

Simultaneous Extraction and Decaffeination Process Optimization of Green Coffee Bean-Based Beverages Using Response Surface Methodology

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Abstract

Coffee is a popular drink worldwide, but some people cannot consume it due to its high caffeine content. However, it contains chlorogenic acid, which benefits the human body. To reduce the caffeine content and maintain phenolic compounds, especially chlorogenic acid and its derivatives in green coffee beans, a simultaneous extraction and decaffeination method using hot water was used. Box Behnken Design-Response Surface Methodology was used in this study to determine the optimum simultaneous extraction and decaffeination process for obtaining minimum caffeine and maximum phenolic and antioxidant activity (IC₅₀) of the decaffeinated green coffee bean powder. The Design Expert software was used to analyze and optimize process parameters. Analysis of Variance (ANOVA) test was conducted to identify significant parameters. Three independent parameters were used, i.e., extraction time (10 to 30 min), decaffeination time (6 to 10 h), and decaffeination temperature (60 to 80 °C), to observe the 3 responses (caffeine content, phenolic content, and IC₅₀). The result revealed that the extraction and decaffeination parameters affected green coffee bean powder-based beverages' caffeine content, total phenolic, and IC₅₀. The lowest caffeine, the highest phenolic, and IC₅₀ were predicted at an extraction time of 10 min, decaffeination time of 8.05 h, and decaffeination temperature of 60 °C with a caffeine content of 1.53 %, total phenolic content of 46.81 mg GAE/g, and IC₅₀ of 29.45 ppm, respectively. The verification showed no significant difference with model predictions at a confidence interval of 5 % based on paired t-tests.

Keywords: Antioxidant activity, Green coffee bean powder, Caffeine, Decaffeination, Total phenolic, Response surface methodology

Introduction

Around the world, coffee is a widely consumed beverage, and its intake has accelerated drastically over the years [1]. In Indonesia, 753,941 tons of coffee were produced in 2020, and 765,415 tons are expected to be produced in 2021. Robusta coffee dominated coffee production in Indonesia in 2020 by contributing up to 71.38 % nationally [2]. Robusta coffee is rich in bioactive compounds, including phenolic acid and alkaloids [3]. The main phenolic fraction of green robusta coffee is chlorogenic acid, reaching up to 14 % [4]. These compounds have some beneficial properties related to their potent antioxidant [5], hepatoprotective [6-8], hypoglycemic [9,10], antiproliferative [8], and antiviral properties in earlier studies [11,12]. Moreover, robusta coffee also contains an alkaloid, which dominates by caffeine content. Robusta coffee has caffeine content 2 times higher than Arabica coffee, which is about 3.41 to 8.13 % [13]. Caffeine has positive effects, including energizing the central nervous system, relaxing smooth muscles, particularly bronchial smooth muscles, and energizing heart muscles [14]. Caffeine will make a person's body feel warmer and fresher if they have a high tolerance for it. Side effects of caffeine, like insomnia, anxiety, increased blood pressure, and an accelerated heart rate, could happen for people with a low caffeine tolerance [14-17]. As a result, it is essential to try to diversify processed coffee products so that consumers can consume processed coffee beverages with less caffeine. The total amount of caffeine is decreased by decaffeination [18].

The decaffeination of caffeine in coffee is commonly conducted with different methods, such as supercritical carbon dioxide, water extraction, and organic solvents [5], with advantages and drawbacks for each method. These technologies differ in cost and solvents used to extract caffeine, but they all share that decaffeination eliminates more than just caffeine [19]. Moreover, water extraction is a more straightforward method to extract and decaffeinate coffee up to 80 % due to the properties of water being safe for further consumption and having a low price [20,21]. The researcher has performed the extraction and decaffeination of coffee beans using water methods since the last 1900 decade in parts of the world, especially in the United States and Europe [22]. By adopting this method, Hall *et al.* [23] found a considerable reduction in the caffeine content of coffee from 42,651.95 to 16,791.13 mg/kg in decaffeinated coffee. In Indonesia, the extraction or decaffeination in separate processes of coffee beans from different locations, like robusta coffee beans from Argopuro, Jember [15,24], green arabica coffee beans from Pangalengan, Garut, and Tasikmalaya [16], and robusta coffee beans from West Lampung [25] was conducted by previous researchers using different methods. However, the simultaneous extraction and decaffeination of green coffee bean powder using hot water and the optimization process has not been widely reported. In this study, to obtain the optimum simultaneous extraction and decaffeination conditions in producing green coffee bean powder-based beverages with low caffeine and high total phenolic and antioxidant activity, the response surface methodology approach was applied. Response Surface Methodology (RSM) is a statistical modeling tool and a mathematical technique that allows for the evaluation of the relationship between various factors and the measured response, independent and dependent variables, and optimizes the conditions to achieve the highest efficiency, intending to reduce unwanted and unnecessary time and cost [26]. Therefore, this study aims to obtain the optimum condition of the simultaneous extraction and decaffeination in producing green coffee bean powder-based beverages with low caffeine and high total phenolic and antioxidant activity.

