OPTIMIZATION OF CONDITIONS OF EXTRACTION PROCESS FROM COFFEE CHERRY PULP (CASCARA) AND APPLICATION TO DRINKING WATER PRODUCT WITH ANTIOXIDANT ACTIVITY

Do Mai Nguyen Phuong *, Hoang Thi Truc Quynh, Le Thi Hong Anh

Department of Post-harvest technology, Food Science & Technology Faculty, Ho Chi Minh City University of Food Industry, 140 Le Trong Tan, Tay Thanh, Tan Phu

*Email: phuongdmn@fst.edu.vn

Received: 30 July 2019; Accepted for publication: 2 November 2019

Abstract. This study explored the optimization of extraction condition of total polyphenols and flavonoids from dried coffee cherry pulp (Cascara) by response surface methodology. The effect of varying the extraction conditions such as solvent (water)-to-material ratio (X1); temperature (X2) and time (X3) were studied. The results demonstrated that the best combination of extraction conditions for dried coffee cherry pulp was at 1:40.77 solid-solvent ratio, 71.59 °C and 48.39 min. At optimal extraction conditions, the total polyphenols content achieved 23.816 mg GAE/g and total flavonoids achieved 11.365 achieved mg QE/g, on a dry basis.

The optimal extraction process was applied to drinking water product from coffee cherry pulps, and sensory methods were implemented according to the ranking test method to determine the mixing formula. Optimal formulation was found comprising of 9 °Bx and 0.01 % acid citric. According to the consumer acceptance test, the final product of this research was rated 4.7 on a scale of 7. The studied product has good organoleptic properties and sterilization standards, ensuring food safety for consumers.

Our research results indicated potential of using dried coffee cherry pulp (Cascara) as promising resources for extracting natural antioxidants.

Keywords: coffee cherry pulp, Cascara, polyphenol, flavonoid.

Classification numbers: 1.2.1, 1.3.1

1. INTRODUCTION

In 2018, Viet Nam is the world’s second – largest exporter of coffee (after Brazil) with nearly 1.9 million tons, about 3.5 billion USD. This accounts for 14 per-cent of the global coffee market. Along with the development of the coffee processing industry, a large number of by-products are released. Over 50 percent of coffee volume is not used in the production of green coffee. Most of coffee wastes are brought directly to landfills or rivers. Handling these is a big challenge. For this reason, efforts have been made to develop methods for coffee waste treatment and management, as well as its utilization as a raw material for the production of
feeds, beverages, vinegar, biogas, caffeine, pectin, pectic enzymes, protein and compost. Coffee waste is emerging as a new feed for producing polysaccharides and monosaccharide [1].

In addition, coffee pulp is rich in carbohydrates, soluble fibers, minerals, proteins, and especially bioactive compounds such as polyphenols with good antioxidant properties, bringing many health benefits [2]. Therefore, the production of polyphenol-rich drinking water products from coffee cherry pulp is a good way to solve the source of coffee waste and diversify the source of beverage products today.

In this study, material ratio parameters: solvent (water)-to-material ratio, temperature and time of extraction process from dried coffee cherry pulp (Cascara) were optimized to capture the highest polyphenol and flavonoid content. Thereby conducting sensory evaluation to have a blending formula that brings the final product to consumers' preference.

2. MATERIALS AND METHODS

2.1. Plant material

The materials used in the study are the arabica coffee collected from the wet (fermented and washed) processing. The coffee sample was dried with humidity 5 ± 0.2 % and ground to about 1.25 to 2 mm in size. The material was vacuumed-packed in PE bags, and stored in a dry place to prevent the absorption of moisture outside.

Dried coffee cherry pulps were purchased at ANKROET Coffee Co., Ltd. Address is 157C Thanh Mau, Ward 7, Da Lat City, Lam Dong.

2.2. Standards and reagents

All chemicals used are of analytical grade. FolinCiocalteu’s phenol reagent, gallic acid, DPPH (1,1-Diphenyl-2-picrylhydrazyl), quercetin, sodium carbonates and pure methanol, BHT and ascorbic acid were purchased from Sigma-Aldrich. All aqueous solutions were prepared using bi-distilled water.

