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Optimising the roasting and extraction process applied to cascara products oriented for use by people with diabetes

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Abstract:

This research aims to utilise the tonnes of coffee pulp discharged annually from Vietnam's Robusta coffee industry. Cascara, a by-product obtained after the coffee beans have been separated, has a high nutrient content and the potential to be used as a raw material in the food industry. However, coffee pulps are often discarded or used as agricultural waste. This study explored the optimisation of roasting and extraction conditions for Cascara using response surface methodology (RSM) to obtain healthier products enriched in total polyphenols and antioxidant activity. The ideal conditions identified by the optimisation process include a roast temperature of 130.798°C, a roast time of 226.153 seconds, a solvent-to-material ratio of 1:38.007, an extraction temperature of 71.581°C, and an extraction time of 46.916 minutes. Additionally, the extract from Cascara tea inhibited the digestive enzyme activity of α-glucosidase, with an IC_{50} value of 99.18 µg/ml. Our research results indicate the potential of using Cascara as a valuable resource for extracting natural antioxidants, especially in products targeted at individuals with type 2 diabetes.

Keywords: **α-glucosidase, cascara, optimisation, polyphenol.**

Classification numbers: **3.1, 3.5, 3.6**

1. Introduction

After Brazil, Vietnam is the most significant producer and exporter of Robusta coffee and the second-largest exporter of coffee overall [1]. The green coffee processing industry produces millions of tonnes of waste annually, including coffee husks. However, only a small portion is utilised in agriculture, the cosmetic and pharmaceutical sectors, or as fuel; the remainder is inadequately used and often released into the environment, causing harm [2]. The coffee pulp contains components beneficial to health, such as caffeine, chlorogenic acid, tannin, rutin, and catechin [3]. Therefore, using coffee cherry pulp to produce beverage products high in polyphenols is an excellent approach to reducing coffee waste and expanding the range of available beverage products.

Diabetes is characterised by abnormally high blood glucose levels, which can lead to other conditions such as cardiovascular, retinal, or neurological diseases. By inhibiting digestive enzymes such as α-amylase and α-glucosidase, type 2 diabetes can be managed [4]. Coffee pulp contains bioactive compounds that influence various pathways involved in the aetiology of type 2 diabetes, thereby reducing the risk of developing the condition [5].

Since the 20th century, scientists from several countries have researched the structure and chemical composition of coffee pulp. Chlorogenic acid, trigonelline, anthocyanin, protocatechuic acid, gallic acid, rutin, and caffeine are the principal bioactive substances present in coffee pods [6]. In specific research, coffee pulp powder has been used to create cookies high in dietary fibre and antioxidant activity [7]. T. Lu, et al. (2023) [8] recently assessed how temperature and roasting duration affect the roasting of coffee pulp.

Accordingly, this research on the topic "Optimising the roasting and extraction process to obtain highly bioactive compounds by response surface method (RSM) applied to Cascara products from coffee pulp oriented for use by people with diabetes" was conducted to determine the best method of roasting and extraction to maximise the product's value. This optimisation helps to improve the position of Cascara tea in the Vietnamese market, where the product is primarily aimed at people with diabetes.

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2. Materials and methods

2.1. Plant materials

The materials used in the study were Robusta coffee pulp collected from the dry processing method. The coffee pulp sample was dried to a humidity level of 5±0.2% and ground to a size of approximately 2-5 mm. The materials were vacuum-packed in PE bags and stored in a dry place to prevent moisture absorption. Cascara was purchased from Lam Dong province, Vietnam.

2.2. Methods

Total phenolic content (TPC): This method involves reducing a phosphowolframate-phosphomolybdate complex to blue products by phenolic compounds. To 5 ml of Folin-Ciocalteu's reagent, 1 ml of extract was added, and the mixture was allowed to react. After 5 minutes, 4 ml of Na_2CO_3 (7.5% w/v) solution was added and shaken. The solution was kept in the dark under ambient conditions for 60 minutes to complete the reaction. The absorbance was then measured at 765 nm. The results are expressed as mg gallic acid equivalents (GAE) on a dry mass basis (mg GAE/g dry basis) [9].

