Optimization of Chlorogenic Acid Extraction from Green Coffee Beans Using Response Surface Methodology

Lai Thi Ngoc Ha¹, Nguyen Viet Phuong², Tran Thi Hoai¹, Dao Thi Viet Ha³ & Hoang Hai Ha¹

¹Faculty of Food Science and Technology, Vietnam National University of Agriculture, Hanoi 131000, Vietnam
²Biotechnology Centre, Food Industrial Collage, Phu Tho 290000, Vietnam
³Scientific Management and International Cooperation Office, Food Industrial Collage, Phu Tho 290000, Vietnam

Abstract

Chlorogenic acid is a natural antioxidant that is widespread in the plant kingdom and can be found at a high content level in green coffee beans. This secondary metabolite in green coffee beans has potent biological properties including antioxidant, anti-inflammatory, anti-cancer, anti-obesity, anti-hypertension, and anticonvulsant. In this study, the extraction of chlorogenic acid from Vietnamese green coffee beans was optimized using the response surface methodology. A second-order polynomial model with three important variables (liquid-to-solid ratio, temperature, and extraction time) was used. A rotatable central composite design consisting of 21 experimental runs with three replicates at the center point was applied to describe the experimental data. The experimental results properly conformed to the constructed model (R² = 0.8549). The optimized conditions were as follows: 40% ethanol (v/v), a liquid-to-solid ratio of 11.77, at 85°C for 64 min. Four extractions were performed in parallel using the optimal conditions to validate the model. The experimental values highly agreed with the predicted value (P <0.05).

Keywords

Phenolic compound, ethanolic extraction, HPLC quantification, validated model

Introduction

Coffee has been consumed for over 1,000 years and today it is one of the most consumed drinks in the world (more than 157 million 60kg bags in 2016-2017) (Statista, 2018). The word “coffee” comes from the name of a region of Ethiopia where coffee was first discovered, ‘Kaffa’. Botanically, coffee belongs to the family Rubiaceae in the genus Coffea. Although the genus Coffea
includes four major subsections, 66% of the world’s production mostly comes from *Coffea arabica* L. and 34% from *Coffea canephora* Pierre ex Froehner (Robusta type) (Mekuria et al., 2004).

Currently, Vietnam is the second biggest producer and exporter of coffee in the world, only after Brazil. According to the USDA Foreign Agricultural Service (2018), coffee is grown in more than 9 provinces in Vietnam. The production of the 2017-2018 season was 29.3 million bags of green coffee beans, of which, 28 million bags were Robusta. Coffee is one of the main agricultural export products of Vietnam and is ranked in the second position after rice. In 2017-2018, Vietnam exported about 25 million bags of green coffee beans with a turnover of 3.5 billion USD (USDA Foreign Agricultural Service, 2018; VietnamNews, January 11, 2019).

Chlorogenic acid (CGA), 5-caffeoylquinic acid (Figure 1), is the ester of quinic acid and caffeic acid. This compound is a natural phenolic antioxidant widespread in the plant kingdom (Clifford, 1999) and well represented in green coffee beans (raw coffee beans). Depending on the species, green coffee beans contain some 3.6-6.0% of CGA on a dry basis, with levels of CGA higher in *Coffea robusta* beans than in *C. arabica* beans (Ky et al., 1997; Clifford, 1999; Perrone, 2008; Liang & Kitts, 2016).

Previous studies have shown that consuming green coffee extract has many beneficial effects on human health such as lowering blood pressure, inhibiting lipid accumulation, increasing body weight, and controlling blood glucose levels (Kozuma et al., 2005; Thom, 2007; Perrone et al., 2008; Iwai et al., 2012). These positive effects of green coffee extract are explained by the presence of CGA in the extract which has several potent biological properties including antioxidant, anti-inflammatory, anti-cancer, anti-obesity, anti-hypertension, and anticonvulsant (Santana-Gálvez et al., 2017). Moreover, recent studies have shown the beneficial effects of CGA on metabolic syndrome. This syndrome is defined as a range of physiological, biochemical, clinical, and metabolic factors that increase the risk of cardiovascular disease and type 2 diabetes. This syndrome is currently considered a global syndrome because of the high cost of treatment and the increasing number of patients, including children and adolescents (Santana-Gálvez et al., 2017). The exploitation of CGA from green coffee beans, a popular agricultural product of Vietnam, not only creates a new source of biologically active compounds applicable in nutraceutical technology but also contributes to increasing the economic value of the coffee plant.