2.3. Analytical methods

2.3.1. Total phenolic content (TPC)

This method is based on the reduction of a phosphowolframate–phosphomolybdate complex to blue products by phenolic compounds. To 5 ml of Folin-Ciocalteu’s reagent, 1 ml extract was added and the mixture was allowed to react. After 5 min, 4 ml of Na₂CO₃ (7.5 % w/v) solution was added and shaken. The solution was kept in the dark under ambient conditions for 60 min to complete reaction. After that the absorbance was measured at 765 nm. The results are expressed as mg Gallic acid equivalents (GAE) on a dry mass basis (mg GAE g dry basis) [3].

2.3.2. Total flavonoid content (TFC)

Total flavonoids content were estimated by Aluminium chloride colorimetric method. To 1 ml extract, 4 ml bi-distilled water and 0.3 ml NaNO₂ (5 % w/v) solution was combined and shaken in 10 ml volumetric flask. After 5 min, 0.3 ml AlCl₃ (10 % w/v) solution was added and
shaken. 6 min later, 2 ml of 1 M NaOH was added and set to mark with bi-distilled water. Then the absorbance was measured at 510 nm. The results are expressed as mg Quercetin (QE) on a dry mass basis (mg QE/g dry basis) [4].

2.3.3. Statistical analysis

The data reported in this paper are the averages of three replicates. The experimental data were analyzed using the statistical software Statgraphics Centurion XV. The significant difference between all the terms was determined by variance evaluation (ANOVA). Not significant (p > 5 %), *: significant (p < 5 %).

Optimal conditions of the extraction process were studied by the surface response method (RSM) using MODDE 5.0 software.

2.4. Experiments

2.4.1. Optimal extraction by RSM surface method

The Central Composite Face (CCF) - Response surface methodology (RSM) was used to optimize the extraction parameters (solvent-to-material ratio (X1); temperature (X2) and time (X3)). All experimental design consisted of seventeen factorial experiments, and three simulates of the center point. The single factor influence was previously examined.

To design an optimal experiment, we first find the experiment at the center of the element to be investigated is the as solvent (water)-to-material ratio; temperature and time. The point at the center selected is the highest total polyphenols and total flavonoids.

The coded and actual values of three variables are given in Table 1. The experiment was designed and its data was processed using MODDE 5.0 software. With values: [0]: value at the center; [-1]: lower value; [+1]: the above values of the parameters from the survey experiments were available from another study.

| Variables                     | Coded levels of variables |
|-------------------------------|---------------------------|
| Solvent-to-material ratio (X1) (g/g) | -1  30  0  40  +1  50 |
| Temperature (X2) (°C)         | -1  60  0  70  +1  80 |
| Time (X3) (min)              | -1  30  0  45  +1  60 |

The process was optimized to maximize the total polyphenols (TPC) (Y1) and the total flavonoids (TFC) (Y2) in the extract. The optimal value is where the total polyphenols and flavonoids content values are maximized.

2.4.2. Sensory evaluation of drinking water products from cascara after applying optimization conditions in the extraction process (Sensory evaluation of final product)

The extract was obtained from the extraction process with the optimized conditions (in Experiment 2.4.1), filtered off the residue and added 0.01 % citric acid and mixed with syrup sugar such that the brix level reached 9° Bx. Syrup sugar (7, 8, 9, 10, 11° Bx) and acid citric (0, 0.01, 0.02, 0.03, 0.04 %) content experiments have been conducted before. The appropriate level
of sugar and citric acid was determined by ranking test (60 person). The ratio of sugar syrup 9° Bx and citric acid 0.01% was assessed to have a higher sensory score than the other; the product had a harmonious sweetness, was not too sour and drown out the typical taste of water from the coffee cherry pulp.

Then, the final product was pasteurized at 83 °C for 30 minutes. Sensory evaluation was conducted by scoring taste to determine the degree of consumer acceptance (60 person) of the product from Cascara and two lines of refreshing health drinks in the market (Dr. Thanh and Oolong Tea plus) was used.

3. RESULTS AND DISCUSSION

3.1. Optimization of extraction parameters

Content of TPC and TFC in the Cascara extract was determined. The results are given in Table 2.

