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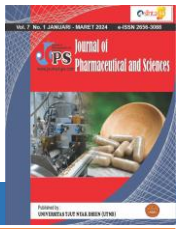

Combined ultrasound and enzymatic-assisted extraction of chlorogenic acid compounds from Arabica Coffee Cascara Kerinci

Ekstraksi kombinasi ultrasonik dan enzimatis untuk senyawa asam klorogenat dari Cascara Kopi Arabika Kerinci

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Abstract

Arabica Coffee Cascara (CKA) has high potential as a source of bioactive compounds such as chlorogenic acid, which has antioxidant activity. The release of active compound can be optimized by combining ultrasonic and enzymatic methods, which are more efficient and environmentally friendly. The aim of this study was to examine the effectiveness of ultrasound-enzymatic-assisted extraction (UEAE) using water as a solvent for chlorogenic acid compound from CKA using Response Surface Methodology Box-Behnken Design (RSM_BBD). Three extraction parameters, such as sonication temperature (35-45°C), enzyme concentration (10-20 mg/g), and enzymolysis temperature (40-60°C), were designed to obtain optimal chlorogenic acid content. Based on the general trend data, results indicated that the highest chlorogenic acid content was 42.55 mg/g of dry extract, and was achieved under specific conditions: a sonication temperature of 40°C, an enzyme concentration of 10 mg/g, and an enzymolysis temperature of 60°C. Verification tests showed a value of 44.17 mg/g dry extract within the model's prediction range with a 95% confidence level. The current quadratic RSM model with the existing factor design is not yet a reliable predictive tool and only serves to explore the range of operational extraction conditions.

***Keywords*: Chlorogenic acid, Coffee Cascara, RSM, Ultrasonic-Enzymatic Extraction, UV-Vis spectrophotometry.**

Abstrak

Limbah Cascara Kopi Arabika (CKA) berpotensi tinggi sebagai sumber senyawa bioaktif seperti asam klorogenat yang memiliki aktivitas antioksidan. Pengoptimalan pelepasan senyawa aktif dapat dilakukan dengan menkombinasi metode ultrasonik dan enzimatis yang lebih efisien dan ramah lingkungan. Tujuan dari penelitian ini adalah untuk melihat efektivitas metode ekstraksi ultrasound-enzymatic-assisted extraction (UEAE) berpelarut akuades untuk senyawa asam klorogenat dari CKA menggunakan Response Surface Methodology-Box-Behnken Design (RSM-BBD). Tiga parameter ekstraksi seperti suhu sonikasi (35-45°C), konsenterasi enzim (10-20 mg/g), dan suhu enzimolisis (40-60°C) dirancang untuk memperoleh kadar asam klorogenat yang optimal. Berdasarkan data trend umum yang dihasilkan yaitu pada kondisi suhu sonikasi 40°C, konsenterasi enzim 10 mg/g, dan suhu enzimolisis 60°C dapat diperoleh kadar asam klorogenat tertinggi sebesar 42,55 mg/g ekstrak kering dan hasil verifikasi menunjukkan nilai sebesar 44,17 mg/g ekstrak kering dan dalam rentang prediksi model dengan tingkat kepercayaan 95%. Model RSM kuadratik dengan desain faktor saat ini belum mampu menjadi alat prediktif yang andal dan hanya mampu untuk mengeksplorasi rantang kondisi ekstraksi operasional.

Kata Kunci: Asam klorogenat, Cascara Kopi, RSM, Ekstraksi Ultrasonik-Enzimatis, Spektrofotometri UV-Vis.

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Introduction

Arabica coffee (*Coffea arabica*) is a coffee plant variety belonging to the genus Coffea and the family Rubiaceae [1]. Based on its development from 2001 to 2022, the expansion of the cultivation area and the productivity of Arabica coffee in Indonesia experienced a significant increase of 10.07% and 2.9% per year, respectively, compared to Robusta coffee-producing regions on Sumatera island is Kerinci Regency, Jambi Province, with a total production of 2,342.75 thousand tons [2]. High coffee production also generates much coffee pulp waste or cascara; 100 kg of ripe coffee cherries content 39 kg after pulping [3].