Materials and methods

Materials and chemicals

The raw material used in this study was green robusta-type coffee beans obtained from the Dampit area, Malang Regency. Then, Robusta coffee beans are dried using an oven at 180 °C for 3 min (up to the first crack), ground using a dry blender, sieved using an 80-mesh sieve, and obtained green coffee bean powder with a characteristic yellowish-green color and a distinctive coffee smell. The chemical characteristics of green coffee bean powder are a caffeine content of 2.16 %, total phenolic content of 53.12 mg GAE/g, and IC₅₀ of 30.77 ppm. The chemical used in this study was methanol, DPPH reagent (2,2-diphenyl-1-picrylhydrazyl), active carbon, gallic acid standards, follin-ciocalteu reagents, CaCO₃, quercetin standards, NaNO₂, AlCl₃, NaOH, acetonitrile (HPLC grade), and formic acid were obtained from Merck KGa, Darmstadt, Germany in analytical grade. The caffeine standard was obtained from Sigma-Aldrich, USA, in HPLC grade.

Green coffee bean powder extraction

The green coffee bean powder was weighed and extracted using the infusion method with demineralized water under the following condition: Green coffee bean powder: Solvent (1:15, w/v), the temperature of 90 °C, and various extraction times (10, 20, and 30 min). Then, the aqueous green coffee bean extract was filtered using coarse filter paper and ready for further processing.

Preparation of activated carbon

For activation of carbon follows the method of Fischer and Kummer [22] with modification. Shortly, the raw activated carbon of 25 % b/v was combined with the 2.5 % w/v of sugarcane, and formic acid of 0.5 % b/v, followed by heating the mixture inside the water bath shaker at 80 °C for 6 h. After that, the active carbon separated from the solvent and was rinsed with water.

Decaffeination of green coffee bean extract

As described previously, the aqueous green coffee bean extract was combined with the activated carbon with a ratio of 1:75 (w/v). After that, decaffeination was performed in a water bath shaker at various temperatures (60, 70, and 80 °C) and various times (6, 7, and 8 h). After the reaction process, it was filtered using coarse filter paper to obtain the aqueous decaffeinated green coffee bean extracts.

Production of functional beverage powders

As described previously, decaffeinated green coffee bean extracts were combined with maltodextrin with a ratio of 100:5 (w/w), then dried using a food dehydrator at 60 °C for 5 h. The dried decaffeinated green coffee bean was reduced in size using a dry blender and filtered with a sieve of 80 mesh, following the method of Fischer and Kummer [22] with modification.

Analysis of caffeine content

The caffeine content in the green coffee bean-based beverage was determined using UPLC (Ultra High-Performance Liquid Chromatography) Methods. Shortly, 1 µL of the prepared sample was injected into a Hypersil Gold column (150 mm × 2.1 mm × 1.9 µm) at 30 °C. The autosampler compartment was set at 16 °C with the mobile phase (1 % formic acid in acetonitrile (A) and 0.1 % formic acid in H₂O (B)) was set to the composition, 0.0 - 1.5 min 85 % B, 1.5 - 4.0 min 15 % B, 4.0 - 5.0 min 15 % B, 5.5 - 7.0 min 85 % B. at a rate of 300 L/min. The operating conditions of MS/MS Triple Quadrupole with ESI (Electrospray Ionization) ionization source were controlled by TSQ Tune software, which operates in positive ionization mode. SRM (Selected Reaction Monitoring) mode was selected for the target compound analysis method. The spray voltage was set at 3.0 kV, the evaporation temperature was 275 °C, the capillary temperature was 300 °C, nitrogen as sheath gas pressure was conditioned at 40 psi, and Aux gas pressure was conditioned at 10 psi for argon gas. Caffeine content was quantified using a standard caffeine curve prepared in advance. Briefly, standard caffeine was dissolved in methanol at concentrations of 0.5, 1, 2, 4, and 8 µg/mL was injected into UPLC to obtain a standard caffeine curve, with linear regression equation: $y = a(x) + b$.

