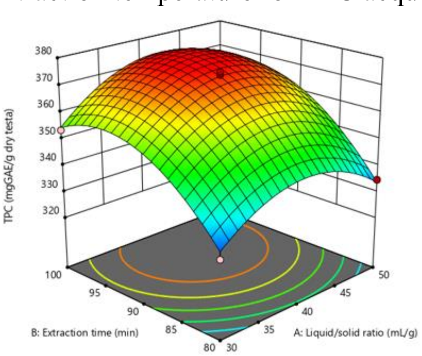
### 3.2. Response surface analysis

Investigating the correlative effects of the experimental factors on TPC in Box-Benhken model and the interactive result three-dimensional (3D) can provide visual imaging between two variables and facilitate the optimal conditions for maximal TPC yields [17]. The response surfaces plots were performed in Figure 1, and from these 3D plots, we can clearly see the active correlation between the two factors of A, B, and C.

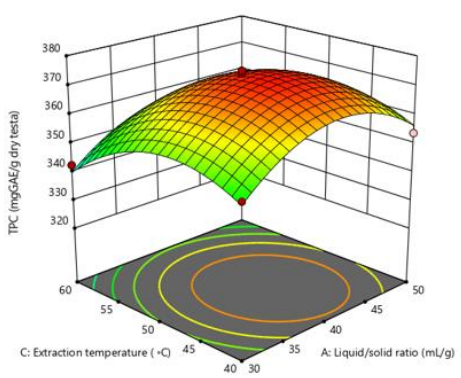
The effect of the extraction time on the TPC yield is shown in Figures 1a, 1c, when the extraction time interacted with the other factors. The maximal extraction yields that could be achieved for TPC when the extraction time was around 90 min, and at low and high levels of the extraction time, the TPC yields was lower. Compared with other heat extraction methods, ultrasound could promote the pervasion of solvents into cashew testa cells, and release intracellular products [18], which can increase the extraction efficiency and shorten extraction time.

For the liquid-solid ratio correlation to the other experimental factors on TPC is shown in Figures 1a, 1b. It was obvious that the yield of TPC increased when increasing the ratio from 30:1-40:1 (mL/g), but the A value over 40:1 (mL/g) appeared to ebb extraction yields. These results have also been reported for the phenolic extraction from different materials [19]. The best liquid-solid ratio for TPC yields is around 40 (mL/g).

The effect of extraction temperature is shown in Figures 1b, 1c, and the yield of TPC increase when the extraction temperature rises from 40 to 50ºC, this might be due to the increase in extraction temperature that accelerated the mass transfer. The TPC was decreased when temperature around 50ºC. The temperature over 50ºC appeared to be disadvantaged on the extraction of TPC, because high temperature increases the thermal decomposition of some heat-sensitive flavonoid molecules, which in turn reduces the TPC [20]. So, the best extraction temperature for TPC acquisition is around 50ºC.




a

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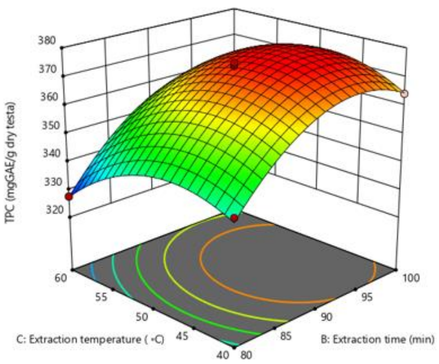


c

**Figure 1.** Response surface plot showing interactive effects of independent variables on the TPC yield. a, b, c, d, e, f: the picture of Response surface with the correlation of the different conditions

### 3.3. Optimization results and verification of method

According to the result, the optimal under correlation of three factors to extract the highest TPC contents from cashew testa were determined as follows: liquid-solid ratio 42.6 (mL/g), extraction time 97.8 (min), and extraction temperature (48.5°C), the TPC was 375.73 mg GAE/g. The results are shown in Table 3. The result of TPC in Vietnamese cashew testa has a little higher than in Sri Lanka samples [21], and the TPC content is better than the total phenolic that extract from other materials in the previous research [21-23].