Table 2. The result of optimal extraction experiment.

|   | X1 | X2 | X3 | Solvent-to-material ratio | Temp | Time | TPC (Y1) (mg GAE/g dry basis) | TFC (Y2) (mg QE/g dry basis) |
|---|----|----|----|---------------------------|------|------|-------------------------------|-----------------------------|
| 1 | -1 | -1 | -1 | 30                        | 60   | 30   | 19.750                        | 8.288                       |
| 2 | 1  | -1 | -1 | 50                        | 60   | 30   | 20.865                        | 9.627                       |
| 3 | -1 | 1  | -1 | 30                        | 80   | 30   | 20.484                        | 9.365                       |
| 4 | 1  | 1  | -1 | 50                        | 80   | 30   | 20.263                        | 9.082                       |
| 5 | -1 | -1 | 1  | 30                        | 60   | 60   | 19.276                        | 8.988                       |
| 6 | 1  | -1 | 1  | 50                        | 60   | 60   | 20.886                        | 9.801                       |
| 7 | -1 | 1  | 1  | 30                        | 80   | 60   | 21.037                        | 10.202                      |
| 8 | 1  | 1  | 1  | 50                        | 80   | 60   | 21.495                        | 9.513                       |
| 9 | -1 | 0  | 0  | 30                        | 70   | 45   | 22.316                        | 10.498                      |
| 10| 1  | 0  | 0  | 50                        | 70   | 45   | 22.782                        | 10.623                      |
| 11| 0  | -1 | 0  | 40                        | 60   | 45   | 22.018                        | 10.421                      |
| 12| 0  | 1  | 0  | 40                        | 80   | 45   | 22.972                        | 11.089                      |
| 13| 0  | 0  | -1 | 40                        | 70   | 30   | 21.892                        | 10.619                      |
| 14| 0  | 0  | 1  | 40                        | 70   | 60   | 23.542                        | 11.198                      |
| 15| 0  | 0  | 0  | 40                        | 70   | 45   | 23.841                        | 11.217                      |
| 16| 0  | 0  | 0  | 40                        | 70   | 45   | 23.962                        | 11.228                      |
| 17| 0  | 0  | 0  | 40                        | 70   | 45   | 24.021                        | 11.284                      |

The regression equation (*) objective function Y1 (TPC content) is expressed:

\[ Y = 23.7997 + 0.3428X1 + 0.3456X2 + 0.2882X3 - 1.1444X1^2 - 1.1984X2^2 \]
According to (*), there is no interaction between material ratio: solvent (X1) and extraction time (X3), between extraction temperature (X2) and extraction time (X3) but the factors independently affect the TPC target function. There is interaction between solvent (X1) and temperature (X2) but not trivial.

Whereas the raw material variables: solvent, temperature and extraction time have a positive effect on the TPC target function (due to the result "+"). The results showed that although the parameters affect recovery of TPC. However, the quadratic coefficient of solvent, temperature and time had relative in inverse ratio to polyphenol content. If continued to increase, TPC yield tended to decrease at higher extraction parameters. Two-dimensional contour plots and three-dimensional response surface plots are presented in Fig. 1.

Regression coefficient is $R^2 = 0.975$ and virtual variation coefficient $Q^2 = 0.811$. The value of $R^2$ and $Q^2$ were nearly close to 1, indicating a degree of correlation between the observed and predicted values, hence suggesting that the model was significant [5].

![Figure 1](image1.png)

**Figure 1.** Graph of surface response of total polyphenols content by contour plot (a) and three-dimensional (b).

The regression equation (***) objective function $Y_2$ (TFC content) is expressed:

$$Y = 11.3286 + 0.1305X_1 + 0.2126X_2 + 0.2721X_3 - 0.8323X_1^2 - 0.6377X_2^2 - 0.4843X_3^2 - 0.3905X_1X_2 - 0.1165X_1X_3$$

![Figure 2](image2.png)

**Figure 2.** Graph of surface response of total flavonoids content by contour plot (a) and three-dimensional (b).
According to the regression equation (**), there is no interaction between the extraction temperature (X3) and the extraction time (X2) but the factors independently affect the TFC target function. There is interaction between solvent (X1) and temperature (X2); between solvent (X1) and temperature (X3) but not trivial (due to the result "-").