Arabica Coffee Cascara (CKA) waste is typically used for beverages (tea), biofuel [4], animal feed, and fertilizer [5]. However, cascara also benefits health and holds potential in the dermatocosmetic industry due to its antioxidant activity. This activity iss primarily influenced by chlorogenic acid [6]. These compounds underpin the rationale for this research, which aims to optimize their extraction by combining ultrasound-assisted extraction (UAE) with enzymes, a method known as ultrasound-enzymatic-assisted extraction (UEAE). The UAE employs an environmentally friendly extraction method that utilizes water as a solvent. This approach enhances the extraction process by improving both yield and performance while reducing extraction time and solvent use [7]. A previous study comparing UAE and maceration with water as a solvent showed that the extraction yield of caffeine and the total phenolic content were 85% higher by the UAE method than by the maceration. The caffeine content obtained with the UAE method was 206.6±0.1 mg caffeine/L, and the total phenolic content was 164.9±5.2 mg GAE/L under optimal extraction conditions: 394 W ultrasonic power for 5.5 minutes at 90°C [8].

To further increase extraction efficiency toward more optimal conditions and lower energy consumption, UAE must be combined with another extraction technique, such as enzymatic-assisted extraction (EAE). Combining these methods is straightforward and can shorten extraction time, reduce operational costs, allow for rapid operation with better extract purity, and enhance the efficiency of bioactive compounds [9]. This enhanced efficiency results from the sonication damaging the surface of the powdered material, which facilitates the binding of cellulase enzymes to the cell wall cellulose and ultimately accelerates the enzymatic reaction rate [10].

Response Surface Methodology (RSM) can optimize the levels of chlorogenic acid extracted from CKA. This method allows for more efficient experimentation by utilizing a reduced number of trials to systematically evaluate the correlation between different parameters [11].

Quantitative Analytical Methods

Materials and Apparatus

Materials include Arabica Coffee Cascara (PT. Cascara Kerinci Agro), chlorogenic acid (MarkHerb), aquadest (PT. Semangat Pagi Luar Biasa), natrium hydroxide (Merck), hydrochloric acid p.a (Merck), and cellulase 10639 U/g (Xi'an Best Bio-Tech). Equipment include grinder, 60 mesh sieve, moisture balance (Ohaus), ultrasound bath (GT Sonic), water bath (B-One), UV-Vis spectrophotometer (BEL Photonics), analytical balance (Fujitsu), 100-1000 µL micropipet (Dragon Lab), blue tip (Liferesources Gilson), whatman filter paper (Cytiva), volumetric flasks (Pyrex), and other laboratory glassware.

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Arabica Coffee Cascara Preparation

Arabica Coffee Cascara (CKA) was collected from PT. Cascara Kerinci Agro, Kerinci, Jambi, Indonesia and has been determined at the Herbarium Jatinangor, Laboratorium Taksonomi Tumbuhan, Jurusan Biologi FMIPA UNPAD with no. 85/HB/11/2025. Then, a grinder was used to grind the CKA into a fine powder, and shieving with a 60-mesh sieve. The fine powder was stored at room temperature until further use.

Loss on Drying (LOD) measurement

The moisture content (LOD) of the powder was determined using a moisture balance. Each sample weighed ±3,000 g, and the measurement was conducted with three replicates.

Ultrasound enzymatic-assisted extraction (UEAE)

Five grams (±5.00 g) of CKA powder were placed in a stoppered erlenmeyer flask and mixed with distilled water, pH 4.5. The mixture was then sonicated (40 kHz, 150 W) at the selected temperature for 20 min. Afterward, cellulase enzyme was added at a designated concentration. The enzymatic reaction was carried out at a selected temperature for 1.5 h. to terminate the enzyme activity, the mixture was heated in a water bath at 90°C for 5 min. The filtrate was then filtered using a Buchner funnel and evaporated until a thick extract was obtained.

Experimental design

In this study, three factors/ parameters with the most significant influence on chlorogenic acid content will be selected. The experimental design can be seen in Table 1. Statistical analysis of the data obtained from RSM using the BBD method was performed in Design-expert 13.

