# EXTRACTION OPTIMIZATION AND ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS FROM AVOCADO PEEL

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**Abstract**

Avocado peel has been considered as a potential source of natural antioxidants in which phenolics are among the most important compounds. Therefore, this study aims to optimize the extraction process of phenolics using response surface methodology and evaluate the corresponding antioxidant activity. From the quadratic model, the optimal condition was determined including the ethanol concentration 54.55% (v/v), the solvent/solute ratio 71.82/1 (mL/g), temperature 53.03 °C and extraction time 99.09 min. The total phenolic content and the total antioxidant capacity at this condition with minor modifications were 26,74 ± 0,04 (mg GAE/g DW) and 188.06 ± 1.41 (mg AAE/g DW), respectively. The significant correlation between total phenolic content and total antioxidant capacity was also confirmed.

***Key words:*** *response surface methodology, central composite rotatable design, total phenolic content, total antioxidant capacity, avocado peel.*

## 1. INTRODUCTION

Phenolic compounds occurring commonly in plants and agricultural by-products have been seen as important natural constituents since they possess various biological effects such as anti-allergenic, anti-artherogenic, anti-microbial, anti-inflammatory, anti-thrombotic, cardioprotective and vasodilatory activities [1]. Many of these effects are considered to be related to their antioxidant activity through different mechanisms, including reduction or scavenging of reactive oxygen species, chelation of transition metal ions, and inhibition of enzymes involved in oxidative stress [2]. Therefore, much attention has been focused on practical aspects of phenolic extraction from agricultural wastes which are effective and inexpensive sources of phenolic antioxidants [3].

In the interest of seeking for a good source of phenolic compounds from local agricultural by-products, we collected several residual products including avocado peels and seeds (*Persea americana* Mill.), grapefruit peels (*Citrus grandis* (L.) Osb. var. *grandis*), peanut shells (*Arachis hypogaea* L.), mung bean (*Vigna radiata* (L.) R. Wilczek) and cowpea (*Vigna unguiculata* Walp. subsp. *cylindrica* (L.) Verdc.) seed pods, manihot stems (*Manihot esculenta* Crantz), and the residual powder of turmeric rhizomes (*Curcuma longa* L.) and elephant yam corms (*Amorphophallus paeoniifolius* (Dennst.) Nicolson). Our screening tests for total content of phenolics have shown that avocado peel is one of the richest sources of phenolics among the tested waste products.

Avocado (*Persea americana* Mills.) belonging to Lauraceae family is widely distributed in most of the tropical and subtropical countries. This fruit is rich in vitamins (C, B and E), potassium, dietary fiber and unsaturated fatty acids such as oleic, linoleic and α-linolenic acids which are highly beneficial to human health. The mainly consumed part, however, is the edible flesh of fresh fruits while other avocado by-products, particularly peels, are usually discarded, raising environmental concerns [4].

The avocado by-products generally showed higher TPC than other fresh fruits, vegetables, and plant extracts, described in the literature as good sources of polyphenols. For instance, the TPC of selected Mediterranean fruits and northern berries ranged from 69 to 4604 mg GAE/100 g and from 1190 to 5080 mg GAE/100 g, respectively, whereas common vegetables such as beetroot and carrots had between 40 and 740 mg GAE/100 g [5]. Although avocado peel has been reported as a potential antioxidant source with larger amounts of phenolics, there have been insufficient data about the optimization of extraction processes which can be applied in practical aspect [4], [6].

The yield of chemical extraction usually depends on many factors of the extraction process as well as on the chemical composition and physical characteristics of the samples [7]. Firstly, solvents play a key role in the extraction process which

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*Received: 18/12/2018, Resived: 12/6/2019; Accepted: 15/6/2019*

is influenced by the solubility of the phenolic compounds varying greatly in different plants. Thus, it is impractical to develop a standard extraction procedure suitable for the extraction of all plant phenols [5]. In general, polar solvents are used for extracting phenolic compounds from plant matrices such as methanol, ethanol, acetone, ethyl acetate, and their combinations, often with different proportions of water. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols. However, ethanol has been known as a good solvent for polyphenol extraction and, most importantly, is safe for human consumption [7]. Secondly, extraction time and temperature, which reflects the conflicting actions of solubilization and analyte degradation by oxidation, also influence the recovery of phenolic compounds. These factors are in turn related to extraction techniques used. While the conventional extraction methods such as maceration and soxhlet extraction have shown low efficiency and potential environmental pollution, ultrasound-assisted extraction, among other methods developed in recent years, is a potentially useful technology which does not require complex instruments and is relatively low-cost. Because of its applicability on both small and large scale, this method has been widely used in the natural product industry. In addition, another factor may affect the yield of phenolic compounds is the solvent-to-sample ratio (or liquid-to-solid ratio, LSR) which is able to enhance phenols yields but it is still needed to obtain an optimized value due to the balance between cost and efficiency [7].

From the above reasons, the ultrasound-assisted extraction method and the aqueous ethanol were used in this current study which aims to optimize four factors of extraction conditions including solvent composition, i.e. the percentage of ethanol in water, liquid-solid ratio, extraction temperature and time. The optimization was conducted using response surface methodology. Also, the correlation between the resulting phenolic contents and their antioxidant activities was elucidated using phosphomolybdenum assays.

## 2. MATERIALS AND METHODS

### Chemicals and equipments

Chemical reagents were used including Folin-Ciocalteu's phenol reagent (2N), phosphomolybdenum (>98%), gallic acid (97.5-102.5%) (Sigma-Aldrich), ascorbic acid (Northest Pharmaceutical Group Co., P.R.C), and other reagents which are of analytical grade.