The exploitation of natural biologically active ingredients in general and CGA from green coffee beans in particular always starts by extracting the active ingredients from the natural materials. So far, many methods have been used, ranging from conventional extraction methods using solvents to modern methods requiring expensive equipment such as supercritical...
CO₂ extraction. However, extraction using solvents is always a low-cost technology to obtain molecules to be used as food additives or nutraceutical products and can be a reasonable strategy for the exploitation of plant materials in developing countries.

Extraction studies can be done by using the one-factor-at-a-time approach (Chirinos et al., 2007; Kossah et al., 2010) or a response surface methodology (RSM) (Silva et al., 2007; Kiassos et al., 2009; Pompeu et al., 2009). The one-factor-at-a-time approach, also known as a single factor experiment, is a classical method in which only one factor is variable at one time while all others are kept constant. This approach has several drawbacks, such as that it is time-consuming, there is an inability to determine the interaction between the variables, it is costly, and it is less effective than other methods (Silva et al., 2007). The RSM is a statistical method that uses data from appropriate experimental designs to determine and solve multivariate equations. This approach can overcome the drawbacks of the one-factor-at-a-time method and has previously been used in the extraction of phenolic compounds from plant sources (Silva et al., 2007; Pompeu et al., 2009; Radojkovic et al., 2012).

The main objective of this study was to optimize the extraction parameters of CGA from Robusta green coffee beans produced in Vietnam by using the RSM. In the first step, the effects of several important factors on the extraction process were investigated in order to determine the intervals of the variables. In the second step, a model was constructed to describe the extraction and the optimized conditions were determined. The resulting extract could be further used as food additives or nutraceutical products.

Materials and Methods

Materials

Green coffee beans were purchased from the Vietnam National Coffee Corporation (VINACAFE). They were produced in the 2016-2017 season in Dak Lak province, located in the Central Highlands of Vietnam. The chlorogenic acid standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol of analytical grade, acetonitrile, and formic acid of HPLC grade were obtained from Merck (Darmstadt, Germany).

Selection of relevant variables and determination of experimental ranges

Effect of ethanol concentration on the extraction of CGA

Ethanol in water was used as an extraction solvent in this study. CGA from the ground green coffee beans was extracted using various ethanol concentrations (0, 20, 40, 60, 80, and 99.5% (v/v)). Dried green coffee bean powder (50µg) was steeped in the extracting solvent (1mL), and shaken for 60min at 40°C. The extract was centrifuged at 3,642g (6,000rpm) for 10min at 4°C. The supernatant was collected and the CGA content analyzed.

Effect of temperature on the extraction of CGA

Dried green coffee bean powder (50µg) was mixed with 1mL of the ethanol solution selected from the concentration experiment described above. The mixture was shaken for 60min at different temperatures (40, 50, 60, 70, 80, and 95°C). The mixture then was centrifuged at 3,642g for 10min at 4°C. The CGA content of the supernatant was analyzed.

Effect of the liquid-to-solid ratio on the extraction of CGA

Dried green coffee bean powder was mixed with 1mL of the ethanol solution chosen from the concentration experiment described above in order to obtain liquid-to-solid ratios of 5/1-25/1 and shaken for 60min at 50°C. The mixture was centrifuged at 3,642g for 10min at 4°C. The supernatant was collected and then the CGA content analyzed.

Effect of extraction time on the extraction of CGA

Dried green coffee bean powder was mixed with 1mL of the ethanol solution chosen from the concentration experiment described above in order to achieve the optimal liquid-to-solid ratio determined in the previous step. The mixture
was shaken for various times ranging from 30 to 120 min at 50°C. The mixture was centrifuged at 3,642 g for 10 min at 4°C. The supernatant was collected and then the CGA content analyzed.