Table 1. BBD experimental design

Label	Factors	-1[a]	0[b]	+1[c]
A	Ultrasonic temperature (°C)	35	40	45
B	Enzyme concentration (mg/g)	10	15	20
C	Enzymatic temperatures (°C)	40	50	60

-1[a] = lowest value of the tested parameters; 0[b] = middle value of the tested parameters; +1[c] = highest value of the tested parameters

Determination of chlorogenic acid

The chlorogenic acid content was determined according to the method described by Navarra, *et al*. [12], with minor modifications in the concentration range. The extract ± 0.20 g was dissolved in distilled water to achieve a concentration of 8000 µg/mL, which was then diluted to 200 µg/mL. The standard, chlorogenic acid was dissolved in ethanol to make stock solutions at concentrations of 100 µg/mL. A series of chlorogenic acid with concentrations ranging from 2 to 12 µg/mL were prepared. Before quantitative analysis, the maximum wavelength of chlorogenic acid was optimised using UV-Vis spectrophotometry. The test solution and each dilution series of the standards were measured for absorbance at the maximum wavelength 325 nm. Subsequently, a standard curve was constructed, and the compound content in the extract was calculated using a linear regression equation. The content of chlorogenic acid was calculated as follows:

$$Chlorogenic\ acid\ content\ \left(\frac{mg}{g\ of\ dry\ extract}\right) = \frac{Concentration\ \left(\frac{\mu g}{mL}\right) x\ Volume\ (mL)\ x\ Dilution\ factor}{mass\ (g)\ x\ 1000} \quad (1)$$

Verification of the model

According to the response model, a verification experiment was conducted using the optimal extraction conditions from the BBD to assess the model's accuracy.

Results and Discussion

Loss on drying

CKA was obtained from PT. Cascara Kerinci Agro in a quantity of 4,000 g. the sample was ground in a grinder to obtain CKA powder, which was then sieved through a 60-mesh sieve to obtain a homogeneous powder with a large surface area. This homogeneity enabling an effective and efficient extraction process [13]. Prior to the extraction process, the powder was tested for moisture loss using a moisture balance. As shown

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in Table 2, the loss on drying of the CKA powder was 8.16±0.30, indicating excellent compliance with the quality standards outlined in the Indonesian Herbal Pharmacopoeia. This value is below the maximum limit of 10% required by the Indonesian Herbal Pharmacopoeia for the fruit peel category [14]. Furthermore, these results align with Ariva's research at 2020, which reported a moisture content of 6.57±0.03% (w/w) for CKA tea [15].

Table 2. Loss on drying of CKA powder results

Sample name	Sample weight (g)	Loss on drying (%)	Average of loss on drying (%)±SD	Remarks
CKA powder	3.00	8.44	8.16±0.30	CKA powder complies with the loss on drying standards outlined in the Indonesia Herbal Pharmacopoeia, which is ≤10%
	3.00	8.20		
	3.00	7.85		

Ultrasound enzymatic-assisted extraction (UEAE)

In the CKA study, the chlorogenic acid content was reported to be 5.59 µg/mL using the infusion method, which involved heating water to 90°C for 10 min [16]. In the modern method, EAE, the chloreogenic acid content obtained was 2.2 mg/ g of cascara pulp and UAE method, the content reached 7.9 mg/g of cascara pulp under extraction conditions of 60°C and 250 W for 35 min using a 50% ethanol [17]. In this study, however, a significantly higher chlorogenic acid content was achieved, ranging from 33.81 to 42.55 mg/g of dry extract. Although the overall extraction yield was not particularly high, the concentration of chlorogenic acid in the obtained extract was relatively high (as shown in Table 3). This suggests that the extraction process effectively concentrated the target compounds. The highest concentration of chlorogenic acid was achieved under ultrasonic temperature 40°C, with an enzyme concentration of 10 mg/g and an enzymatic temperature of 60°C. The synergistic mechanism between UAE and EAE may contribute to the elevated chlorogenic acid content obtained.