Ultrasound extraction was conducted using Elma S100 (Elmasonic, Germany). The molecular absorption spectra and absorbance at specific wavelengths were recorded with UV-visible spectrophotometer V630 (Shimadzu, Japan). The other laboratory equipments were utilized including analytical balance GR-200 (A&D, Japan), centrifuge Z326K (HERMLE Labortechnik GmbH, Germany), waterbath WNE and heating oven (Memmert, Germany), micropipette Biopette (Labnet, USA), and other analytical glassware.

### Samples and sample preparation

Ripe avocado fruits (*Persea americana* Mills.) were purchased from local suppliers in Quang Tri province, Viet Nam, between April and August. The peels were then manually separated from the flesh and cleaned under the flow of tap water.

Fresh avocado peels (AP) were chopped into small pieces, roughly about 1 × 1 cm, and dried in heating oven at 50 °C. They were ground and the resulting powder was sieved through stainless steel sieve (aperture size 2 mm). This powder was stored in sealed plastic bags in the dark, at room temperature without exceeding a storage duration of 4 weeks, and was mixed well before using for experiments. The dried weight (DW) determination of samples was followed the instruction of Vietnam National Standards (TCVN) No. 9738 (ISO 1572) regulation.

### Evaluation of total phenolic content (TPC)

Phenolic measurements were conducted using the Folin-Ciocalteu's phenol reagent according to TCVN 9745-1:2013 regulation. Briefly, 1 mL of filtered extract was mixed with 5 mL Folin-Ciocalteu's reagent (diluted 1:10 with distilled water) and subsequently adding 4 mL of 7.5% sodium carbonate in water after about 3 to 8 min. The mixture was then mixed well and the absorbance was measured at 765 nm after keeping at room temperature within 60 min. Extraction solvents were used instead of extracts in case of blank samples. The results are expressed as miligram of gallic acid equivalents (mg GAE) per gram of dry weight (g DW).

### Evaluation of total antioxidant capacity (TAC)

TAC was measured using phosphomolybdenum method (PM) according to Prieto *et al.* (1999) [8]. Briefly, 0.3 mL of filtered extract was mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium sulfate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After the samples had

cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. The results are expressed as miligram of ascorbic acid equivalents (mgAAE) per gram of dry weight (gDW).

**Preliminary studies**

According to the literature data and the practical aspect in manufacturing process, four variables were chosen including solvent composition of ethanol-water (%EtOH, %), liquid-solid ratio (LSR, mL/g), temperature (T, °C), and time (t, min). The preliminary studies were followed the model described by Pradal *et al.* (2016) with some modifications [9]. The procedure was carried out step by step in which the previous results was subsequently used for the next experiments to obtain central point (Cp) values of all four variables applied in the main optimization studies (**Table 1**).

**Table 1.** Preliminary study design

| Step | Variables | %EtOH | LSR | T | t | Cp values | Unit |
|---|---|---|---|---|---|---|---|
| 1 | Solvent composition | **30 – 80**[a] | 40/1 | 50 | 50 | **C** | **%** |
| 2 | Liquid-solid ratio | C | **10/1 – 140/1**[b] | 50 | 50 | **R** | **mL/g** |
| 3 | Temperature | C | R | **30 – 70**[c] | 50 | **T** | **°C** |
| 4 | Time | C | R | T | **30 – 150**[d] | **t** | **min** |

*[a, b, c, d] values varied with a 10-unit in each step ranging from the lowest value to the highest value. The cp value was assigned when the resulting TPC value were the highest among screening experiments.*

All experiments were conducted using ultrasound extraction method and 0.5 g of dried AP powder. The resulting extracts were centrifuged at 4 °C and 5000 rpm in 15 min. After filtering the supernatant solutions, the filtered extracts were evaluated for their TPC and TAC.

**Optimization studies**

**Response surface methodology (RSM**) presented by Box and Wilson, with a four-variable and five-level central composite rotatable design (CCRD), was employed to optimize extraction conditions for the highest TPC from dried AP powder [10].

A model for a second-order interaction presents the following terms:

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} X_i X_j + \varepsilon$$

Where k is the number of variables, $\beta_o$ is the constant term, $\beta_i$ represents the coefficients of the linear parameters, $\beta_{ii}$ represents the coefficients of the quadratic parameters, $\beta_{ij}$ represents the coefficients of the interaction parameters, and ε is the residual associated to the experiments. This polynomial quantifies relationships among the measured response Y and a number of experimental variables $X_1 \ldots X_k$ [10], [11].

**Mathematical–statistical treatment of data**

All experiments were conducted in triplicate to present data as mean values.

Experimental model design, statistical analysis of the experimental results, and all related graphs were performed using Minitab® v.17.0 software (Minitab Inc., USA) [11]. Data were also analyzed using Microsoft Excel 2013 and SPSS 23.0 if applicable.

## 3. RESULTS AND DISCUSSION

### 3.1. Results of optimization studies

The operating ranges for all the factors were chosen by a set of preliminary measurements according to the **Table 1**, the resulting values were the corresponding central point values of the CCRD model which are shown in **Table 2** (coded level of zero).

Traditionally, optimization in extraction method has been performed by changing one factor at a time on an experimental response, called one-variable-at-a-time. Its major drawback is that it does not include the interactive effects among the variables studied. Consequently, this method cannot estimate the complete effects of the parameter on the response. Therefore, response surface methodology has been considered as an effective solution to overcome the above problem since it is well applied when a response or a set of responses of interest are influenced by various variables [10].

Central composite design (CCD) is probably the most popular class of experimental designs, which allow for efficient estimation of second-order response surfaces. This design is rotatable (CCRD) when each experimental factor is represented at the five levels of coded units including -α, -1, 0, 1, α. As the result, it ensures constant variance at

points that are equidistant from the center point, and therefore provides equal precision of response estimation in any direction of the design. The α value is determined in a full factorial CCD as $\alpha = (2^k)^{0,25}$, since k = 4, α = 2 in this study [11]. After selecting design and measuring the central point of each factor, the experimental variables and the levels at which they were tested are shown in **Table 2**.

