Bulletin of the *Transilvania* University of Braşov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 13(62) No. 1 – 2020
https://doi.org/10.31926/but.fwiafe.2020.13.62.1.10

THE OPTIMIZATION OF THE CONDITIONS OF DECAFFEINATION FROM COFFEE PULP (*COFFEA ROBUSTA*) USING THE RESPONSE SURFACE METHODOLOGY

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Abstract: *The study focused on determining the optimal extraction conditions of caffeine from coffee pulp ("Coffea robusta") using the hot water extraction method and response surface methodology (RSM). The extraction process consisted of three independent factors, namely, solvent/material ratio (30/1-50/1), extraction temperature (70-90^{o}C) and extraction time (90-150 min) and they strongly influenced the caffeine extraction efficiency (CEE, %). The results of the research indicated that the optimum extraction conditions were a solvent/material ratio of 38.47/1, extraction temperature of 82.19^{o}C and extraction time of 120.55 minutes, CEE value obtained 88.52%. The predicted values of CEE were in agreement with the experimental values, thus this indicated the suitability of RSM in optimizing the extraction conditions.*

Key words: *Caffeine, coffee pulp, extraction, RSM, yield.*

1. Introduction

Vietnam is currently the world's largest exporter of robusta coffee as well as the world's second-largest exporter of coffee beans after Brazil. In 2016, the total production of Vietnam is about 1.636.500 tons coffee beans [16] and annually exports about 450.000 tons of dried coffee pulp. This coffee pulp is mainly used as a fuel for drying fruit, coffee bean or organic fertilizer for cultivating coffee trees. This usage causes serious environmental pollution. Coffee pulp is the first waste product obtained during the processing of coffee beans. Coffee pulp constitutes about 28% of the dry weight of the whole berries. In coffee pulp, caffeine makes up 0.51-1.3%. Caffeine (1, 3, 7, trimethyxanthine) is found in coffee beans, tea leaves, cocoa seeds and more than 60 other plants [3],

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[8]. The presence of caffeine in coffee pods does not have any good effect on the digestive system of animals. Ramanavičienė et al. [12] investigated the direct effect of caffeine on Gram-negative bacteria cultures. The result showed that the high caffeine concentration in the culture medium can inhibit the growth of bacteria.

Caffeine is the cause of mutation in bacteria through DNA repair inhibition. Caffeine concentration of 0.1% is able to inhibit the protein synthesis in both bacteria and yeast. They are completely inhibited by a caffeine concentration of 1%. In addition, low caffeine concentration of 10^{-2} M can either inhibit phosphodiesterase or delay the cell division. For yeast, caffeine concentration over 10 mM can even lead to mutation. At low concentrations of caffeine, yeast can grow and reproduce as *E. coli* and other bacteria because they have anti-caffeine ability. Besides, caffeine also affects the lactose fermentation and indole synthesis by *E. coli* [7]. The effects of caffeine on filamentous fungi including *Trichoderma* and other pathogens showed that caffeine could inhibit the growth and development in fungi [14]. *A. niger* and *A. carbonarius* were inhibited by a caffeine concentration of less than 1%. However, if caffeine concentration is 4%, some bacteria still survive and continue to multiply such as *A. westerdijkiae*, *A. ochraceus* and *A. steynii* [2]. Therefore, it is quite important to remove caffeine from the coffee pulp before using them to produce animal feed or ethanol. The process of removing caffeine is also considered the pre-treatment process of coffee pulp before producing ethanol or animal feed.