The main components of CKA cell walls include cellulose (24 – 26%), hemicellulose (25 – 29%), and lignin (23 – 33%). These components create a complex matrix that binds compunds such as chlorogenic acid within vacuoles and intercellular spaces, making conventional extraction methods often inefficient [18]. The UAE can help increase the release of chlorogenic acid into the solvent. When ultrasonic waves at a frequency of 40 kHz are emitted into a liquid, microbubbles form, leading to micro-explosions (cavitation) that can disrupt the structure of cell walls and membranes. This process increases the permeability of the tissue by breaking hydrogen bonds and allowing the solvent to penetrate more effectively, thereby opening the pores in the lignocellulose matrix. Additionally, ultrasonic vibrations reduce the size of cascara particles, increasing their surface area and exposing them to more solvent. The extraction time (20 min) enhances solvent penetration and compound release.

However, if the duration is extebded, it may lead to the degradation or excessive damage of chlorogenic acid. Temperature also plays a crucial role in this process. At 60°C, it affects solvent viscosity, compound solubility, and stability of thermolabile compounds. For most solids, solubility tends to increase with temperature. A more dilute solvent can penetrate plant powdered more easily and quickly, improving the interaction between the solvent and plant powdered, thereby accelerating the extraction process. The increased mobility of enzyme molecules and water molecules, which move rapidly and collide forcefully with the surface of the plant powdered, also enhances the diffusion of solutes into the solvent.

However, this effect decreases after reaching 60°C, as excessively high temperatures can damage the activity and effectiveness of cellulase, hindering the complete breakdown of cell walls. As a result, the release of compounds will be limited. The use of cellulase enzymes aids in hydrolyzing the β-1,4-glycosidic bonds in cellulose. This process damages the cell walls and increases tissue permeability, allowing chlorogenic acid bound in pectin or lignin and trapped within cells or intercellular spaces to be more easily released and made accessible to the solvent [17,19].

CKA extract, which is high in chlorogenic acid, is beneficial for natural cosmetics due to chlorogenic acid known antioxidant and anti-inflammatory properties [19].

Statistical analysis and model fitting

The determination of chlorogenic acid content in CKA Kerinci was conducted using UV-Vis spectrophotometry. Linier regression equations were established for standard curve: y = 0.0584x + 0.0032 (r =

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0.9948). Content were assessed for 15 treatment samples obtained from the extraction (see Table 3), with two repititions for each sample, resulting in the chlorogenic acid content ranged from 45.0837 – 56.7318 mg/g of dry extract.

The data were processed using Design-expert 13 software, which provided model fitting and statistical analysis for the experiment. A quadratic model was recommended for optimizing process conditions in relation to the content of chlorogenic acid. The relationship between the response values and the factors were defined as follows:

Chlorogenic acid content (mg/g of dry extract) = 49.70 – 0.45 A – 0.50 B + 2.40 C – 0.69 AB – 0.49 AC + 0.01 BC – 2.93 A^2 – 0.24 B^2 + 2.89 C^2 (2)

Table 3. Box-Behnken response surface design and corresponding response values

Run	Ultrasonic temperature (°C) A	Enzyme concentration (mg/g) B	Enzymatic temperatures (°C) C	Yield (%)	Chlorogenic acid content (mg/g of dry extract)[1]
1	40	10	60	31.90	42.55
2	40	20	60	31.60	40.08
3	40	20	40	30.16	35.96
4	40	15	50	32.54	38.43
5	35	10	50	33.90	33.81
6	45	10	50	36.10	34.99
7	35	20	50	36.40	35.85
8	40	15	50	38.60	36.17
9	40	10	40	32.00	38.46
10	45	20	50	39.10	34.95
11	35	15	40	29.36	36.08
12	45	15	60	36.80	37.68
13	45	15	40	33.64	35.33
14	35	15	60	35.40	39.89
15	40	15	50	30.80	37.23

[1]each run was repeated twice

In this research, the analysis of variance for the regression model was used to determine the p-values for the chlorogenic acid regression models, which are 0.0866. These values suggest that the effects are not statistically significant ($p > 0.05$). This implies that the model is not adequately explaining the variability in the response across the range of factors tested. Additionally, the p-values for lack of fit are 0.3422. Since this value is also insignificant, it indicates that the recommended quadratic model is sufficiently consistent with the experimental data with low error and minimal influence from unknown factors.