**Table 2.** Tested levels of experimental factors

| Name of variable | Coded factor | Uncoded factor | Unit | Coded Levels and Corresponding Absolute Levels | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | - α | -1 | 0 | +1 | + α |
| Solvent composition | $X_1$ | $U_1$ | % | 40 | 50 | 60 | 70 | 80 |
| Liquid-solid ratio | $X_2$ | $U_2$ | mL/g | 50/1 | 60/1 | 70/1 | 80/1 | 90/1 |
| Extraction temperature | $X_3$ | $U_3$ | °C | 30 | 40 | 50 | 60 | 70 |
| Extraction time | $X_4$ | $U_4$ | min | 30 | 60 | 90 | 120 | 150 |

*α = 2, the absolute levels of factors were calculated as $U_i = u_i.\triangle U_i + U_{cp}$, where $U_i$ is the "real" level, $u_i$ varies in the range of -2, -1, 0, +1, +2; $\triangle U_i$ is the difference between two adjacent absolute values, and $U_{cp}$ is the absolute value measured at the central point (level zero).*

The experimental matrix was generated using Minitab v.17.0. The TPC measurement at the central point (level 0 in coded unit) was replicated seven times at different stages. The total number of experimental trials was calculated using the equation: $N = 2^k + 2k + cp$, where k = 4 and cp = 7, thus N = 31 trials. Experiments were conducted following this experimental matrix showing TPC values ranging from 19.55 to 25.29 (mg GAE/ g DW) and TAC values ranging from 139.42 to 175.14 mg AAE/g DW (**Table 3**).

**Table 3.** Central composite rotatable design and the corresponding TPC and TAC results

| No. | Uncoded | | | | Coded | | | | TPC (mg GAE/ g DW) | TPC predicted (mg GAE/ g DW) | TAC (mg AAE/ g DW) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $U_1$ | $U_2$ | $U_3$ | $U_4$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ | | | |
| 1 | 50 | 60/1 | 40 | 60 | -1 | -1 | -1 | -1 | 22.26 ± 0.21 | 21.67 ± 1.16 | 153.39 ± 0.69 |
| 2 | 70 | 60/1 | 40 | 60 | +1 | -1 | -1 | -1 | 20.19 ± 0.52 | 19.31 ± 1.16 | 145.82 ± 1.26 |
| 3 | 50 | 80/1 | 40 | 60 | -1 | +1 | -1 | -1 | 22.61 ± 0.28 | 22.50 ± 1.16 | 158.11 ± 2.47 |
| 4 | 70 | 80/1 | 40 | 60 | +1 | +1 | -1 | -1 | 19.84 ± 0.13 | 20.00 ± 1.16 | 145.67 ± 1.43 |
| 5 | 50 | 60/1 | 60 | 60 | -1 | -1 | +1 | -1 | 22.72 ± 0.34 | 22.26 ± 1.16 | 156.45 ± 0.62 |
| 6 | 70 | 60/1 | 60 | 60 | +1 | -1 | +1 | -1 | 20.74 ± 0.50 | 20.57 ± 1.16 | 146.08 ± 1.23 |
| 7 | 50 | 80/1 | 60 | 60 | -1 | +1 | +1 | -1 | 23.15 ± 0.05 | 22.87 ± 1.16 | 164.04 ± 1.01 |
| 8 | 70 | 80/1 | 60 | 60 | +1 | +1 | +1 | -1 | 21.31 ± 0.39 | 21.04 ± 1.16 | 149.08 ± 1.17 |
| 9 | 50 | 60/1 | 40 | 120 | -1 | -1 | -1 | +1 | 22.61 ± 9.20 | 22.33 ± 1.16 | 156.19 ± 1.50 |
| 10 | 70 | 60/1 | 40 | 120 | +1 | -1 | -1 | +1 | 20.37 ± 0.07 | 20.82 ± 1.16 | 146.70 ± 1.07 |
| 11 | 50 | 80/1 | 40 | 120 | -1 | +1 | -1 | +1 | 22.74 ± 0.28 | 23.08 ± 1.16 | 158.43 ± 1.36 |
| 12 | 70 | 80/1 | 40 | 120 | +1 | +1 | -1 | +1 | 21.50 ± 0.18 | 21.42 ± 1.16 | 150.32 ± 1.33 |
| 13 | 50 | 60/1 | 60 | 120 | -1 | -1 | +1 | +1 | 22.95 ± 0.15 | 22.97 ± 1.16 | 161.77 ± 1.06 |
| 14 | 70 | 60/1 | 60 | 120 | +1 | -1 | +1 | +1 | 22.56 ± 0.47 | 22.13 ± 1.16 | 158.77 ± 2.12 |
| 15 | 50 | 80/1 | 60 | 120 | -1 | +1 | +1 | +1 | 23.16 ± 0.18 | 23.50 ± 1.16 | 169.60 ± 1.51 |
| 16 | 70 | 80/1 | 60 | 120 | +1 | +1 | +1 | +1 | 21.76 ± 0.04 | 22.52 ± 1.16 | 152.28 ± 0.95 |
| 17 | 40 | 70/1 | 50 | 90 | -2 | 0 | 0 | 0 | 23.16 ± 0.04 | 23.48 ± 1.16 | 166.33 ± 2.26 |
| 18 | 80 | 70/1 | 50 | 90 | 2 | 0 | 0 | 0 | 20.09 ± 0.22 | 20.14 ± 1.16 | 145.70 ± 2.48 |
| 19 | 60 | 50/1 | 50 | 90 | 0 | -2 | 0 | 0 | 19.69 ± 0.13 | 20.67 ± 1.16 | 140.87 ± 1.42 |
| 20 | 60 | 90/1 | 50 | 90 | 0 | 2 | 0 | 0 | 22.50 ± 0.10 | 21.89 ± 1.16 | 173.29 ± 3.42 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | 60 | 70/1 | 30 | 90 | 0 | 0 | -2 | 0 | 21.26 ± 0.24 | 21.57 ± 1.16 | 143.41 ± 0.39 |
| 22 | 60 | 70/1 | 70 | 90 | 0 | 0 | 2 | 0 | 23.18 ± 0.05 | 23.25 ± 1.16 | 166.75 ± 1.29 |
| 23 | 60 | 70/1 | 50 | 30 | 0 | 0 | 0 | -2 | 19.55 ± 0.06 | 20.67 ± 1.16 | 139.42 ± 1.14 |
| 24 | 60 | 70/1 | 50 | 150 | 0 | 0 | 0 | 2 | 23.55 ± 0.13 | 22.81 ± 1.16 | 168.45 ± 2.43 |
| 25 | 60 | 70/1 | 50 | 90 | 0 | 0 | 0 | 0 | 24.62 ± 0.18 | 24.43 ± 1.16 | 172.15 ± 2.58 |
| 26 | 60 | 70/1 | 50 | 90 | 0 | 0 | 0 | 0 | 24.64 ± 0.09 | 24.43 ± 1.16 | 173.87 ± 1.56 |
| 27 | 60 | 70/1 | 50 | 90 | 0 | 0 | 0 | 0 | 24.21 ± 0.14 | 24.43 ± 1.16 | 169.65 ± 2.79 |
| 28 | 60 | 70/1 | 50 | 90 | 0 | 0 | 0 | 0 | 24.82 ± 0.20 | 24.43 ± 1.16 | 173.62 ± 1.40 |
| 29 | 60 | 70/1 | 50 | 90 | 0 | 0 | 0 | 0 | 23.66 ± 0.37 | 24.43 ± 1.16 | 167.79 ± 2.11 |
| 30 | 60 | 70/1 | 50 | 90 | 0 | 0 | 0 | 0 | 25.29 ± 0.15 | 24.43 ± 1.16 | 175.14 ± 3.31 |
| 31 | 60 | 70/1 | 50 | 90 | 0 | 0 | 0 | 0 | 23.75 ± 0.27 | 24.43 ± 1.16 | 169.05 ± 2.31 |