Meanwhile, the ratio of raw material ratio: solvent, temperature and extraction time have a positive influence on the results of the TFC target function (due to the result "+"). The results showed that although the parameters affect recovery of TFC. However, the quadratic coefficient of solvent, temperature and time had relative in inverse ratio to flavonoid content. If continued to increase, TFC yield tended to decrease at higher extraction parameters. Two-dimensional contour plots and three-dimensional response surface plots are presented in Fig. 2.

Regression coefficient is $R^2 = 0.992$ and virtual variation coefficient $Q^2 = 0.953$. The value of $R^2$ and $Q^2$ were nearly close to 1, indicating a degree of correlation between the observed and predicted values, hence suggesting that the model was significant [5].

Optimal extraction conditions are found by RSM. The optimal results of the optimization model verification are in Table 3:

Table 3. Predicted and experimental values under optimum conditions based on the multiple responses of TPC and TFC.

| Solvent-to-material ratio | Temp  | Time  | Predicted value | Experimental value |
|---------------------------|-------|-------|-----------------|-------------------|
|                           |       |       | TPC (mg GAE/g)  | TFC (mg QE/g)     |
|                           |       |       | TPC (mg GAE/g)  | TFC (mg QE/g)     |
| 1: 40.7733                | 71.5914 | 48.3857 | 23.8703         | 11.3831           |
|                           |       |       | 23.816          | 23.816            |

Experimental results show that the average TPC ($p = 0.479 > 0.05$) and TFC ($p = 0.489 > 0.05$) when tested by student test ($n = 5$) there are no significant difference between the predicted value and the experiment.

3.2. The result of sensory evaluation of drinking water product from *cascara* after applying optimization conditions in the extraction process

The results evaluated of final product with two healthy herbal tea lines, Dr Thanh and Oolong tea plus, as shown in the Figure 3.

![Figure 3](image-url)  
*Figure 3. Graph of the degree of consumer acceptance by sensory evaluation, final product (a), Dr. Thanh (b) and Oolong tea plus (c).*
With an average score of 4.7125 on a scale of 7, drinking water from Cascara is receiving positive reviews from consumers. At the same time, there is no difference in priority between final product and Dr Thanh tea product ($\alpha = 0.05$), showing that the product has been accepted by the consumer as a refreshing drink that are healthy.

4. CONCLUSIONS

Research results showed that three factors: the ratio of materials: solvent (water), temperature and time affect the extraction from coffee cherry pulp (cascara). The optimization process offers optimal conditions: the ratio of solvent material 1:40.7733, the extraction temperature of 71.5914 °C, the extraction time of 48.39 minutes. After the control experiment was performed, the total polyphenols and flavonoids content were 23.816 mg GAE/g dry basis and 11.365 mg QE /g dry basis, respectively. Applying the optimization conditions of extraction process to drinking water products from coffee cherry pulps and implementing sensory methods according to the ranking test method to determine the mixing formula: syrup ratio 9°Bx, citric acid content is 0.01 %.

Acknowledgements. The research funding from the Research Foundation of Ho Chi Minh University of Food Industry was acknowledged.

REFERENCES

1. Heeger A., Kosińska-Cagnazzo Cantergiani E., and Andlauer W. - Bioactives of coffee cherry pulp and its utilisation for production of Cascara beverage, Food chemistry 221 (2017) 969-975.
2. Geremu M., Tola Y. B., and Sualeh A. - Extraction and determination of total polyphenols and antioxidant capacity of red coffee (Coffea arabica L.) pulp of wet processing plants, Chemical and Biological Technologies in Agriculture 3 (2016) 25.
3. Sulaiman C. and Balachandran I. - Total phenolics and total flavonoids in selected Indian medicinal plants, Indian journal of pharmaceutical sciences 74 (2012) 258.
4. Al-Farsi M. A. and Lee C. Y. - Optimization of phenolics and dietary fibre extraction from date seeds, Food Chemistry 108 (2008) 977-985
5. Duc C. T. and Van K. - Finding optimal conditions for hydrophilic polymer polymerization process based on acrylic acid using ammonium sulfate initiator by experimental planning method, Journal of Science and Technology 49 (4) (2011) 95-99.