Analysis of total phenolic content

The colorimetric method analyzed total phenolic content with Folin Ciocalteu as a reagent [27]. Shortly, 0.5 mL of coffee extracts were put into a test tube, followed by adding 2.5 mL of 10 % Folin Ciocalteu reagent and 2.0 mL of 7.5 % Na₂CO₃, then vortexed to homogenize and incubated at room temperature for 15 min. The aliquot measured absorbance at a wavelength of 517 nm in triplicates for each sample with 3 replications of analysis. The total phenolic content was calculated using the gallic acid standard curve in concentrations of 0, 20, 40, 60, 80, and 100 mg/L in distilled water with the following formula (Eq. (1)):

$$TPC = \frac{C \times f}{M \times V} \quad (1)$$

whereas: TPC (total polyphenol content in mg GAE/g), C (gallic acid concentration in mg/ml), f (dilution factor), V (extract volume in mL), and M (weight of the extracted sample in grams).

Analysis of antioxidant activity (IC₅₀)

The free radical activity was measured using the Atanassova *et al.* [28] method with DPPH (1,1-diphenyl-2-picrylhydrazyl) as radicals. Briefly, the coffee extracts were diluted in absolute ethanol at 100, 200, 300, 400, and 500 ppm concentrations. Then, 1 mL of sample from each dilution was put into a test tube, added with 4 mL of 0.004 % DPPH solution in ethanol, and vortexed until homogeneous, followed by incubation for 1 h at room temperature and in a dark room. The aliquot measured absorbance at a wavelength of 517 nm in triplicates for each sample with 3 replications of analysis. The free radical activity of DPPH in percentage (%) is calculated by linear regression $y = a(x) + b$ by stating the y value as 50 and the x value as IC₅₀.

Experimental design and statistical analysis

The extraction time, decaffeination time, and decaffeination temperature were optimized using the Response Surface Methodology (RSM) with a Box Behnken design (BBD) in the Design Expert 13.0 software (trial version, MN, USA). The upper, center, and lower limits of extraction time, decaffeination time, and decaffeination temperature in this study are listed in **Table 1**, and these ranges are based on a preliminary study.

Table 1 The range of independent factors in producing green coffee bean powders-based beverages.

Independent Factors	Unit	Lower Limit (-1)	Center (0)	Upper Limit (+1)
Extraction time	min	10	20	30
Decaffeination time	h	6	8	10
Decaffeination temperature	°C	60	70	80

A second-order polynomial equation was used to fit the green coffee bean-based beverage responses, and the multiple data regression was used to create an empirical model based on the independent variables. The general second-order polynomial equation is written in Eq. (2). Utilizing Design-Expert software, the accuracy of the polynomial model was examined to determine correlation R^2 , adjusted- R^2 , adequate precision, and lack of fit for each response (caffeine content, total phenolic, and IC_{50}).

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (2)$$

Y is the predicted responses (caffeine content, total phenolic, and IC_{50}); β_0 is the intercept coefficient; x_i and x_j are independent variables; β_i , β_{ii} , and β_{ij} are the linear, quadratic, and interaction coefficient of variables i and j , respectively.

Verification of model

The optimum condition of software prediction was verification for 3 times in the laboratory. The software prediction and verification results were evaluated by a paired t-test using Minitab 17 software (trial version, CA, USA).

Results and discussion

Model fitting

The experiment was designed to evaluate the effect of extraction time, decaffeination time, and decaffeination temperature on the green coffee bean powder-based beverage quality and determine the optimum condition using response surface methodology based on the caffeine content, total phenol, and antioxidant activity. The experimental data in Box-Behnken Design was presented in **Table 2**. The experimental data served as the basis for the response surface regression analysis. **Table 3** displays the prediction of a second-order polynomial of the responses.

Table 2 The green coffee bean powder-based beverage quality using BBD-RSM.

Std	Independent Factor			Response		
	Extraction time (min)	Decaffeination time (h)	Decaffeination temperature (°C)	Caffeine content (%)	Total phenolic content (mg GAE/g)	IC_{50} (ppm)
1	10	6	70	1.50	43.39	59.39
2	30	6	70	1.85	49.25	60.43
3	10	10	70	1.67	35.77	68.50
4	30	10	70	1.60	42.06	82.58
5	10	8	60	1.60	49.38	32.34
6	30	8	60	1.58	54.16	60.13
7	10	8	80	1.58	50.26	71.50
8	30	8	80	1.62	46.51	42.28
9	20	6	60	1.58	38.10	53.42
10	20	10	60	1.56	50.03	51.13
11	20	6	80	1.69	46.78	56.40
12	20	10	80	1.79	44.57	75.50
13	20	8	70	1.74	53.21	62.88
14	20	8	70	1.74	51.78	63.54