**3.2. Statistical analysis of the model**

From the results in **Table 3**, the statistical significance of the terms of the model can be evaluated using the analysis of variance (ANOVA) shown in **Table 4**.

**Table 4.** ANOVA table for the full quadratic model

| Source of variation | DF | Adj SS | Adj MS | *F-ratio* | *p*-Value |
|---|---|---|---|---|---|
| **Model** | 14 | 69.7142 | 4.9796 | 9.66 | < 0.0001 |
| Linear | 4 | 30.0879 | 7.5220 | 14.60 | < 0.0001 |
| $X_1$ | 1 | 16.7691 | 16.7691 | 32.54 | < 0.0001 |
| $X_2$ | 1 | 2.2271 | 2.2271 | 4.32 | 0.054 |
| $X_3$ | 1 | 4.2362 | 4.2362 | 8.22 | 0.011 |
| $X_4$ | 1 | 6.8556 | 6.8556 | 13.3 | 0.002 |
| Square | 4 | 38.3850 | 9.5963 | 18.62 | < 0.0001 |
| $X_1^2$ | 1 | 12.2161 | 12.2161 | 23.71 | < 0.0001 |
| $X_2^2$ | 1 | 17.6609 | 17.6609 | 34.27 | < 0.0001 |
| $X_3^2$ | 1 | 7.3035 | 7.3035 | 14.17 | 0.002 |
| $X_4^2$ | 1 | 12.9130 | 12.9130 | 25.06 | < 0.0001 |
| 2-Way Interaction | 6 | 1.2413 | 0.2069 | 0.40 | 0.867 |
| $X_1 X_2$ | 1 | 0.0188 | 0.0188 | 0.04 | 0.851 |
| $X_1 X_3$ | 1 | 0.4516 | 0.4516 | 0.88 | 0.363 |
| $X_1 X_4$ | 1 | 0.7145 | 0.7145 | 1.39 | 0.256 |
| $X_2 X_3$ | 1 | 0.0469 | 0.0469 | 0.09 | 0.767 |
| $X_2 X_4$ | 1 | 0.0069 | 0.0069 | 0.01 | 0.909 |
| $X_3 X_4$ | 1 | 0.0025 | 0.0025 | 0.00 | 0.945 |
| **Error** | 16 | 8.2452 | 0.5153 | | |
| Lack-of-fit | 10 | 6.1719 | 0.6172 | 1.79 | 0.247 |
| Pure error | 6 | 2.0732 | 0.3455 | | |
| **Total** | 30 | 77.9593 | | | |

The regression equation expressed in uncoded units was given using Minitab v.17.0 as follows:

$$\mathbf{TPC = -51{,}9 + 0{,}577\ U_1 + 1{,}185\ U_2 + 0{,}481\ U_3 + 0{,}1127\ U_4 - 0{,}00654\ U_1^2 - 0{,}00786\ U_2^2 - 0{,}00505\ U_3^2 - 0{,}000747\ U_4^2 - 0{,}00034\ U_1.U_2 + 0{,}00168\ U_1.U_3 + 0{,}000704\ U_1.U_4 - 0{,}00054\ U_2.U_3 - 0{,}000069\ U_2.U_4 + 0{,}000042\ U_3.U_4}$$

where the unit of TPC is mg GAE/g DW and all variables are presented as actual values.