However, the predicted R-squared values are -0.6967, while the adjusted R-squared values are 0.6244. These results indicate that the models are relatively unstable and less reliable. In addition, the correlation coefficients (R^2) for chlorogenic acid was 0.8659. These values indicate a poor correlation between the experimental response values and the model predictions, as the proposed model accounts for only 86.59% of the variability in the responses. Additionally, these values are both less than 1, suggesting that the model is not well-suited to the experimental data. The coefficients of variation (CVs) was 3.90, which highlight significant discrepancies between the observed and predicted values, leading to low accuracy and reliability in the experiments. Moreover, the measured adequate precision values exceeded 4, with values of 6.5556, indicating good reproducibility [20,21].

Based on this data, the model produced is not statistically significant due to several factors. The minimum and maximum ranges of the factors were too narrow, resulting in response variation not being optimally captured. Additionally, there were high experimental data variability, and the interactions between factors were not strong enough to establish a stable quadratic model [22].

Interaction analysis of each factor in the model

The 3D response surface illustrates the relationship between factor interactions and the content of chlorogenic acid and caffeine. In the 3D plot, the colors represent different levels of content: blue indicates lower levels, while orange to red indicates higher levels. Additionally. A contour plot can show the strength of interactions among various factors through contour lines [20,22].

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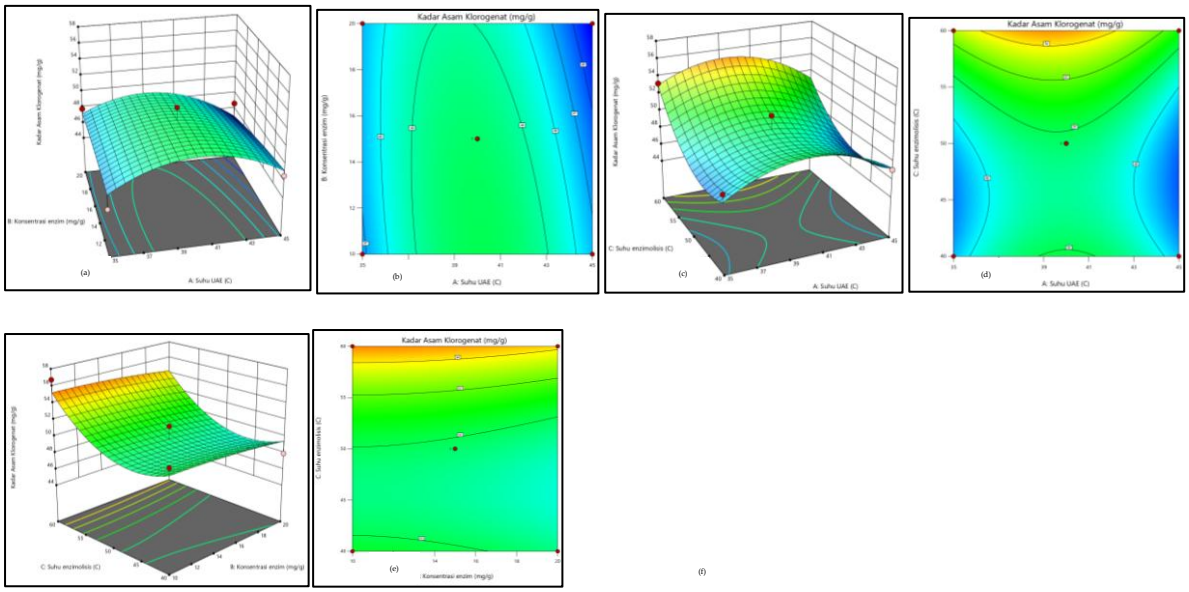

Figure 3. Response surface diagram and contour diagram of the interaction of various experimental factors to chlorogenic acid content

The analysis of the 3D diagrams and contour liness presented in Fugures 3a-b shows that as the ultrasonic temperature and enzyme concentration increase, the content of chlorogenic acid and caffeine decreases. In Figures 3c-d, the 3D diagrams exhibit a curved shape, indicating a two-way concavity. This suggests that the interaction between the enzymatic temperature and the ultrasonic temperature has opposing effects. Specifically, when the enzymatic temperature is decreased, the levels of chlorogenic acid and caffeine rise. Conversely, if the enzymatic temperature is decreased and the ultrasonic temperature is increased, the levels decline. Sonication works by generating small cavitation bubbles at the microscopic level, which break down cellulose and hemicellulose components.