Some caffeine extraction methods are commonly used in decaffeination studies such as caffeine extraction by supercritical carbon dioxide from coffee beans [9], caffeine extraction from guarana with methylene chloride [6], extraction of caffeine from natural matter using a bio-renewable agrochemical solvent [4], etc. Among them, the study of Tello et al. [15] on the caffeine extraction from robusta coffee husks using supercritical carbon dioxide was outstanding. The results showed that extraction efficiency was 84% and the purity of caffeine reached up to 94%. Previous studies on caffeine extraction focused on the recovery of caffeine with high purity or optimal extraction conditions. However, economic benefit, as well as the feasibility of commercialization, was not mentioned. In this study, we employed the conventional extraction method using hot water. This method can dramatically reduce caffeine content in the initial material, it is quite easy to practice and does not have high equipment requirements. In addition, this is a cheap and non-polluting method. Moreover, we only focused on the removal of caffeine from materials as much as possible with the lowest cost and high efficiency; the purity of extracted caffeine was not mentioned in this study. Therefore, the extraction method by hot water was chosen.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

The Robusta coffee pulp was collected from the PongDrang commune, KrongBuk district, in the Dak Lak province. Ripened berries are bright red, free of physical damage and fungal infection. After harvesting, the pulp was removed and dried at 60°C until having reached a 5-8% moisture content. Then pulp was crushed into fine powder (0.5-1mm), packed in polyethylene bags and stored in room conditions.

2.2. Chemicals and Reagents

The caffeine reagent was purchased from Sigma-Aldrich (USA). Other chemicals originated from China and India; they were of analytical reagent grade.

2.3. Extraction Process of Caffeine Compound

Coffee pulp powder (5g) was extracted in a water bath system (Memmert WNB14, Germany) at various extraction conditions with distilled water as solvent (Table 1). The mixture was filtered for removal of the residue by means of the vacuum filtration system, and then the caffeine content in the extract was analyzed.

Coded level and actual values of independent factors Table 1

Independent factors	Symbols	Coded levels		
		-1	0	1
Solvent/material ratio [v/w]	X_1	30/1	40/1	50/1
Extraction temperature [°C]	X_2	70	80	90
Extraction time [min]	X_3	90	120	150

2.4. Determination of Caffeine Content

A 100ppm stock standard of caffeine was prepared by dissolving caffeine in dichloromethane in a volumetric flask and a standard curve was built. The received extracts were then filtered through a vacuum filter. After that, the filtrate was made up to 1.000mL by distilled water (V_{dm}). Then, 5mL (V_x) of filtrates was shaken with 5mL of dichloromethane in a separating funnel. The dichloromethane layer containing caffeine was collected and made up to 25mL (V_o). The extract was stored for further experiments. The caffeine content was determined by using a UV-Vis Spectrometer (Genesis 10S) and the absorbance was determined at 275nm [10].

$$CC = C \cdot \frac{100}{10^6} \cdot \frac{V_{dm}}{m} \cdot \frac{V_0}{V_x} \cdot \frac{100}{100-w} \; [g/100g]$$

$$CEE = \frac{CC}{CM} \cdot 100 \; [\%]$$

where:

CC is the caffeine content in extract solution;
CEE - caffeine extraction efficiency;
CM - caffeine content contained in the initial material;
m - mass of material;
w - moisture of material;
C - absorbance of extract.

2.5. Experimental Design

Response surface methodology (RSM) was used as well as optimization studies for determining the response value and analyzing the various effects. Central Composite Face design (CCF) was chosen in this case with three variables and three levels of variation (Table 1). This model was built according to the determined three main factors, including solvent/material ratio (X_1), extraction temperature (X_2) and extraction time (X_3). They were used to find the optimum extraction conditions of the caffeine content from coffee pulp powder. Thus, the response was CEE (Y). The validity of models was confirmed by comparing the experimental and the predicted values.

The regression equation was calculated as follows:

$$Y = a_0 + a_1 X_1 + a_2 X_2 + a_3 X_3 + a_{11} X_1^2 + a_{22} X_2^2 + a_{33} X_3^2 + a_{12} X_1 X_2 + a_{13} X_1 X_3 + a_{23} X_2 X_3$$

2.6. Statistical Data Analysis

Modde software version 8.0 (Umetrics AB company, Sweden) was used to design the experiment and analyze the data. The optimal extraction conditions were performed by contour plots and three dimensional (3D) response surfaces.