The regression equation expressing the correlation between experimental results and theorical model (TPC and predicted TPC) inferred from Minitab was as TPC = 0.000 + 1.000 ×$TPC_{predicted}$ with the coefficient of determination $R^2$ of 0.8942 (> 0.80), indicating that the model approximates the data at the design points. Furthermore, the fitting test results (**Table 5**) has shown that the predictive power of the developed model for new observations may be 87.59%, based on the predicted $R^2$ value. The calculated *p*-value for lack-of-fit is greater than 0.05, thus, it can be assumed that the model adequately represents the experimental results at a 95% confidence level [10], [11], [12].

**Table 5.** Model fitting test results

| Model parameter | Value |
|---|---|
| $R^2$ | 89.42% |
| Adjusted $R^2$ | 89.06% |
| Predicted $R^2$ | 87.59% |
| *p*-value for lack-of-fit | 0.698 |

### 3.3. Effects of the factors

One of the advantages of coding factors is that they eliminate any pseudo effect caused by the use of different measurement units. Consequently, the absolute values of the coded coefficients show the magnitude of the response resulting from one unit change in a factor in one specific term, with all other terms remain unchanged. That correlation, however, is applicable in linear effects only since the interactions between two factors, if it is significant, will affect to the response depending on the value of each factor itself [11].

The coded coefficients shown in **Table 6** revealed that the effects of factor $X_2$ (linear coefficient) and the interactions between all four factors $X_1$, $X_2$, $X_3$, $X_4$ (interaction coefficients) are statistically insignificant ($p > 0.05$). By contrast, the other coefficients are statistically significant ($p < 0.05$) and illustrated in **Figure 1** in descending order of absolute values. Also, it is worth noting that the negative or positive mark exhibits the corresponding impact of each term toward the response, i.e. decreasing or increasing, respectively [11].

**Table 6.** Coded coefficients

| Term | Coefficient | Value | SE | *p*-value |
|---|---|---|---|---|
| Constant | $\beta_0$ | 24.427 | 0.271 | < 0.0001 |
| $X_1$ | $\beta_1$ | -0.836 | 0.147 | < 0.0001 |
| $X_2$ | $\beta_2$ | 0.305 | 0.147 | 0.054 |
| $X_3$ | $\beta_3$ | 0.420 | 0.147 | 0.011 |
| $X_4$ | $\beta_4$ | 0.534 | 0.147 | 0.002 |
| $X_1^2$ | $\beta_{11}$ | -0.654 | 0.134 | < 0.0001 |
| $X_2^2$ | $\beta_{22}$ | -0.786 | 0.134 | < 0.0001 |
| $X_3^2$ | $\beta_{33}$ | -0.505 | 0.134 | 0.002 |
| $X_4^2$ | $\beta_{44}$ | -0.672 | 0.134 | < 0.0001 |
| $X_1$ x2 | $\beta_{12}$ | -0.034 | 0.179 | 0.851 |
| $X_1 X_3$ | $\beta_{13}$ | 0.168 | 0.179 | 0.363 |
| $X_1 X_4$ | $\beta_{14}$ | 0.211 | 0.179 | 0.256 |
| $X_2 X_3$ | $\beta_{23}$ | -0.054 | 0.179 | 0.767 |
| $X_2 X_4$ | $\beta_{24}$ | -0.021 | 0.179 | 0.909 |
| $X_3 X_4$ | $\beta_{34}$ | 0.012 | 0.179 | 0.945 |

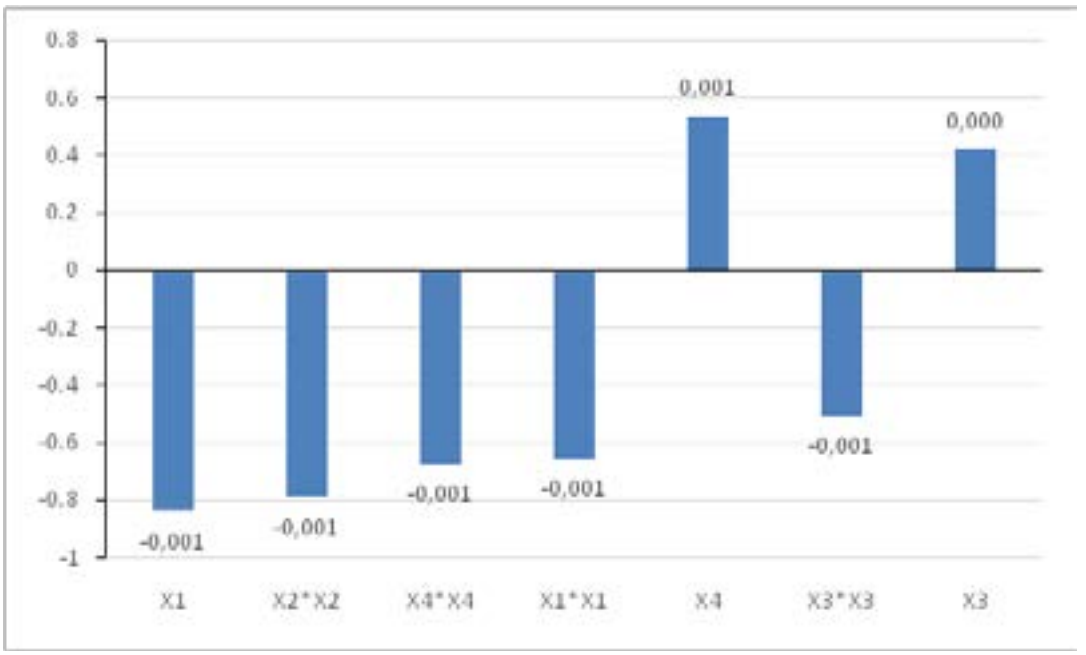
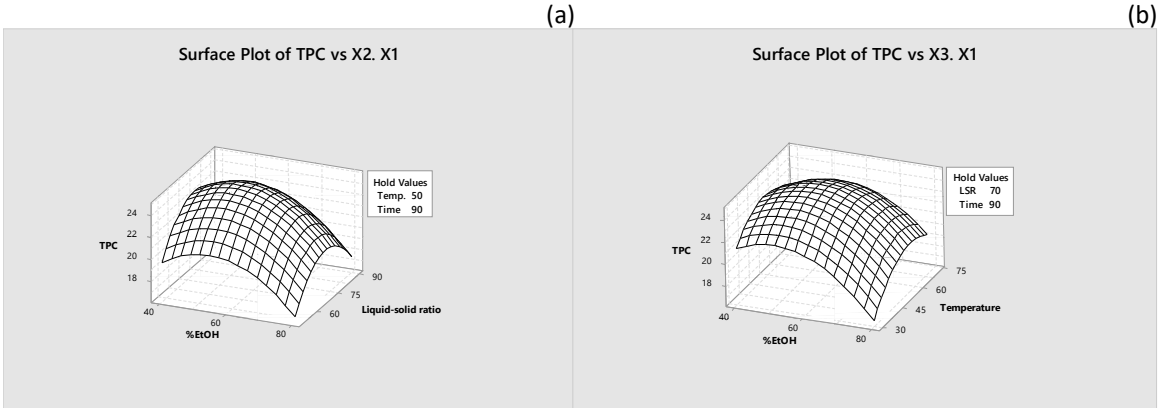