However, to achieve the desired effects of sonication, an optimal temperature must be maintained. While raising the ultrasonic temperature can accelerate the chemical reactions involved in the sample treatment, excessively high temperature can have adverse effects. These high temperatures can reduce gas solubility in the liquid, causing cavitation bubbles to burst rapidly and resulting in mechanical effects that make the extraction process ineffective [23].

Figures 3e-f illustrate that higher temperatures during enzymatic hydrolysis, particularly at low enzyme concentrations, can lead to increased levels of chlorogenic acid. As the temperature rises, both molecular motion and reaction rates also increase [24]. However, optimal enzyme activity is only achieved at specific concentrations; at higher concentrations, enzyme performance may decline or stagnate. This decline can occur because enzymes compete for substrates, or due to barriers on the surface of the sample's cell wall that hinder enzyme access to the tissue [25].

Verification of the model

Further experiment were conducted to verify the optimum conditions identified by RSM. The recommended conditions were an ultrasonic temperature of of 40°C, an enzyme concentration of 10 mg/g, and an enzymatic temperature of 60°C. these conditions were deemed optimal as they yielded the highest desirability value of 0.871, which is very close to 1. A desiribility value near 1 indicates that the chosen parameters are effective for achieving suitable extraction conditions and confirms the accuracy of the optimal conditions [26].

Verification was performed to assess the reliability of the predictions generated by RSM. The results of the verification are shown in Table 4. Analysis revealed that the difference between the predicted and actual results was 6.20%. Although the error percentage for chlorogrnic acid slightly exceeds the general tolerance limit of 5%, the actual values remain within the prediction interval (PI) range. The agreement between the model's predictions and the verification results at a specific point is important. However, it is not sufficient to conclude that the model as a whole is valid or reliable. When experimental results under specific conditions (such as a temperature, concentration) align with the predictions of a model, it suggests that the model can accurately describe the behavior of the system at that point. However, this only proves local consistency and

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does not confirm the model's global vlaidity. If the range of variables (temperature and concentration) is narrow, the model may seem accurate across that entire range. In reality, it may just be "lucky" because it hasn't been tested in non-linear or extreme regions. A model that appears reliable within a limited range might fail when applied outside of it, despite matching results at one point within that range. The need for comprehensive evaluation: to assess the overall validity of the model, it should be tested at multiple points, especially at the extremes of the range and in non-linear areas [27,28].

Table 4. Verification of response surface results

Respond	Prediction result (mg/g of dry extract)	Actual result (mg/g of dry extract)	95% PI low	95% PI high	Error Percentage (%)
Chlorogenic acid content	41.43	44.17	37.55	45.30	6.20

Conclusions

This study developed an RSM-BBD optimization model for the extraction of chlorogenic acid using UEAE. The resulting RSM model was insignificant ($p > 0.05$) and had a negative predicted R-squared. This indicates that the model was not able to accurately describe the relationship between the factors and the responses. Despite these limitations, the model still serves as a useful initial reference for identifying optimal extraction conditions. Under the optimal conditions of 40°C ultrasonic temperature, 10 mg/g enzyme concentration, and 60°C enzyme temperature, a chlorogenic acid content of 42.55 mg/g of dry extract was obtained. To develop a more robust and statistically valid predictive model, it is recommended to repeat the experiment with a wider range of factors and to increase the number of replicate central points in future studies. This will help capture variations in responses more effectively and improve the modeling process.

Conflict of Interest

The authors confirm that there are no personal, financial, or institutional interests that could have influenced the conduct, analysis, or reporting of this research.

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