3. Results and Discussions

3.1. Optimization of the Extraction Process

A preliminary study was conducted to determine the effect of some factors in wide range. Three main factors (solvent/material ratio, extraction temperature and extraction time) were selected as pertinent. The multivariable linear regression was employed to calculate and estimate the constants, coefficients of linear, quadratic and interaction effects (Table 3). The constants and coefficients were inserted into the regression equation which was obtained below. Table 2 showed that the CEE of coffee pulp powder varied from 68.54 to 87.17%. This proved that the independent factors (solvent/material ratio, extraction temperature, extraction time) affect CEE.

In addition, as shown in Table 3, the p_{value} of the regression equation model is lower than 0.05. This confirmed that the model was significant. Furthermore, the lack of fit ($p>0.05$) was not significant for this model indicating the good predictability of the responses by the models.

$$Y = 86.41 - 2.4 X_1 + 2.36 X_2 - 6.8 X_1^2 - 5.16 X_2^2 - 0.92 X_3^2 + 0.52 X_1 X_2 + 0.72 X_2 X_3$$

In addition, the coefficient of determination ($R^2=0.995$) showed that this model can explain more than 99.5% of the actual data in the response. The adjusted coefficient of determination ($R^2_{adj}=99.1\%$) was quite close to the received R^2 value, indicating that the measured and predicted values had a high fitting precision proving the feasibility of the experimental method. It also indicated the close agreement between the experimental and the predicted values in Table 2. Besides, Eriksson et al. [5] pointed out that the Q^2 parameter indicates the accuracy of the prediction and estimates the predictive power of the model. The Q^2 value for CEE in this case (0.968) should be regarded as good (Q^2>0.5 and R^2-Q^2<0.2-0.3). Therefore, this model is quite accurate for predicting the response. Based on the regression equation, CEE depends on all parameters such as solvent/material ratio, extraction temperature and extraction time.

For the linear term, the extraction time (X_3) was removed from the regression equation because it was not statistically significant (p_{value}>0.05). Only solvent/ material ratio (X_1) and extraction temperature (X_2) affect CEE. However, the extraction temperature (X_2) is the positive strongest influence (The coefficient of X_2 has the maximum value in the linear term) and the solvent/material ratio (X_1) is a negative influence on CEE.

Table 2

Experimental design matrix and predicted results for CEE

Run	Independent factors						Responses Y, CEE [%]	
	X_1	X_2	X_3	Solvent/ material ratio [v/w]	Extraction temperature [°C]	Extraction time [min]	Experimental value	Predicted value
1	-1	-1	-1	30	70	90	75.19	74.9107
2	1	-1	-1	50	70	90	69.07	69.0367
3	-1	1	-1	30	90	90	76.74	77.1256
4	1	1	-1	50	90	90	73.62	73.3417
5	-1	-1	1	30	70	150	72.66	72.7437
6	1	-1	1	50	70	150	68.54	67.9596
7	-1	1	1	30	90	150	78.02	77.8587
8	1	1	1	50	90	150	75.08	75.1647
9	-1	0	0	30	80	120	81.78	81.7514
10	1	0	0	50	80	120	76.66	77.4674
11	0	-1	0	40	70	120	78.08	78.8894
12	0	1	0	40	90	120	83.63	83.5994
13	0	0	-1	40	80	90	85.36	85.5654
14	0	0	1	40	80	150	84.82	85.3934
15	0	0	0	40	80	120	86.51	86.4078
16	0	0	0	40	80	120	86.70	86.4078
17	0	0	0	40	80	120	86.15	86.4078
18	0	0	0	40	80	120	86.42	86.4078
19	0	0	0	40	80	120	87.05	86.4078
20	0	0	0	40	80	120	87.17	86.4078

Regarding the quadratic effect, all variables are negative effects on CEE. The influence of extraction time and temperature is lower than that of the solvent/material ratio which also has the strongest influence on CEE. This result is similar to the study of Tello et al. [15] who extracted caffeine from coffee husk using supercritical carbon dioxide and they also pointed out that the extraction temperature is the main factor that has the strongest influence on the extraction process. In addition, Shalmashi et al. [13] noticed that the important influence on the black tea leaf extraction process was the extraction temperature.