To further check the reliability of the BBD method, confirmative experiments were conducted three times under these optimal conditions (A: 42.6 mL/g, B: 97.8 min, C: 48.5°C). The predicted results of the TPC suited the experimental values (Table 3), which were obtained using three factors at the optimum extraction and confirmed by the CV at 0.55.

**Table 3:** The content of total phenolic of extracts prepared under three optimal conditions and ultrasound methods.

| Response | Optimum Extraction Conditions | | | Maximum Value | | % Difference (CV) |
|---|---|---|---|---|---|---|
| | A (mL/g) | B (min) | C (°C) | Experimental (mg/g) | Predicted* (mg/g) | |
| Y | 43 | 98 | 48 | 373.66 | 375.73 | 0.55 |

### 3.4 Quantitative results of main compounds in cashew testa from seven locals

An analysis was conducted on five phenolic compounds, which are the main components present in the extract of cashew testa collected in Bình Phước, including catechin, epicatechin, epicatechin gallate, epigallocatechin gallate (EGCG), and caffeine using the HPLC method. The quantitative results for these compounds are shown in Table 4, and the chromatograms obtained during the analysis are depicted in Figure 2. The results indicate that the predominant component is epicatechin with a concentration of 44.539 mg/g DW, followed by epicatechin gallate with a concentration of 11.256 mg/g DW. The concentrations of these two compounds are significantly higher compared to the others. EGCG has the lowest concentration (2.677 mg/g DW) among the four phenolic catechins, while caffeine has a lower concentration than the four phenolic catechins (1.335 mg/g DW).

The main components in the extract from cashew testa in Bình Phước, Vietnam, differ from those found in cashew testa from other countries such as Indonesia, India, and Sri Lanka. According to previous research, the main components found in cashew testa from these countries are polyphenols of catechin and epicatechin. The highest concentrations were found in India with amounts of 60 and 75 mg/g DW, followed by Sri Lanka with amounts of 47.289 and 28.291 mg/g DW, and Indonesia with amounts of 5.7 and 4.46 mg/g DW, respectively [1, 9, 24]. The catechin content in the Vietnamese sample is lower compared to other countries, while the epicatechin content is lower than in India's cashew testa but higher than the cashew testa of Sri Lanka and Indonesia. All these results are presented in Table 4.

**Table 4:** Comparison of the main components of cashew testa from different areas (mg/g DW)

| Areas | Catechin | Caffein | EGCG | Epicatechin | Epicatechin gallate | Reference |
|---|---|---|---|---|---|---|
| Binh Phuoc | **4.279** | **1.335** | **2.677** | **44.539** | **11.526** | |
| Indonesia | 5.7 | - | - | 4.46 | - | [1] |
| India | 60 | - | - | 75 | - | [9] |
| Sri Lanka | 47.289 | - | - | 28.291 | - | [24] |