***Figure 1. The statistically significant coefficients in descending order of absolute values expressed in coded units***

As shown in **Figure 1**, the most significant factor toward TPC is obviously the solvent composition ($X_1$) which has negative effect toward the response. Extraction time ($X_4$) and temperature ($X_3$) are less significant in terms of linear coefficients. However, they are the only two coefficients which possess positive impacts toward the response, i.e. the increasing in coded units of these terms will result in the increasing of the response. All other coefficients of quadratic terms ($X_2^2$, $X_4^2$, $X_1^2$, $X_3^2$ sorted in descending order of absolute values), have showed the negative impacts, meaning that there are maximums of the response when these terms keep increasing in their coded values.

The visualization of the predicted model equation in uncoded units can be obtained by the surface response plots and the corresponding contour plots (**Figures 2 and 3**, respectively). From these plots, the maximum TPC is observed (> 24 mg GAE/g DW) when the solvent composition (%EtOH in water) ranges from 43% to 65%, the liquid-solid ratio ranges from 63/1–82/1 (mL/g), the extraction temperature ranges from 41 to 65 (°C), and the extraction time ranges from 67 to 132 (min).

(a) (b)



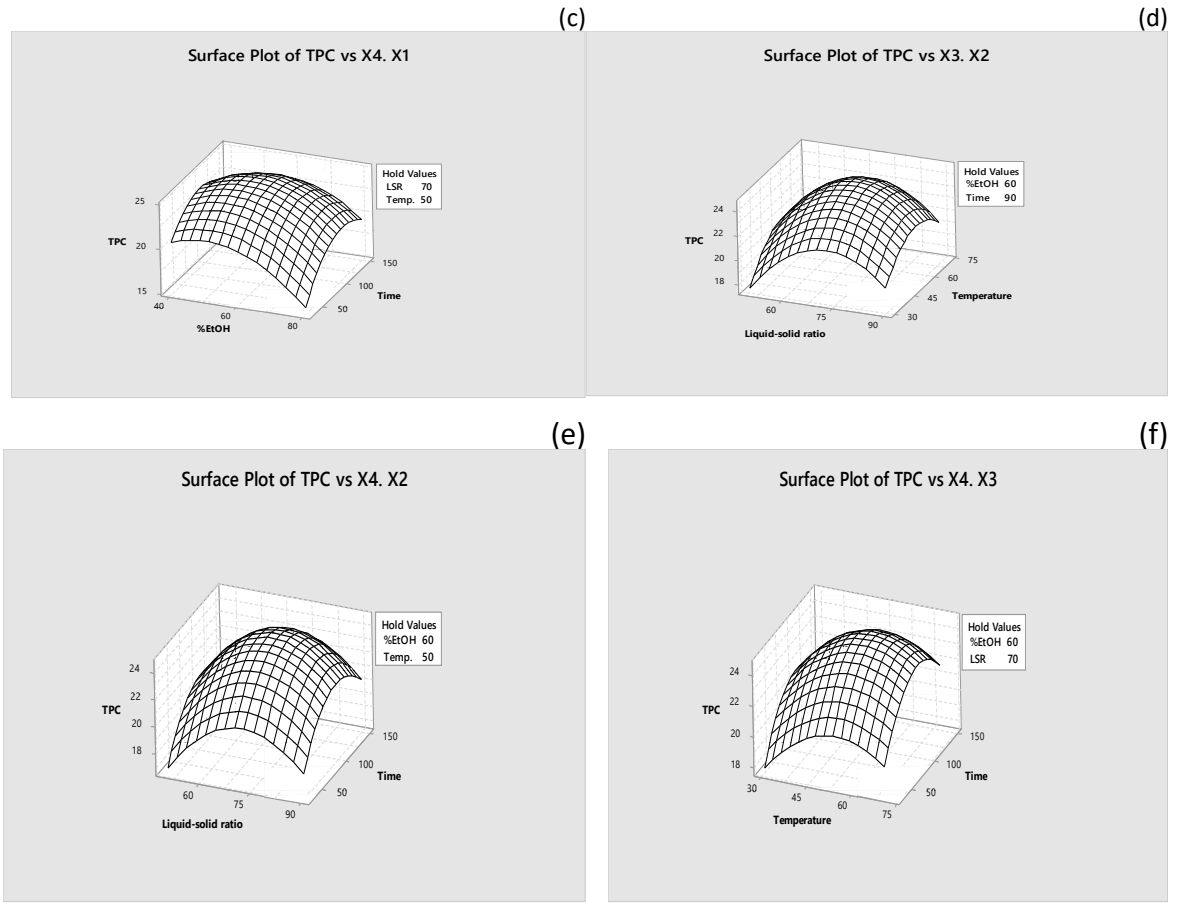
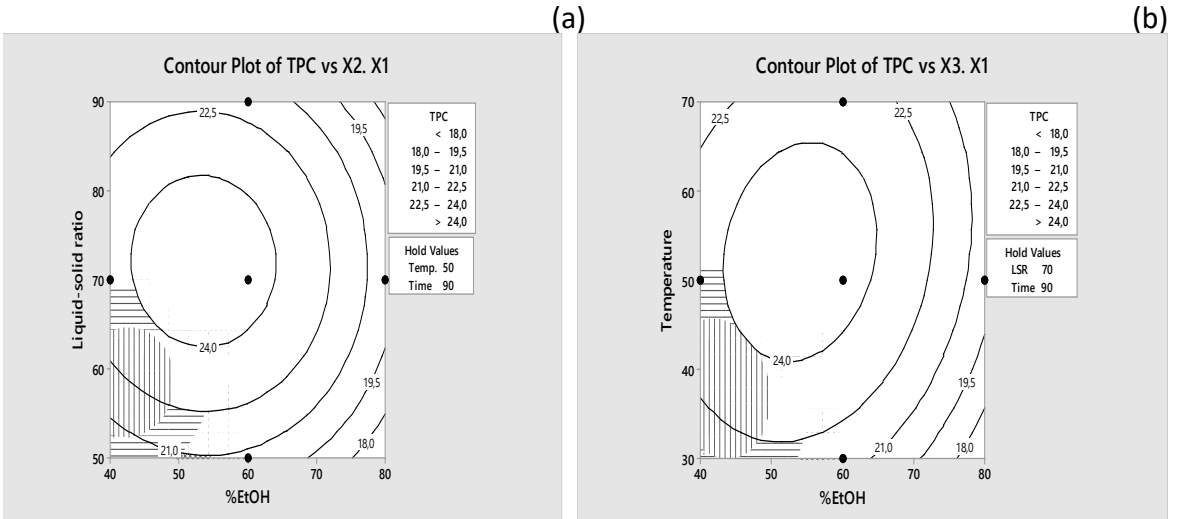