α-*glucosidase inhibition assay:* The *in vitro* method to investigate the inhibitory activity of the α-glucosidase enzyme is based on the principle that when α-glucosidase encounters the α-D-glucose bond, it breaks this link to release the sugar D-glucose. Therefore, substrates with α-linked D-glucose sugar, such as p-nitrophenyl-α-D-glucopyranoside and maltose, are used to develop a test method for α-glucosidase enzyme inhibitory activity in the laboratory [10]. After roasting and extracting under optimal conditions, the coffee pulp was obtained in a crude form and then dissolved with phosphate buffer according to the α-glucosidase inhibitory assay procedure.

Statistical analysis: The data reported in this paper are the averages of three replicates. The experimental data were analysed 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 RSM using Design-Expert 13.

2.3. Design of experiments

Optimal roast by response surface method: The weight of one roasted batch was 200 g. The equipment used was a rotary cage roaster. The heating principle is based on air convection, and the temperature is expressed as the temperature measured inside the centre of the rotating cage. Based on previous measurement experiments, coffee pulp only changes significantly in the temperature range from 100-180°C, and designing the experimental plan according to central composite design (CCD) helps expand the range of considerations to understand the changes. The roasting has been affected by several factors. The roasting factors have been fully classified into the following categories: temperature (X_1) and time (X_2) [8]. These are shown in Table 1.

Table 1. Coded and actual levels of five variables.

Variables	Coded levels of variables				
	-	*-1*	*0*	*+1*	+
Temperature (X_1) (°C)	83	100	140	180	197
Time (X_2) (s)	125	150	210	270	295

The process was optimised to maximise the extract's TPC (Y_1). The optimal value is where the total polyphenols and antioxidant activity content values are maximised. The regression model was a second-order equation, as shown in Equation (1).

$$Y_1 = \beta_0 + \beta_1.X_1 + \beta_2.X_2 + \beta_{12}.X_1.X_2 + \beta_{11}.X_1^2 + \beta_{22}.X_2^2 + \varepsilon \quad (1)$$

Optimal extraction by response surface method: Box-Behnken RSM was used to optimise the extraction parameters [solvent (water)-to-material ratio (X_1), temperature (X_2), and time (X_3)]. All experimental designs consisted of seventeen factorial experiments and five simulations of the centre point. The single-factor influence was previously examined. The coded and actual values of the three variables are given in Table 2. The experiment was designed, and its data were processed using Design-Expert software. Values: [0]: value at the centre; [-1]: lower value; [+1]: higher value of the parameters from the survey experiments were available from another study.

Table 2. Coded and actual levels of three variables.

Variables	Coded levels of variables		
	-1	*0*	*+1*
Solvent (water)-to-material ratio (X_1) (g/g)	10	30	50
Temperature (X_2) (°C)	50	70	90
Time (X_3) (minute)	10	40	70

The process was optimised to maximise the extract's TPC (Y_2). The optimal value is where the total polyphenols and antioxidant activity content values are maximised. The regression model was a second-order equation, as shown in Equation (2).

$$Y_2 = \beta_0 + \beta_1.Z_1 + \beta_2.Z_2 + \beta_3.Z_3 + \beta_{12}.Z_1.Z_2 + \beta_{23}.Z_2.Z_3 + \beta_{13}.Z_1.Z_3 + \beta_{11}.Z_1^2 + \beta_{22}.Z_2^2 + \beta_{33}.Z_3^2 + \varepsilon \quad (2)$$

3. Results and discussion

3.1. Optimisation of roast parameters

Two independent variables, roast temperature (X_1) and roast time (X_2), were selected as the critical variables and designated at five distinct levels: +α, +1, 0, -1, -α. The design of CCD for two variables with one predicted value of total polyphenol (Y_1) is shown in Table 3.

Table 3. The result of an optimal roast experiment.