Std	Independent Factor				Response	
	Extraction time (min)	Decaffeination time (h)	Decaffeination temperature (°C)	Caffeine content (%)	Total phenolic content (mg GAE/g)	IC ₅₀ (ppm)
15	20	8	70	1.75	45.87	59.71
16	20	8	70	1.77	48.34	54.51
17	20	8	70	1.81	48.50	63.54
Prediction	10	8.05	60	1.53±0.00 ^a	46.81±0.00 ^b	29.45±0.00 ^c
Verification	10	8.05	60	1.42±0.14 ^a	48.02±1.07 ^b	30.91 ^c

Remarks: The same letters in the same column indicate no significant difference by paired t-test

Table 3 Quadratics model and significance of the green coffee bean powder-based beverage.

Coefficient	Caffeine content	Total phenolic	IC ₅₀
Intercept			
β ₀	1.76	49.54	60.84
Linear			
β ₁	0.0381 ^{ns}	1.65 ^{ns}	1.71 ^{ns}
β ₂	-0.0004 ^{ns}	-0.6372 ^{ns}	6.01 ^{**}
β ₃	0.0454 ^{ns}	-0.4422 ^{ns}	6.08 ^{**}
Cross product			
β ₁₂	-0.1033 [*]	0.1098 ^{ns}	3.26 ^{ns}
β ₁₃	0.0144 ^{ns}	-2.13 ^{ns}	-14.25 ^{***}
β ₂₃	0.0292 ^{ns}	-3.53 ^{ns}	5.35 [*]
Quadratic			
β ₁₁	-0.0829 [*]	-0.8574 ^{ns}	-0.3292 ^{ns}
β ₂₂	-0.0245 ^{ns}	-6.06 [*]	7.22 ^{**}
β ₃₃	-0.08528 [*]	1.40 ^{ns}	-8.95 ^{**}

Equation: $Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{x_1x_2} + \beta_{x_1x_3} + \beta_{x_2x_3} + \beta_{x_1x_1} + \beta_{x_2x_2} + \beta_{x_3x_3}$
 Significance remarks: ^{ns} $p > 0.05$; ^{*} $0.01 < p < 0.05$; ^{**} $0.001 < p < 0.01$; ^{***} $p < 0.001$

The mathematical model selection of each response (caffeine content, total phenol, and antioxidant activity (IC₅₀)) of green coffee bean powder affected by the extraction and decaffeination process was listed in **Table 4**. Based on **Table 4**, all the observed responses were followed by the quadratic model with a significant model range of 0.0013 to 0.3135 [29]. Solano *et al.* [30], reported the lowest p -value in the model's significance, indicating that the model was highly significant. Moreover, all the responses had $R^2 \geq 0.6536$, adjusted $R^2 \geq 0.2083$, adequate precision ≥ 4.1399 , and lack of fit ≥ 0.0287 . The high R^2 and adjusted R^2 demonstrated that the quadratic model adequately explained the variance of all response data [31,32]. Furthermore, adequate precision measures the signal-to-noise ratio, where a ratio ≥ 4 indicates an adequate signal [33,34]. In this study, the lack of fit ≥ 0.05 , except in caffeine content, indicated that the selected model was high in accuracy and precision related to pure error [30].

Table 4 Mathematical model selection for each response of green coffee bean powder.

Parameters	Mathematical model	Significance model	R ²	Adjusted-R ²	Adequate precision	Lack of fit
Caffeine content	Quadratic	0.0467 ^s	0.8293	0.6099	5.6946	0.0287 ^s
Total Phenolic	Quadratic	0.3135 ^{ns}	0.6536	0.2083	4.1399	0.1073 ^{ns}
IC ₅₀	Quadratic	0.0013 ^s	0.9440	0.8720	15.1750	0.3570 ^{ns}

Remarks: ^s: significant, and ^{ns}: not significant

Caffeine content

The decaffeinated green coffee bean powder-based beverages ranged from 1.50 to 1.85 %, as presented in **Table 2**. The independent factors used in this experiment significantly affected the caffeine content of the green coffee bean-based beverage. **Figure 1** showed that the contour plot and 3D-the surface of caffeine content affected by factors interaction in this experiment showed a quadratic effect. **Figure 1(A)** and **Table 3** revealed that the decreasing extraction time from 30 to 10 min and increasing decaffeination time from 10 to 6 h at fixed decaffeination temperature significantly decreased the caffeine content of the green coffee bean powder. Fuller and Rao [35] reported that the dissolution of caffeine into water increased rapidly up to 180 min, then it increased slowly when the time was increased, and a steady state condition occurred at 400 min. Moreover, Anggriawan *et al.* [29] stated that the longer steamed process produced low caffeine content in the final product, which caused the caffeine compounds in the coffee powder lead to leach into the water as solvents.