***Figure 2. Surface response plots from the optimal model***



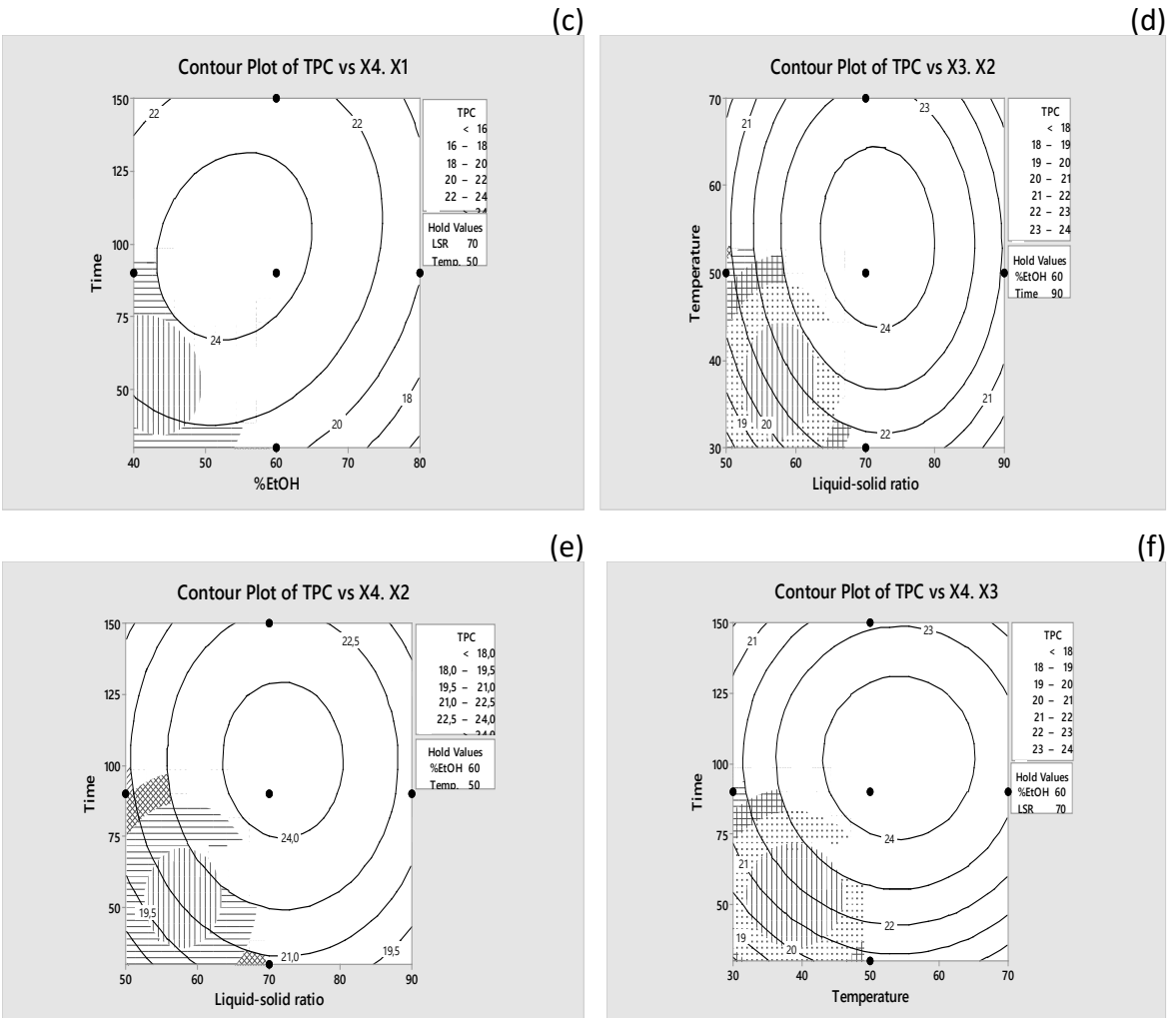



***Figure 3. Contour plots from the optimal model***

**3.5. Optimal extraction conditions for the predicted TPC maximum**

From the analyzing of experimental data, the response optimization performed by Minitab v.17.0 with maximum goal for TPC resulted in the optimal extraction conditions as follows: solvent composition: 54.55% ethanol in water, liquid-solid ratio: 71.82/1 mL/g, extraction temperature: 53.03 °C, extraction time: 99.09 min; the maximum TPC estimated based on 95% prediction interval was between 23.22 and 26.46 mg GAE/g DW.

To confirm this prediction, the actual experiment was conducted, however, those conditions were slightly modified according to the practical aspect of laboratory instruments. In particular, extraction conditions were set as follows: solvent composition: 55% ethanol in water, liquid-solid ratio: 72/1 mL/g, extraction temperature: 55 °C, extraction time: 99 min; the resulting TPC was 26.74 ± 0.04 mg GAE/g DW. This extract also showed significant total antioxidant capacity of 188.06 ± 1.41 mg AAE/g DW.

The TPC in this current study was significantly higher than reported TPCs by Wang *et al.* (2010) from different strains grown in USA and Mexico, ranging from 4.3 to 13.9 mg GAE/g DW. The used solvent system was 10 mL of acetone/water/acetic acid (70:29.7:0.3, v/v/v) for each 0.5 g of material, equivalent to 20/1 (mL/g) of liquid-solid ratio. However, the authors did not describe clearly about other extraction conditions such as extraction temperature and time, as well as did not explain why this solvent system was used.

By contrast, Rodríguez-Carpena *et al.* (2011) reported relatively high TPCs from peels of two avocado varieties collected in Spain using three solvents

including ethyl acetate, acetone/water (70:30, v/v), methanol/water (70:30, v/v) [5]. The TPC values ranging from 32.93 to 172.18 mg GAE/g in which the highest TPC belongs to the use of 70% acetone followed by 70% methanol and ethyl acetate. The remarkable difference of that study from this current report is that samples were frozen without drying at –80 °C prior to being extracted two times using a homogenizer, so those TPCs were expressed as mg GAE per 100 g fresh matter. This might reasonably explain the high value of resulting TPCs. Also, these organic solvents such as ethyl acetate, acetone and methanol are considered "non-green"/unsafe to human health, thus limit their applications in industrial manufacturing compared to that of ethanol or water [13].

**3.6 Analyzing the correlation between TPC and corresponding TAC**

The evaluation of the relationships between total phenolic content and total antioxidant capacity of the corresponding extract were conducted by calculating Pearson's correlation coefficient using Minitab. This calculated coefficient was of 0.941 (> 0.8) with *p*–value smaller than 0.001 demonstrating the strong correlation between these two terms, i.e. the higher TPC is extracted, the higher TAC will probably be [14].