No.	X_1	X_2	Temp. (°C)	Time (s)	Total phenolic content (Y_1) (mg GAE/g dry basis)
1	0	-	140	125	32.51
2	-1	1	100	270	38.12
3	0	0	140	210	39.26
4	0	0	140	210	38.85
5	-1	-1	100	150	28.82
6	0	+	140	295	31.93
7	1	-1	180	150	33.67
8	0	0	140	210	39.26
9	0	0	140	210	38.98
10	+	0	197	210	27.03
11	-	0	83	210	32.47
12	1	1	180	270	24.42
13	0	0	140	210	38.87

The regression coefficients were calculated, and the linear regression model in Design-Expert checked their statistical significance. The outcomes of experiments such as Y_1 are indicated in Table 4. The effect of the regression coefficients in the models might not be significant if the P-value is greater than 0.05 and should be removed from the regression equation.

Table 4. The regression coefficients with the response value.

Source	Coefficient	Standard error	F-value	p-value
Intercept	39.04	0.1218	862.00	<0.0001
X_1	-2.06	0.0959	461.09	<0.0001
X_2	-0.0963	0.0962	1.00	0.3502
$X_1.X_2$	-4.64	0.1362	1160.09	<0.0001
$X_1.X_1$	-4.53	0.1021	1969.78	<0.0001
$X_2.X_2$	-3.35	0.1030	1058.99	<0.0001

A check of the fit of these models indicated that the determination coefficient (R^2) value was 0.9984. The importance of probability with Fisher's statistic was also low ($F=4.09 < F_{0.05;3,4}=6.59$). This indicates that the goodness of fit of the models is significant enough to use for optimising the roasting process. The regression Equation (3) for the objective function Y_1 (TPC content) is expressed as:

$$Y_1 = 39.04 - 2.06X_1 - 4.64X_1X_2 - 4.53X_1^2 - 3.35X_2^2 \quad (3)$$

A response surface plot indicates the relationship between multiple input variables and one output variable, as shown in Fig. 1. The effects between the main variables (β_1, β_2, β_3) and their interactions (β_{12}, β_{23}, β_{13}) on the response surface values are shown in Fig. 1. As the roasting temperature increases, the roasting time is reduced, and the total polyphenol value is highest.

As shown in Fig. 1, the relationship between roasting temperature and roasting time is a quadratic curve, and the maximum total polyphenol value obtained was 39.297 mg GAE/g dry basis. Optimal conditions were determined as a roasting temperature of 131°C and a roasting time of 226 seconds.

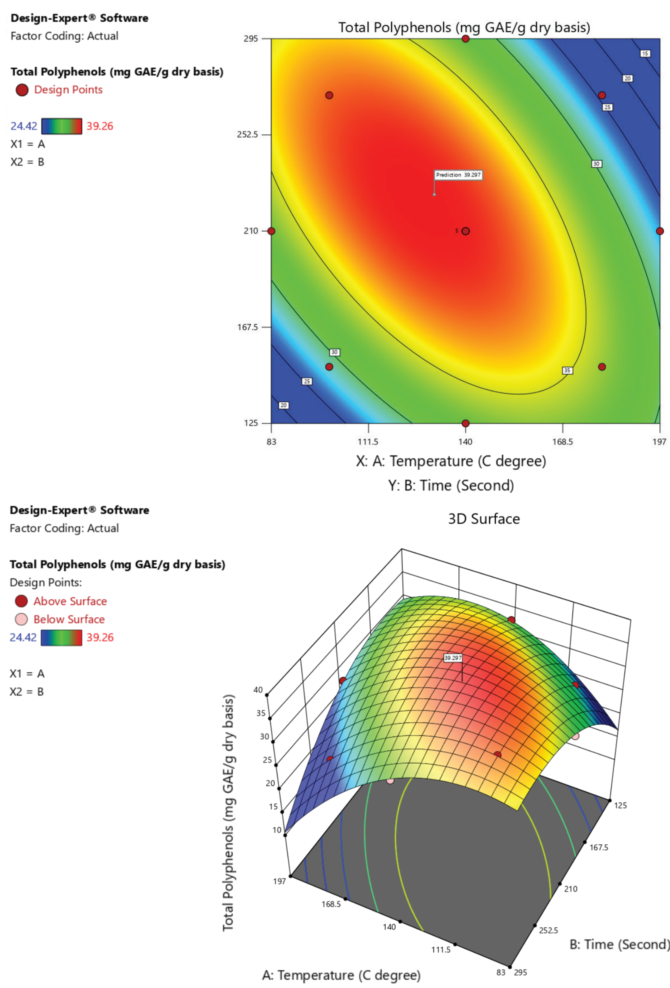

Fig. 1. Graph of the surface response of total polyphenols content by contour plot and three-dimensional plot.