For the interactions between factors, there is the interaction between the solvent/material ratio and extraction temperature (X_1X_2); extraction temperature and time (X_2X_3). CEE did not depend on the interaction between the solvent/material ratio and extraction time (X_1X_3). These interactions are positive effects; however, the level of influence of X_2X_3 is stronger than that of X_1X_2 and this influence depended on the presence of the extraction temperature.

Table 3

Analysis of variance and regression equation coefficients of the model

Y	Coefficient	Std. Err	p_{value}
Constant	86.4073	0.203903	1.31739e-22
X_1	-2.142	0.187563	4.64792e-7
X_2	2.355	0.187563	1.90632e-7
X_3	-0.0859954	0.187563	0.656404*
X_1X_1	-6.79819	0.357669	3.52872e-9
X_2X_2	-5.16319	0.357669	5.0513e-8
X_3X_3	-0.92818	0.357669	0.026716
X_1X_2	0.522503	0.209702	0.0319001
X_1X_3	0.272498	0.209702	0.222946*
X_2X_3	0.724998	0.209702	0.0061507
N=20	R^2=0.995	R^2_{adj}=0.991	Q^2=0.968
Regression equation model: p_{value} = 0.000			
Lack of fit (F=3.67): p_{value}=0.09*			

*: p_{value}>0.05, insignificant.

3.2. Response Surface Plots

The three-dimensional response surfaces of CEE as a function of solvent/material ratio, extraction temperature and extraction time were given in Figure 1. According to Figure 1 (A and A'), CEE increased when the solvent/material ratio and the extraction temperature increased. This is similar to the results of studies by Bermejo et al. [4] and Tello et al. [15].