The single factor of extraction time in quadratic showed that increasing the extraction time to 20 min increased the caffeine content, and it gradually decreased when the extraction time increased to 30 min, as revealed in **Figures 1(A)** and **1(B)**. The trend in this study was in agreement with the previous study reported by Syakfanaya *et al.* [36], which reported that the caffeine content of the *Coffea canephora* bean increased from 2.89 to 4.85 mg/g in extraction time 15 to 45 min, and decreased when extraction time prolonged to 60 min with the caffeine content of 4.23 mg/g during the simultaneous extraction. The decrease in the caffeine content might be due to the prolonged extraction time, which leads to increased temperature in the extraction medium and degrades the caffeine [35,36]. In quadratic terms, a similar trend with extraction time was found in decaffeinated temperature. It confirmed that increasing the decaffeinated temperature to 70 °C elevated the caffeine content, and it was gradually reduced when the decaffeinated temperature increased to 80 °C (**Figures 1(B)** and **1(C)**). Damayanti *et al.* [38] stated that the increase in decaffeination temperature causes the mass density of both water as a solvent and coffee powder solids to be more tenuous so that it has a larger intermolecular space, thereby increasing the solubility of caffeine in coffee powder to dissolve into the extraction medium. Sharif *et al.* [39] also reported that caffeine's solubility and diffusion coefficient in the extraction medium was higher at 90 °C than at 30 °C during decaffeination. However, using high temperatures (75 - 95 °C) for 2 - 3 min significantly increased caffeine levels in green tea, and with the increase in time after that, caffeine content tended to be stable [40].

Compared with the raw material, the simultaneous extraction and decaffeination using hot water successfully reduced the caffeine content in decaffeinated green coffee bean powder-based beverages by as much as 14.35 to 30.56 %. This finding is in line with Anggriawan *et al.* [29], who reported that the caffeine content in green coffee beans decreased by 28.20 % than the raw material after the steam pretreatment process. Moreover, this study's caffeine percentage was lower than Sinaga *et al.* [18], who reported decreased caffeine content from 2.6 % in green bean coffee to 1.04 % in decaffeinated green bean coffee using hot water as a solvent, meaning caffeine content decreased by 60 %. Boiling in the decaffeination process can enlarge the pores of the coffee network so that the solvent quickly diffuses into the tissue and dissolves the caffeine into the solvent, and easy to capture by activated carbon [18]. In addition, the boiling process can also break down the complex bonds of caffeine and chlorogenic acids into small sizes, making it easy to move and capture by activated carbon, causing the caffeine content to decrease [25]. Furthermore, the simultaneous extraction and decaffeination process by hot water as a solvent in this study was considered a method to reduce the caffeine content to produce a low price of green coffee beans powder-based beverages. Hence, the reduction result is lower than that of Sinaga *et al.* [18] due to the stability of caffeine in the samples. Caffeine has a thin, flat surface that makes it easily hydrated, which causes water

to split and become somewhat nonpolar. Despite this, the loss in caffeine concentration is still just a tiny amount during processing [41]. The exact mechanism of removal of caffeine during the extraction and decaffeination process is still not known clearly. However, the mechanism that might occur in this study is that the water used as the solvent penetrates the network of pores in the coffee powder to improve the diffusion of caffeine into the solvent due to a rise in temperature during the simultaneous extraction and decaffeination process. Additionally, the water-soluble caffeine will be drawn in and affixed to the pores of the activated carbon, significantly reducing the amount of caffeine in the coffee powders [18,25,42].