The TPC of the coffee pulp steadily increased with temperature during roasting, but it dropped as temperature was raised further. At high temperatures, the composition of total polyphenols may vary due to the breakdown of chlorogenic acid and an increase in ferulic acid, caffeic acid, and p-coumaric acid. However, if the temperature is increased excessively, the organic materials inside the coffee pulp will begin to break down, lowering the TPC.

An essential factor that affects the TPC is roasting time. The graph's findings demonstrate that the TPC rises. Given the interaction between roasting time and roasting temperature, the time is extended until it reaches its maximum range between 220 and 230 seconds. With more roasting time, it gradually drops.

Response surface method identifies optimal roast conditions. The optimal results of the optimisation model verification are in Table 5.

Table 5. Predicted and experimental values under optimum conditions based on the multiple responses of total phenolic content (mg GAE/g dry basis).

Temp. (°C)	Time (s)	Predicted value	Experimental value
		Total phenolic content (mg GAE/g dry basis)	*Total phenolic content (mg GAE/g dry basis)*
130.798	226.153	39.297	39.38

3.2. Optimisation of extraction parameters

The TPC in the coffee pulp extract was determined. The results are given in Table 6.

Table 6. Results of optimal extraction experiment.

No.	X_1	X_2	X_3	Solvent to material ratio (g/g)	Temp (°C)	Time (min)	Total phenolic content (Y_2) (mg GAE/g dry basis)
1	0	-1	1	30	50	70	39.12
2	0	0	0	30	70	40	42.05
3	0	1	-1	30	90	10	38.64
4	0	-1	-1	30	50	10	38.12
5	0	0	0	30	70	40	41.88
6	0	0	0	30	70	40	41.96
7	-1	1	0	10	90	40	38.98
8	0	1	1	30	90	70	39.42
9	0	0	0	30	70	40	42.13
10	1	1	0	50	90	40	40.24
11	1	-1	0	50	50	40	39.32
12	-1	0	1	10	70	70	40.08
13	-1	-1	0	10	50	40	38.98
14	0	0	0	30	70	40	41.91
15	1	0	1	50	70	70	41.28
16	-1	0	-1	10	70	10	39.21
17	1	0	-1	50	70	10	39.92

A check of fit of these models pointed out that the determination coefficient (R^2) value was 0.9973. The probability values with Fisher's statistic were also low ($F=2.58<F_{0.05;4,4}=6.39$). This indicates that the goodness of fit of the models is significant enough to use for optimising the extraction process. The outcomes of the experiment are shown in Table 7.

Table 7. Regression coefficients with the response value.

Source	Coefficient	Standard error	F-value	p-value
Intercept	41.99	0.0481	291.91	<0.0001
X_1	0.4388	0.0381	132.93	<0.0001
X_2	0.2175	0.0381	32.67	0.0007
X_3	0.5012	0.0381	173.50	<0.0001
$X_1.X_2$	0.2300	0.0538	18.26	0.0037
$X_1.X_3$	0.1225	0.0538	5.18	0.0570
$X_2.X_3$	-0.0550	0.0538	1.04	0.3408
$X_1.X_1$	-0.6542	0.0525	155.57	<0.0001
$X_2.X_2$	-1.95	0.0525	1384.49	<0.0001
$X_3.X_3$	-1.21	0.0525	531.46	<0.0001

The regression Equation (4) for the objective function Y_2 (TPC) is expressed as:

$$Y_2 = 41.99 + 0.4388X_1 + 0.2175X_2 + 0.5012X_3 + 0.23X_1X_2 - 0.6542X_1^2 - 1.95X_2^2 - 1.21X_3^2 \quad (4)$$

According to Equation (4), there is no interaction between the material ratio: solvent (X_1) and extraction time (X_3), nor between extraction temperature (X_2) and extraction time (X_3), but the factors independently affect the TPC target function. 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 the recovery of TPC, the solvent, temperature, and time quadratic coefficients had a relatively inverse ratio to polyphenol content. TPC yield tended to decrease at higher extraction parameters if continued to increase. Two-dimensional contour plots and three-dimensional response surface plots are presented in Fig. 2.