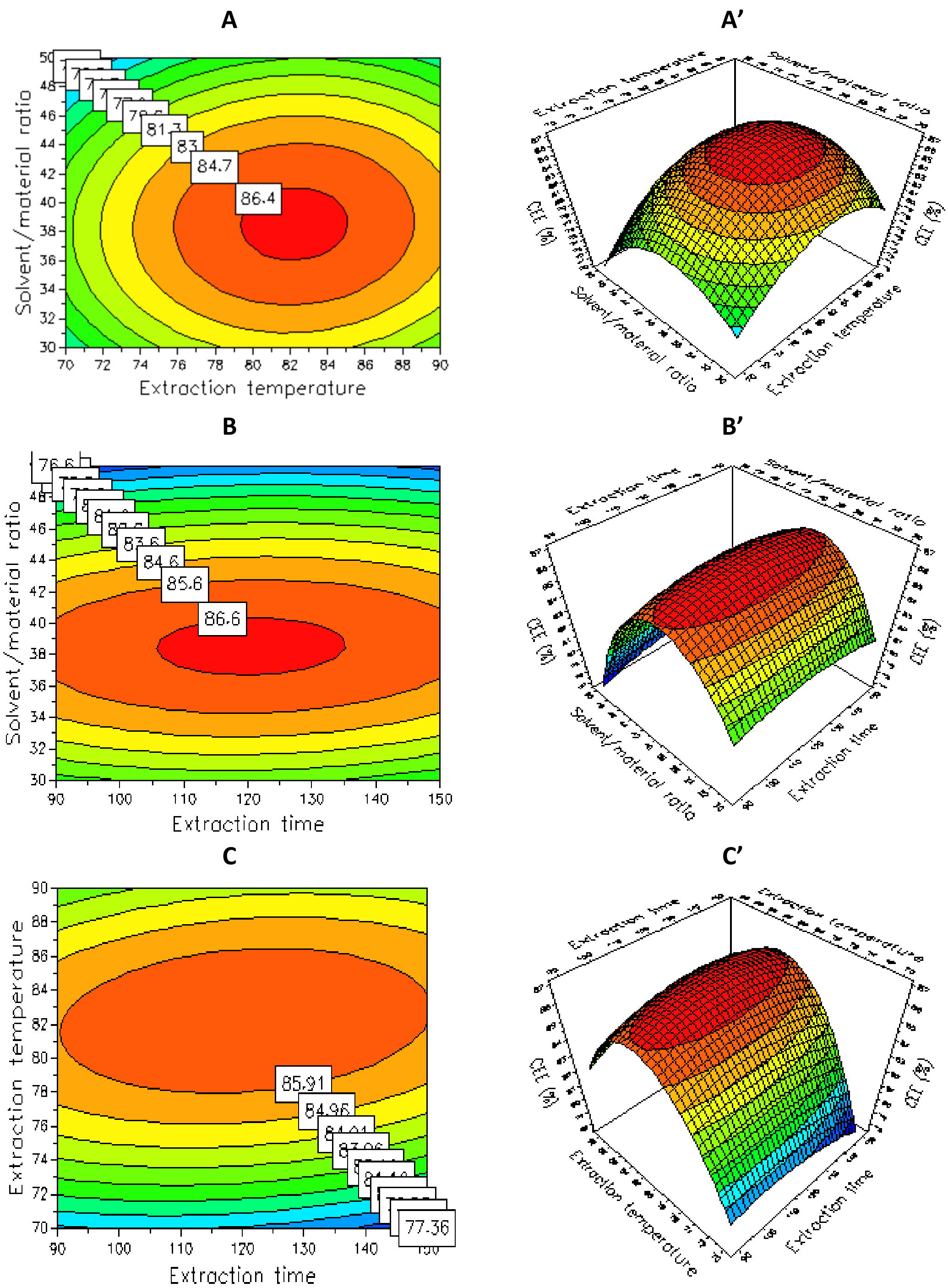

Fig. 1. *Contour (left) and response surface (right) plots of solvent/material ratio, extraction temperature and extraction time and its mutual interaction to CEE*

The same observation was found in the case of the interaction of the solvent/material ratio and extraction time (Figure 1b and 1b') or the interaction of extraction time and extraction temperature (Figure 1c and 1c'). When the solvent/material ratio and extraction time increase, the CEE also increases.

Extending the extraction time can improve the CEE, soluble compounds easily diffuse into the solvent. Besides, the CEE increases proportionally with increasing extraction temperature. The high temperature can reduce the viscosity of the solvent, rupture the cell wall and easily release caffeine compound [1], [11]. However, the increases in these factors have a limitation; the limitation causes the degradation of bioactive compounds mainly because they are heat-sensitive compounds and easily oxidized on longer exposure to the ambient atmosphere.

3.3. The Determination and Validation of Optimal Conditions

The RSM model proved that the optimum conditions for maximizing CEE were a solvent/material ratio of 38.47/1, an extraction temperature of 82.19°C and an extraction time of 120.55 min (Table 4). The predicted theoretical value and the actual value at the optimal conditions are 86.83% and 88.52%, respectively. This indicated that experimental and the predicted results were quite similar. Hence, the suggested model could be used to predict the response value.

Table 4

Results of optimal conditions

Factors	X_1 [v/w]	X_2 [°C]	X_3 [min]	Y [%]
Predicted result	38.47/1	82.19	120.55	86.83
Experimental result				88.52 ± 0.49

4. Conclusion

In general, the caffeine extraction was optimized by RSM. The results pointed out that the CEE significantly depended on the solvent/material ratio, the extraction temperature, extraction time and the interaction between these factors. The experimental values were in agreement with the predicted values and this indicated the suitability of the models. Hence, this model can be used to optimize the caffeine extraction from coffee pulp powder in experimental conditions.

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