Agricultural by-products have increasingly attracted scientists and manufacturers for the ability of their antioxidant compounds in preventing the oxidative damage involved in many chronic diseases such as cardiovascular disorder and cancer. For instance, products manufactured from mangosteen (*Garcinia mangostana*) have begun to be used as a botanical dietary supplement in the United States due to their potent antioxidant properties, especially the pericarps [15]. In addition, the study of adding grape (*Vitis vinfera* L.) seed extract as a simple dietary supplement on healthy volunteers has shown that it could reduce up to 33% the urine redox potential, reflecting an overall increase in antioxidant status. It is also worth noting that grape extract used in this study is known as a concentrated source of polyphenols and has been a commercially available product, named exGrape® [16]. Those examples demonstrated the potential applications of agricultural "wastes" in healthcare.

In 2008, the global production of avocados was about 3.2 million tons, mostly from Latin America and the Caribbean and with the European Union being the major importer [5]. Because of its high nutritional value resulting in the globally increasing demand for avocados, there is a great prospect for Vietnam which have suitable climatic conditions, especially on the highlands in Lam Dong province or in the Mekong Delta areas, to embark on a large-scale plantation of avocado for export [17]. Although avocados are mostly consumed fresh as a salad fruit or juice, they have also been used in the oil, cosmetic, soap, and shampoo industry, as well as processed foods derived from it, such as guacamole, frozen products and avocado paste [18]. Therefore, the current study on the efficient recovery of phenolics from avocado peels, a by-product from processed-food industrials, would encourage further applications for these residues, e.g. instant teas enriched with polyphenols, hence promote the cultivation of avocados and enhance the farmers' additional income.

## 4. CONCLUSION

Four factors related to the extraction conditions including solvent composition (%EtOH), liquid-solid ratio, extraction temperature and extraction time were optimized using respond surface methodology with the central composite rotatable design. The optimal conditions were determined at 54.55% ethanol in water (%EtOH), 71.82/1 (mL/g), 53.03 (°C) and 99.09 (min), respectively, and resulting TPC was of 26.74 ± 0.04 mg GAE/g DW with some minor modifications. The strong correlation between TPC and the corresponding TAC were also confirmed. To our knowledge, this is the first time the phenolic extraction optimization of avocado peels was conducted toward four factors at the same time using RSM and CCRD, and the phosphomolybdenum method was used to determine the antioxidant effect of avocado peel extract.

**Acknowledgment**

The authors acknowledge the financial support from Hue University of Medicine and Pharmacy, Hue University, Vietnam, to conduct this research.

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