The gradient in concentration between the solid and liquid phases drives the extraction process. When the solvent/material ratio increases (using more solvent), the amount of extractant increases, and the extraction time decreases [11], the concentration difference is significant. However, if the

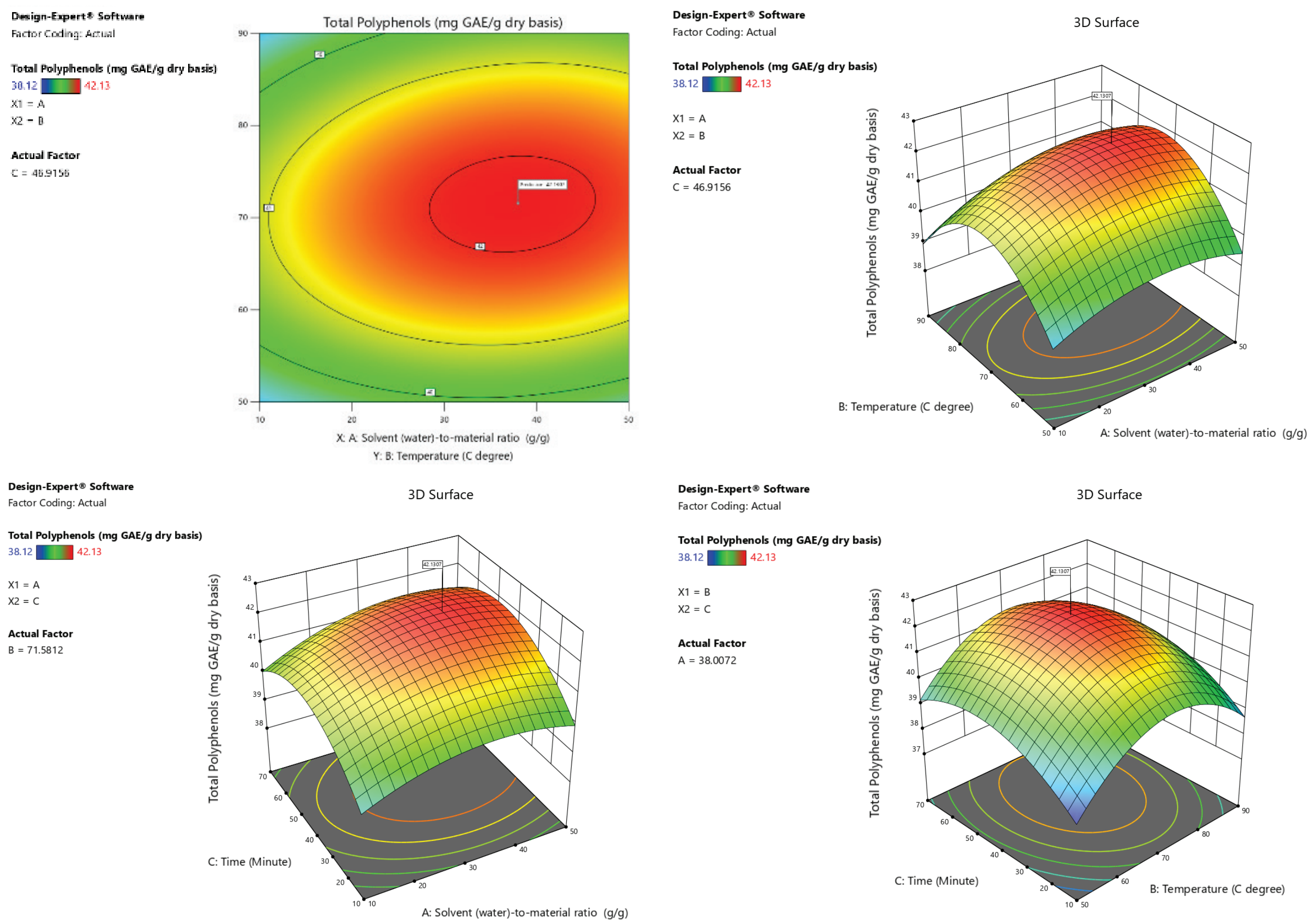

Fig. 2. Graph of the surface response of total phenolic content by contour plot and three-dimensional plot.

solvent is used in excess, the extract will become diluted, necessitating concentration or other treatments to remove the solvent. It is crucial to choose an appropriate solid-to-liquid ratio for the extraction process. The extraction efficiency decreases with increasing suspension density because there is less uniform convection when the solvent-to-coffee pulp ratio is smaller. The graph illustrates this: as the solvent/coffee pulp ratio rises, so does the extraction efficiency, but it is at its highest range of 30 to 40.

The extraction efficiency is significantly influenced by the extraction temperature, according to the findings of earlier studies on the extraction of compounds from plant materials [12]. The bubble-bursting process will become more intense as the temperature rises because more bubbles will form, and the system's viscosity will decrease. Furthermore, the functional characteristics of polyphenols are significantly influenced by temperature. As a result, there is a suitable temperature value to boost the extraction yield without changing the properties of the polyphenol that can be extracted.

The study considered how different extraction time values affected the extracts' content. The graph's findings demonstrate that the variety of substances grew. The extraction time increased simultaneously and peaked around 45-50 minutes in terms of the interaction between time, solvent/coffee pulp ratio, and temperature. After that, the extraction time gradually decreased as the extraction time increased due to polyphenol compounds being oxidised by prolonged high-temperature exposure.

Response surface method identifies optimal extraction conditions. The optimal results of the optimisation model verification are in Table 8.