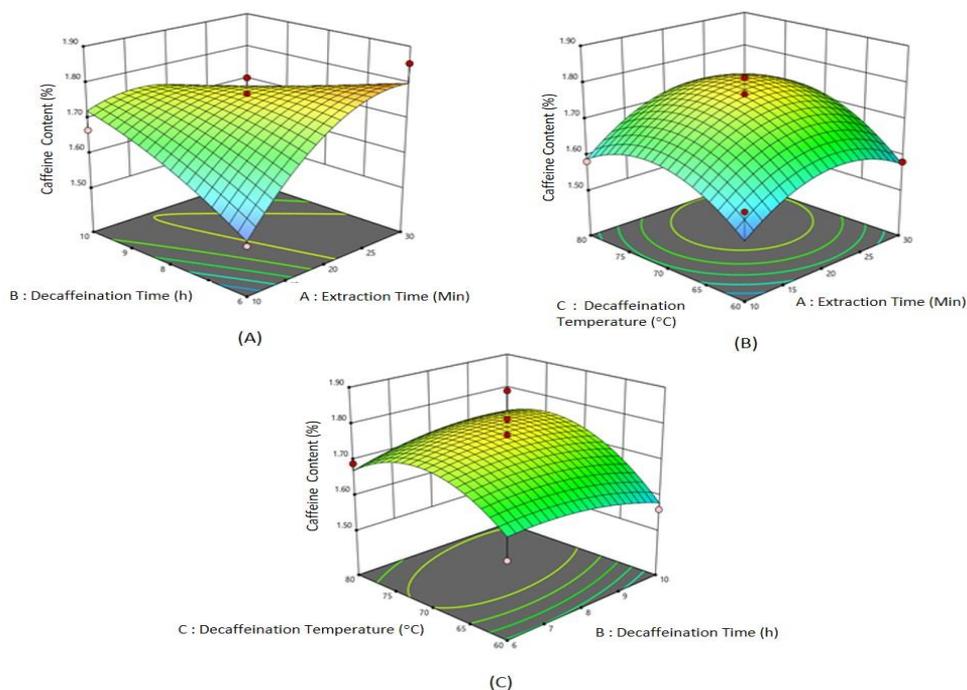


Figure 1 Contour plot and the 3D surface of the interaction of extraction time and decaffeination time (A), the interaction of extraction time and decaffeination temperature (B), and the interaction of decaffeination time and decaffeination temperature (C) on the caffeine content of the green coffee bean powder-based beverage.

Total phenolic content

The total phenolic content in decaffeinated green coffee bean powder-based beverages ranged from 35.77 to 54.16 mg GAE/g (**Table 2**). The results were in the range of the TPC data of shell and pulp coffee processed using the UAE (Ultrasonic assisted extraction) method reported by Solano *et al.* [30], which is 27.09 to 41.16 mg GAE/g. The total phenolic content in this study was only affected by decaffeination time (B^2) in quadratic terms, as presented in **Table 4**. **Figures 2(A)** and **2(B)** revealed that increasing extraction time from 10 to 30 min tends to increase the total phenolic content of the green coffee bean-based beverage. Moreover, the total phenolic content increased significantly with the increasing decaffeination time until 8 h, and then it gradually decreased when the decaffeination time increased to 10 h, as revealed in **Figures 2(A)** and **2(C)**. The increase in total phenolic content with increasing decaffeination time is caused by increasing the solubility of phenolic compounds in water during decaffeination [43-45]. However, the longer decaffeination time decreased the total phenolic content due to the degradation of the bioactive compounds at high temperatures [46]. In another independent factor, total phenolic content increased insignificantly with increasing decaffeination temperature from 60 to 80 °C, as revealed in **Figures 2(B)** and **2(C)**.

Compared with the raw materials, the total phenolic content of the green coffee bean powder-based beverage decreased by 2.52 to 32.66 %, except at Std. 6 and Std.13 listed in **Table 2**, which increased by 0.17 - 1.92 %. The decreased total phenolic content in decaffeinated green coffee bean powder-based beverages may be caused by the more prolonged extraction and decaffeination processes and the higher decaffeination temperature, which can alter and degrade the bioactive compounds in green coffee bean powder, particularly chlorogenic acid. Sinaga *et al.* [18] reported that the boiling process at high temperatures at a specific time during the decaffeination of green coffee beans degraded their chlorogenic

acid. Like other phenolic compounds, chlorogenic acid is highly susceptible to damage due to high-temperature extraction, and decaffeination temperatures cause phenol damage and reduce the total phenol content in the decaffeination of green coffee beans powder-based beverages [47]. Additionally, polyphenols have hydrophilic qualities that allow them to dissolve in moisture during the decaffeination process and be degraded [29]. The chlorogenic acids dominated the phenolic compound in coffee beans and their derivative products [48]. The conversion or degradation of chlorogenic acids is known to occur quickly, especially when water is present. 5-CQA has been found to isomerize into 3-CQA and 4-CQA when heated in water [49]. Perhaps the isomerization of chlorogenic acid into other compounds causes a decrease in its bioactivity ability as a phenolic compound, resulting in a decrease in phenolic content when tested. The phenomena support the results of this study.