Response surface procedure for CGA extraction from green coffee beans

The RSM used a three-factor and central composite rotatable design (CCRD) consisting of 21 experimental runs with eight factorial points, six axial points (two axial points on the axis of each design variable at a distance of 1.68 from the design center), and three replicates at the center point and maximal and minimal factorial points. The design variables were the liquid-to-solid ratio \((x_1)\), the temperature \((^\circ C; x_2)\), and the time of extraction (min; \(x_3\)). Each variable was coded at five levels -1.68, -1, 0, 1, and 1.68. Extractions were carried out in 2 mL Eppendorf tubes placed in a water bath. Extractions were terminated by centrifugation at 3,642 g for 10 min at 4°C. The obtained extracts were analyzed by HPLC-DAD. The experimental data were fitted to the following second-order polynomial model:

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \sum_{j=2}^{3} \beta_{ij} x_i x_j
\]

where, \(Y\) is the measured response (CGA content of the green coffee beans), \(\beta_0, \beta_i, \beta_{ij}\), and \(\beta_{ij}\) are the regression coefficients for the intercept, linear, quadratic, and interactions terms, respectively, and \(x_i\) and \(x_j\) are the coded or standardized values of the independent variables.

The optimal conditions of the CGA extraction process were determined using the JMP 10 software. Four experimental replicates were performed at the optimized conditions and the experimental and predicted values were compared.

HPLC-DAD analysis and quantification of CGA

Quantifications of CGA in the extracts were performed using HPLC (Shimadzu system, Japan) equipped with a LC-10Ai pump, a DGU-20A3 degasser, a SPD-20A diode array detector, and a CBM-20A interface. The method was modified from Lai et al. (2013). A 20µL aliquot of a CGA extract was manually injected onto a reversed-phase Kinetex EVO C18 column (150 x 4.6mm i.d.; 5µm particle size) equipped with a guard column of the same type (Phenomenex, CA, USA). The mobile phases were A (0.1% formic acid in water) and B (acetonitrile). The flow rate was 1 mL min\(^{-1}\), and the column temperature was set at 35°C. The 32 min gradient was as follows: 0 min, 0% B; 2 min, 0% B; 5 min, 15% B; 12 min, 15% B; 22 min, 50% B; 25 min, 100% B; 30 min, 100% B; 35 min, 0% B; and 37 min, 0% B. The monitoring system was set at 325 nm for the quantification of CGA. The chlorogenic acid in the extract was identified by its retention time as compared to an authentic standard and was quantified using five-point calibration curves (\(y = 52965x - 31348; R^2 = 0.9998\)).

Statistical analysis

The experimental results were analyzed using the SAS 9.0 software (SAS Institute, Cary, NC) and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) and Duncan’s multiple range test were used to determine the differences amongst the means. \(P\)-values <0.05 were considered to be significantly different. In the RSM experiment, multiple linear regression analysis was performed using the software JMP 10 (SAS Institute, Cary, NC).

Results and Discussion

Determination of the relevant variables and experimental ranges

Effect of ethanol concentration

Water-ethanol mixtures were used as the extraction solvent in this study. The selection of ethanol as the extraction solvent was justified by the fact that ethanol is a food grade solvent, and is less toxic and is more abundant as compared to acetone, methanol, and other organic solvents (Kiassos et al., 2009; Chew et al., 2011). The
use of ethanol at the different concentrations in water was chosen because binary-solvent systems have demonstrated higher yields of polyphenols as compared to mono-solvent systems (Zhou et al., 2011; Wang et al., 2013; Lai et al., 2014). In this study, the ethanol concentration showed a significant effect on the extracted CGA quantity ($P < 0.0001$). Indeed, the extracted CGA increased with an increase in the ethanol concentration, reached its highest value ($37.86 \pm 1.23 \text{ mg g}^{-1} \text{ dry weight (DW)}$) at 40% ethanol, and then began to decrease (Figure 2). The effect of ethanol concentration in extraction mediums on the yields of phenolic compounds has been observed in various studies. Chew et al. (2011) reported that the highest total phenolic content of Centella was achieved at a 60% ethanol concentration. The optimized ethanol concentration for the extraction of piceatannol from the sim seed was 79% (Lai et al., 2014). The impact of the ethanol concentration is due to its effect on the polarity of the extraction solvent and the resulting solubility of the phenolic compounds. The general principle is “like dissolves like”, which means that solvents only extract phytochemicals that have a similar polarity to that of the solvent. An ethanol concentration of 40% might have a similar polarity as CGA. This concentration was then selected for further experiments.