Table 8. Predicted and experimental values under optimum conditions based on the multiple responses of total phenolic content (mg GAE/g dry basis).

Solvent-to-material ratio	Temp. (°C)	Time (min)	Predicted value	Experimental value
			Total phenolic content (mg GAE/g dry basis)	*Total phenolic content (mg GAE/g dry basis)*
1 : 38.007	71.581	46.916	42.131	42.83

3.3. α-Glucosidase inhibition assay

This study used acarbose as positive evidence when investigating α-glucosidase enzyme inhibitory activity with an IC_{50} of 135.41 µg/ml. When extracted in ethanol, the IC_{50} value of dried coffee pulp was 99.18±0.00 µg/ml (Table 9), which is lower than that of the control acarbose, indicating that the extract of coffee pulp has higher α-glucosidase inhibitory activity than the control sample.

Table 9. Results of IC_{50} (µg/ml) α-glucosidase inhibitory activity.

Extraction solvent	Sample	IC_{50} (µg/ml)
Ethanol 90%	The coffee pulp	99.18±0.00
Acarbose	135.41±0.01	

4. Conclusions

The results of the research indicated that three variables, the ratio of materials to solvent (water), temperature, and time, influence the extraction from the coffee cherry pulp (Cascara), while two variables (roast temperature and roast time) affect the roasting of coffee pulp. The roast temperature of 130.798°C, the roast time of 226.153 seconds, the solvent material ratio of 1:38.007, the extraction temperature of 71.581°C, and the extraction time of 46.916 minutes are the ideal conditions provided by the optimisation process. The IC_{50} value following the control experiment was 99.18 µg/ml, suggesting that people with diabetes can use the product.

CRediT author statement

Thi-Ngoc-Mai Tran: Conceptualisation, Methodology, Formal analysis and investigation, Writing - Original draft preparation, Writing - Reviewing and Editing, Validation; Anh-Tan Duong: Conceptualisation, Methodology, Writing - Reviewing and Editing, Validation, Resources, Supervision.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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