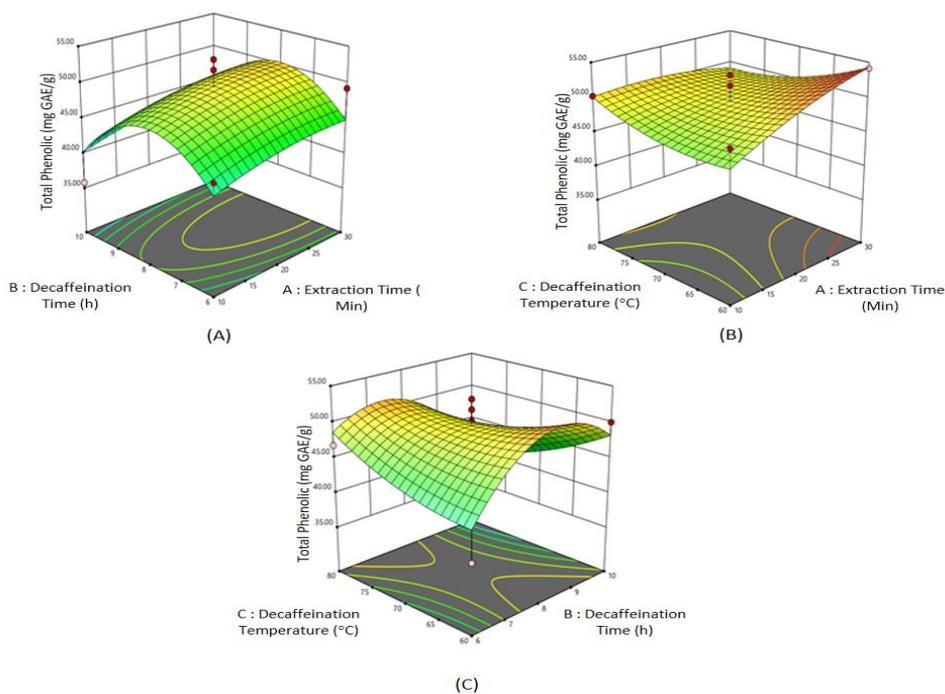


Figure 2 Contour plot and the 3D surface of the interaction of extraction time and decaffeination time (A), the interaction of extraction time and decaffeination temperature (B), and the interaction of decaffeination time and decaffeination temperature (C) on the total phenolic content of the green coffee bean powder - based beverage.

Antioxidant activity (IC_{50})

The antioxidant activity (IC_{50}) in decaffeinated green coffee bean powder-based beverages ranged from 32.34 to 82.58 ppm, as shown in **Table 2**. The result was aligned with the IC_{50} of decaffeinated coffee from Garut, Tasikmalaya, and Pengalengan, West Java, Indonesia (55.74 to 75.23 ppm) [16]. The IC_{50} in this study was affected by decaffeination time (B), decaffeination temperature (C), the interaction between extraction time and decaffeination temperature (AC), the interaction between decaffeination time and decaffeination temperature (BC), decaffeination time (B^2) and decaffeination temperature (C^2) in a quadratic term. **Figure 3(A)** shows that the IC_{50} of antioxidant activity increases insignificantly with the extraction time from 10 to 30 min and decaffeination time from 6 to 10 h at fixed decaffeination temperature. Furthermore, increasing the extraction time from 10 to 30 min and decaffeinated temperature from 60 to 70 °C significantly increases the IC_{50} of antioxidant activity at the fixed decaffeination time, as revealed in **Figure 3(B)**. In addition, **Figure 3(C)** shows that the IC_{50} of antioxidant activity increases with the increasing decaffeination time from 6 to 10 h and decaffeination temperature from 60 to 70 °C at the fixed extraction time. Kusumocahyo *et al.* [50] reported that the IC_{50} and total phenolic content have a reverse correlation: the high total phenolic content caused the lower IC_{50} in another. Muchtaridi *et al.* [16] stated that decaffeination not only decreases the concentration of caffeine but also decreases the concentration of chlorogenic acid. Moreover, chlorogenic acid, a phenolic acid in coffee bean extracts, can play an essential role as an antioxidant.

The IC_{50} in green coffee bean powder-based beverages increased by 4.85 to 62.74 % than the raw material in this investigation. The increase in IC_{50} in green coffee bean powder-based beverages was positively correlated with the decrease in their total phenolics content and caffeine content, as explained before. The degradation of phenolic compounds, especially chlorogenic acid and caffeine content in green bean coffee during decaffeination, caused a significant decrease in their radical scavenging ability and increased IC_{50} value. Budryn *et al.* [51] demonstrated that a decrease in the total polyphenol content of 45 % of coffee beans during processing could reduce the ability of radical scavenging up to 60 %. Babova *et al.* [52] also reported that higher caffeine content and chlorogenic acid in green coffee beans were correlated with higher antioxidant activity, as indicated by the lower IC_{50} . According to Liao *et al.* [53], roasted coffee beans' antioxidant activity positively correlated with chlorogenic acid. The lower antioxidant activity in roasted coffee is caused by chlorogenic acid degradation.