**Effect of temperature**

The extraction temperature had a significant effect on the CGA extraction from green coffee beans ($P = 0.0072$, Figure 3). As shown in Figure 3, the extracted chlorogenic acid quantity increased when the temperature went up. This effect of temperature was in accordance with studies on piceatannol extraction from passion seeds (Lai et al., 2016) and on phenolic extraction from areca husks (Chen et al., 2012). An increase in the extraction temperature may increase the solubility of CGA in the solvent and decrease the viscosity of the solvent. The combination of these two phenomena enhanced the overall extraction efficiency. However, in comparison to other phenolic compounds, whose extraction yields decreased when the extraction temperature increased after having reached the highest value, the extracted CGA quantity increased continuously with increased extraction temperature. This indicated that CGA is a thermo-resistant phenolic compound and a high-temperature range could be used in the RSM experiment. Moreover, as the extracted CGA quantity did not change significantly when the temperature increased from 50°C to 90°C, 50°C was then used in the determination of the liquid-to-solid ratio and temperature effect on CGA extraction from green coffee beans.

![Figure 2](image_url)  
**Figure 2.** Effect of ethanol concentration on the chlorogenic acid content of green coffee beans

*Note: Columns with different letters (a, b, or c) are significantly different ($P < 0.05$).*
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Effect of the liquid-to-solid ratio

The impact of the liquid-to-solid ratio on the extraction of CGA from green coffee beans is presented in Figure 4. The results of the one-way analysis of variance showed that the liquid-to-solid ratio had a significant effect on the CGA extraction (P = 0.0002). The extracted CGA quantity initially increased when the ratio increased from 5/1 to 10/1 and then remained fairly constant. A similar effect of the liquid-to-solid ratio on extraction yield was reported for the extraction of CGA from *Inula helenium* (Wang *et al.*, 2013) and the extraction of phenolic compounds from *Inga edulis* leaves (Silva *et al.*, 2007). The ratio of 10/1 gave the highest CGA content. This ratio was, hence, chosen as the central value in the RSM experiment.

Effect of extraction time

The amounts of CGA extracted from green coffee beans as a function of extraction time is presented in Figure 5. The CGA content of the coffee beans increased markedly during the first hour with the rate of 0.70 mg g⁻¹ DW per min and then remained constant. This result agreed with other studies on the extraction of phenolic compounds from plant materials. Indeed, the kinetics of phenolic extraction from *Inga edulis* leaves could be divided into two extraction phases: a fast one, which made up the first 20 min, and a slow one, which accounted for the rest of the studied time (Silva *et al.*, 2007). Thus, the choice of a long extraction time led to no significant effect on the variable “time”. According to our results, 45 min and 20 min were chosen as the central value and variation of the extraction time, respectively, in the RSM experiment in order that the variable time in the RSM experiment covered both phases of extraction.

**Modelization and optimization of chlorogenic acid extraction from green coffee beans by RSM**

The CGA extraction from green coffee beans was further optimized through the RSM approach. Based on the primary results, a fixed ethanol concentration (40%, v/v) was chosen, while three factors, namely the liquid-to-solid ratio, temperature, and time, were considered as variables in the model. Their ranges are presented in Table 1. The experimental design of a five-level, three-variable CCRD and the experimental results of the extraction are shown in Table 2. By applying multiple regression analysis, the relation between the tested independent variables and the response was explained by Equation 1, in which $x_i$ were the standardized or coded variables.
To fit the response function and experimental data, the linear and quadratic effects of the independent variables, as well as their interactions in the response, were evaluated by analysis of variance (ANOVA) and regression coefficients were determined (Tables 3 and 4). The ANOVA of the regression model showed that the model was highly significant or useful due to a very low probability value ($P < 0.0017$) (Table 3). The fitness of the model was judged by the coefficient of determination ($R^2$). In this study, the $R^2$ value for the regression model of the CGA content of green coffee bean was 0.8549, which was close to 1, suggesting that the predicted second order polynomial model defined the CGA extraction process from green coffee beans well and that 85.49% of variation for the CGA content was attributed to the three studied factors (Bharathi et al., 2011).