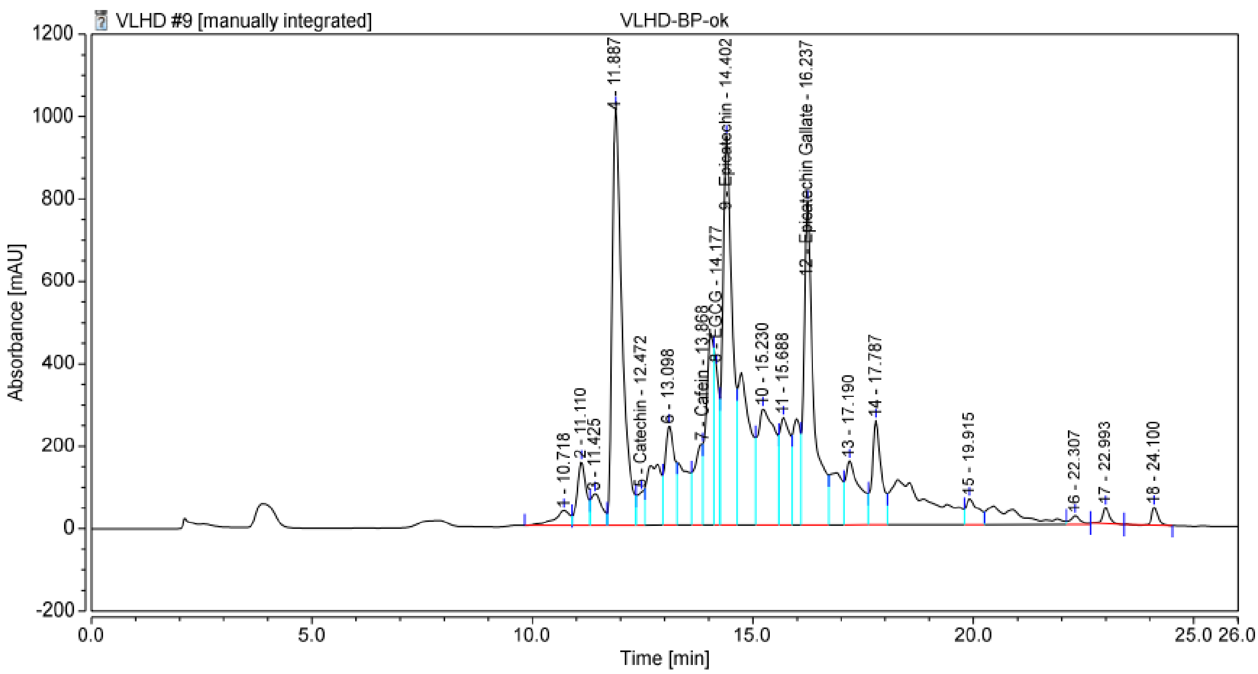


**Figure 2**. Representative HPLC-DAD chromatograms of the ethanolic extract from Binh Phuoc sample

## 4. Conclusions

Optimal three conditions to yield TPC and the ultrasonic-assisted technique could be used as an effective method to extract phenolics from cashew testa. The results showed good effect of three parameters including liquid-solid ratio, extraction time, extraction temperature on the yield TPC. Besides, the present study also analyzed the five main compounds in cashew testa including catechin, epicatechin, epicatechin gallate, EGCG, and caffein by HPLC, in with the highest content in the cashew testa of seven samples are epicatechin, next is epicatechin gallate, and the lowest content is caffein. From this study, cashew testa become the rich source of epicatechin and epicatechin gallate, provides constructive information to further investigate cashew testa to create new application for cashew testa, such as modulation of epicatechin and epicatechin gallate.

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# TỐI ƯU HÓA CÁC ĐIỀU KIỆN CHIẾT XUẤT TỔNG HÀM LƯỢNG PHENOLIC TỪ VỎ LỤA HẠT ĐIỀU (*ANACARDIUM OCCIDENTALE* LINN.) CÓ SỰ HỖ TRỢ CỦA SIÊU ÂM


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**Tóm tắt:** Tối ưu hóa các điều kiện chiết trên nguyên liệu là vỏ hạt điều (*Anacardim occidentale* Linn.) đã được tiến hành với ba yếu tố chiết xuất bao gồm tỷ lệ dung dịch-chất rắn, thời gian chiết và nhiệt độ chiết, hàm mục tiêu là tổng hàm lượng phenolic (TPC). Kết quả cho thấy hàm lượng TPC cao nhất đạt 375.73 mg axit gallic /g trọng lượng khô (mg GAE/g DW) khi sử dụng dung môi là methanol, tỷ lệ dung dịch-chất rắn là 42.6 (mL/g), thời gian chiết là 97.8 (phút), và nhiệt độ chiết là 48.5°C. Phân tích định lượng năm thành phần chính của vỏ hạt điều bao gồm Catechin, Epicatechin, Epigallocatechin gallate (EGCG), Epicatechin gallate tại bảy địa phương ở Việt Nam đã được thực hiện bằng phương pháp HPLC. Kết quả cho thấy rằng Epicatechin (25.495-44.539 mg/g DW) có hàm lượng cao nhất, tiếp theo là Epicatechin gallate (7.492-13.501 mg/g DW), Catechin (2.625-4.279 mg/g DW), EGCG (1.609-2.168 mg/g DW), và caffein(1.335-1.168 mg/g DW).



**Từ khóa.** tổng hàm lượng phenolic, vỏ hạt điều, Epicatechin, Catechin.

*Ngày nhận bài: 20/01/2024*

*Ngày chấp nhận đăng: 07/10/2024*