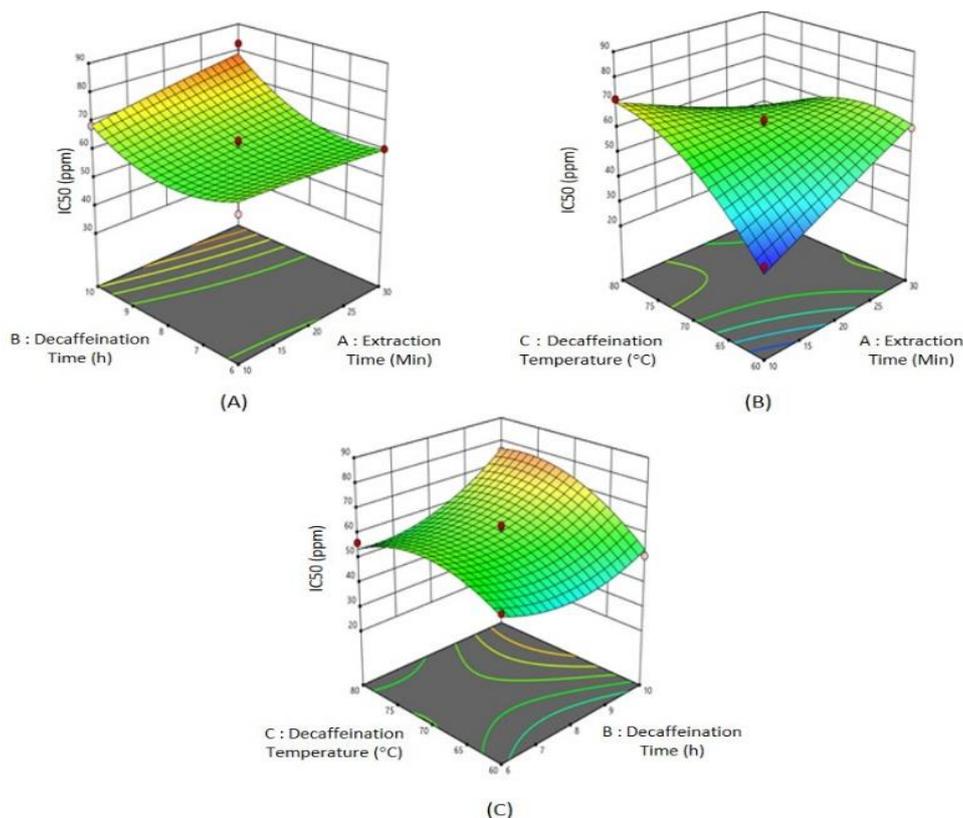


Figure 3 Contour plot and the 3D surface of the interaction of extraction time and decaffeination time (A), the interaction of extraction time and decaffeination temperature (B), and the interaction of decaffeination time and decaffeination temperature (C) on IC_{50} of green coffee bean powder-based beverage.

Verification of the model

The extraction time of 10 min, decaffeination time of 8.05 h, and decaffeination temperature of 60 °C are predicted as the ideal conditions for the extraction and decaffeination process of green coffee bean powder-based beverage with the following responses: The caffeine content of 1.53 %, total phenolic content of 46.81 mg GAE/g, and IC_{50} of 29.45 ppm and the desirability value is 0.822. The desirability value near 1 indicates that predicting the ideal point has a higher level of validity [30]. Three replications were used to verify the optimal condition and obtained caffeine content of 1.42 ± 0.14 %, total phenolic content of 48.02 ± 1.07 mg GAE/g, and IC_{50} of 30.91 ppm. The experimental values under ideal conditions agreed closely with the predicted values (Table 2), and a paired t-test revealed no statistically significant differences between them at $p > 0.05$. It proves the validation experiment supports the best optimum of validity and predictions.

Conclusions

An experimental design was developed to analyze the effect of the decaffeination process on coffee-based beverage quality. The extraction time, decaffeination time, and decaffeination temperature significantly affected the caffeine content, total phenolic content, and IC₅₀ of coffee powder-based beverages. The optimum conditions were obtained in 10 min of extraction time, 8.05 h of decaffeination time, and 60 °C of the decaffeination temperature with the caffeine content of 1.42±0.14 %, total phenolic content of 48.02±1.07 mg GAE/g, and IC₅₀ of 30.91 ppm. The predicted response value agrees reasonably well with the experimental findings. This study is expected to contribute to the making process of green coffee bean-based functional beverages with low caffeine content.

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