The effects of the liquid-to-solid ratio, temperature, and time of extraction on the CGA content are presented in Table 4 and Figure 6. As illustrated by Table 4, the temperature and time of extraction showed significant linear effects for the CGA content ($P < 0.0002$ and 0.0166, respectively). Among them, temperature appeared to be the most affecting factor of the
CGA extraction process from the green coffee beans since its coefficient had the highest value (4.7786). As shown in Figure 6, the extracted CGA quantity increased when the temperature went up. This agreed with the results of our primary experiment about the effect of temperature on the CGA extraction as previously mentioned.

Concerning the extraction time, this factor had a significant linear (P = 0.0166) effect on the CGA content of green coffee beans. In run 14 (Table 2), a high quantity of CGA (29.90 mg g⁻¹ DW) was observed when the time of extraction was only 11.40 min. When the extraction time increased from 11.40 to 78.60 min, the CGA content increased slightly (from 29.90 mg g⁻¹ DW for run 14 to 30.50 mg g⁻¹ DW, the average value for runs 15A, 15B, and 15C). This would mean that an important quantity of CGA was extracted during the first minutes of the extraction. Accordingly, the maximal rates of extraction of phenolic compounds from agrimony, sage, and savory leaves were found to take place during the first minutes of their extractions (Kossah et al., 2010).

Table 1. Variables and experimental ranges

| Variables           | Central value | Variation* |
|---------------------|---------------|------------|
| Liquid/solid ratio  | 10            | 3          |
| Temperature (°C)    | 65            | 20         |
| Time (min)          | 45            | 20         |

Note: * Variation corresponds to a unit of standard value.

Table 2. Rotatable central composite design setting in the coded form (x₁, x₂, and x₃) and real values of the independent variables (X₁, X₂, and X₃), and experimental results for the response variable (CGA content of green coffee beans)

| Run  | x₁    | x₂    | x₃    | Liquid-to-solid ratio | Temperature (°C) | Time (min) | Chlorogenic acid (mg g⁻¹ DW) |
|------|-------|-------|-------|-----------------------|------------------|------------|-----------------------------|
| 1A   | 1     | 1     | 1     | 13                    | 85               | 65         | 35.85                       |
| 1B   | 1     | 1     | 1     | 13                    | 85               | 65         | 33.75                       |
| 1C   | 1     | 1     | 1     | 13                    | 85               | 65         | 36.94                       |
| 2    | -1    | 1     | 1     | 7                     | 85               | 65         | 32.45                       |
| 3    | 1     | -1    | 1     | 13                    | 45               | 65         | 30.80                       |
| 4    | -1    | -1    | 1     | 7                     | 45               | 65         | 25.44                       |
| 5    | 1     | 1     | -1    | 13                    | 85               | 25         | 33.26                       |
| 6    | -1    | 1     | -1    | 7                     | 85               | 25         | 23.76                       |
| 7    | 1     | -1    | -1    | 13                    | 45               | 25         | 20.31                       |
| 8A   | -1    | -1    | -1    | 7                     | 45               | 25         | 19.58                       |
| 8B   | -1    | -1    | -1    | 7                     | 45               | 25         | 18.91                       |
| 8C   | -1    | -1    | -1    | 7                     | 45               | 25         | 17.29                       |
| 9    | 1.68  | 0     | 0     | 15.04                 | 65               | 45         | 30.62                       |
| 10   | -1.68 | 0     | 0     | 4.96                  | 65               | 45         | 28.98                       |
| 11   | 0     | 1.68  | 0     | 10                    | 98.6             | 45         | 43.35                       |
| 12   | 0     | -1.68 | 0     | 10                    | 31.4             | 45         | 21.60                       |
| 13   | 0     | 0     | 1.68  | 10                    | 65               | 78.6       | 33.39                       |
| 14   | 0     | 0     | -1.68 | 10                    | 65               | 11.4       | 29.90                       |
| 15A  | 0     | 0     | 0     | 10                    | 65               | 45         | 31.05                       |
| 15B  | 0     | 0     | 0     | 10                    | 65               | 45         | 30.07                       |
| 15C  | 0     | 0     | 0     | 10                    | 65               | 45         | 30.38                       |

Y = 30.77 + 1.56x₁ + 4.78x₂ + 2.42x₃ + 0.41x₁²x₂ + 0.62x₁x₃ + 1.07x₂x₃ – 1.16x₁² – 0.21x₂² – 0.51x₃² (Equation 1)
Table 3. Analysis of variance for the response surface quadratic model of CGA content of green coffee beans

| Source of variance | DF | Sum of square | Mean square | F ratio |
|--------------------|----|--------------|-------------|---------|
| Model              | 9  | 769.18549    | 85.4651     | 7.2035  |
| Error              | 11 | 130.50734    | 11.8643     | P = 0.0017 |
| Total              | 20 | 899.69283    |             |         |

Note: * Degrees of freedom.

Table 4. Parameter estimates of the predicted second-order model for the responses (CGA content of green coffee beans)

| Term                   | Estimate  | Standard Error | t Ratio | Probability>|t| |
|------------------------|-----------|----------------|---------|-------------|
| Intercept              | 30.765081 | 1.984491       | 15.50   | <.0001*     |
| Ratio (7/1 - 13/1)     | 1.5552511 | 0.856331       | 1.82    | 0.0967      |
| Temperature (45°C - 85°C) | 4.7786034 | 0.856331       | 5.58    | 0.0002*     |
| Time (25min - 65min)   | 2.4157213 | 0.856331       | 2.82    | 0.0166*     |
| Ratio*Temperature      | 0.4127129 | 1.103759       | 0.37    | 0.7156      |
| Ratio*Time             | -0.622287 | 1.103759       | -0.56   | 0.5842      |
| Temperature*Time       | -1.072287 | 1.103759       | -0.97   | 0.3522      |
| Ratio*Ratio            | -1.160569 | 1.019493       | -1.14   | 0.2791      |
| Temperature*Temperature| -0.214813 | 1.019493       | -0.21   | 0.8370      |
| Time*Time              | -0.508263 | 1.019493       | -0.50   | 0.6279      |

Figure 6. Response surface for the CGA content in the function of the liquid-to-solid ratio, temperature, and time of extraction

The negative quadratic effect of \( x_1 \), \( x_2 \), and \( x_3 \) indicated that there was a maximum CGA content at a certain liquid-to-solid ratio, temperature, and time. The optimum conditions of the CGA extraction from green coffee beans was acquired using JMP 10. The software was set to search the optimum desirability of the response, meaning the maximum CGA content of the green coffee beans. The optimum conditions were as follows: liquid-to-solid ratio, 11.77, temperature, 85°C, and time of extraction, 64min as shown in Figure 7. In order to examine the validity of the model, the extraction was completed with four replicates under these conditions. The measured values (34.49, 35.75, 35.40, and 36.19 mg g\(^{-1}\) DW) laid within a 95% mean confidence interval of the predicted value (32.99-40.11 mg g\(^{-1}\) DW). These results confirmed the predictability of the model. The second-order polynomial model can thus be effectively applied to predict the CGA content of extracts from green coffee beans.
Figure 7. Desirability and response in function of the liquid-to-solid ratio, temperature, and time of extraction

Conclusions

The RSM was successfully employed to describe and to optimize the CGA extraction process from green coffee beans. The optimized extraction conditions were as follows: 40% ethanol (v/v), a liquid-to-solid ratio of 11.77, at 85°C for 64 min. This study should be considered as the first step for the production of CGA-rich products to be used as nutraceuticals from green coffee beans.

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