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Simultaneous extraction and decaffeination process optimization of green tea-based beverage using Box Behnken design-response surface methodology

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Abstract

Green tea is a popular beverage consumed worldwide after water, but some people cannot consume it due to its high caffeine concentration. One of the easiest methods to reduce caffeine and retain the bioactive compounds in green tea is through the simultaneous extraction and decaffeination process using hot water. Thus, this work aimed to achieve high flavonoid and low caffeine with antioxidant activity (IC_{50}) in green tea powder-based beverages through simultaneous extraction and decaffeination processes by the Box Behnken design-response surface methodology. Three independent factors were studied, namely extraction time (10 to 30 mins), decaffeination time (1 to 5 hrs), and decaffeination temperature (50 to 100°C), with three observed responses (total flavonoid, $IC_{50,}$ and caffeine content). Process parameters were analyzed and optimized using the Design Expert program. The analysis of variance (ANOVA) was used to detect significant independent factors in each response. The results demonstrated that the extraction and decaffeination parameters influenced the total flavonoid, $IC_{50,}$ and caffeine content of green tea powder-based beverages. The extraction time of 29.17 mins, decaffeination time of 5 hrs, and decaffeination temperature of 50°C was predicted to produce the green tea powder-based beverages with the lowest caffeine and $IC_{50,}$ and high total flavonoid, with total flavonoid of 100.18 mg QE/g, IC_{50} of 32.64 ppm and the caffeine content of 1.54 mg/g, respectively. Based on paired t-tests, the verification revealed no significant difference with model predictions with a confidence interval of 5%. In conclusion, the optimized simultaneous extraction and decaffeination process utilizing the Box Behnken design-response surface methodology successfully produced green tea powder-based beverages with reduced caffeine content, enhanced flavonoid levels, and potent antioxidant activity, offering a promising solution for individuals seeking a healthier alternative to traditional green tea consumption.

1. Introduction

Tea is a type of drink widely consumed after water by the world's population. Tea derived from the *Camellia sinensis* plant is generally consumed in three types: green, black, and oolong tea. Among the three types of tea that have the most significant effect on human health is green tea (Cabrera *et al.*, 2006). It is estimated that around 2.5 million tonnes of tea leaves are produced annually worldwide, of which 20% is green tea widely consumed in Asia, parts of North Africa, America, and Europe (Chacko *et al.*, 2010).

Green tea contains catechins, antioxidants, and theanine (Chacko *et al.*, 2010; Sun *et al.*, 2010; Das *et al.*, 2019). Numerous previous studies reported that green tea and its contents have various human health advantages, such as preventing cancer, cardiovascular disease, infectious disease, diabetes, and obesity (Cabrera *et al.*, 2006; Chacko *et al.*, 2010; Vuong *et al.*, 2011). Dried green tea leaves have significant quantities of alkaloids (caffeine), which can reach 5% (w/w). This is higher than the caffeine content of coffee beans, which can range between 2% (w/w) (Vuong *et al.*, 2013). However, the caffeine concentration in green tea beverages is usually one-third to one-half that of coffee beverages since less green tea than coffee is used to manufacture the respective beverages (Vuong *et al.*,

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2012; Vuong *et al.*, 2013). Caffeine has several beneficial physiological effects related to improved cognitive function, neuromuscular coordination, mood elevation, and anxiety alleviation (Glade, 2010). On the other hand, caffeine can produce undesirable side effects, such as gastrointestinal irritation and sleepiness in some people (Vuong *et al.*, 2013). In the case of coffee powder -based beverages, many methods have been developed for decaffeinated instant green tea powders to provide convenience and health benefits (Kraujalytė *et al.*, 2016; Ye *et al.*, 2007). Furthermore, due to health concerns, some people are sensitive to caffeine (Vuong *et al.*, 2013), so various methods have been developed to remove the alkaloid and remaining catechins from green tea powders (Sun *et al.*, 2010), including organic solvents (Senol and Aydin, 2006; Dong *et al.*, 2011), activated carbon (Ye *et al.*, 2007), supercritical CO_2 extraction (Sun *et al.*, 2010; Bermejo *et al.*, 2016), microwave-enhanced vacuum ice water (Lou *et al.*, 2012), montmorillonite (Shiono *et al.*, 2017), and so on. However, these processes have drawbacks, such as the risk of solvent residues, expensive equipment, long production times, and difficulty scaling up for commercial production. Therefore, a simple, cheap, and safe method is needed to remove caffeine from green tea powder during decaffeination. In one investigation, the simple hot water blanching process successfully removed caffeine from green tea leaves (Liang *et al.*, 2007). The advantages of using water as a solvent in this method were simple, low price, and safe for further consumption. So, this method was adopted and further used in this study. However, the optimum simultaneous extraction and decaffeination process conditions to produce green tea powder-based beverages have never been described. Hence, this study aimed to find the optimum conditions for green tea powder-based beverage production using Box Behnken response surface methodology (BBD-RSM) with low caffeine concentrations, IC_{50} of antioxidant activity, and high flavonoid content.

2. Materials and methods

2.1 Materials

The raw material used in this study was green tea (grade 1 taken from the top 3 shoots) from the planting site in the Ciwidey area of Bandung and harvested in July 2019. Then, a sorting process was carried out to separate low-quality green tea, followed by air drying until dry and continued size reduction using a dry grinder, and then sieved using an 80-mesh sieve, which has a characteristic as follows: total flavonoid content of 125.957 mg QE/g, IC_{50} of 27.45 ppm, and caffeine content of 30.3 mg/g. The chemicals used in this study were quercetin standards, $NaNO_2$, $AlCl_3$, NaOH, acetonitrile (HPLC grade), methanol, DPPH reagent (2,2 -diphenyl-1-picrylhydrazyl), and formic acid was obtained from Merck KGa, Darmstadt, Germany in analytical grade. Demineral water, distilled water, and maltodextrin were purchased from the local chemical supplier in Malang, Indonesia, in the technical grade.

2.2 Method of the green tea powder-based beverage production

2.2.1 Extraction of green tea powder

The green tea powder was weighed and extracted using the infusion method with de-mineral water as a solvent: solvent (1:15 b/v), 90°C, and various extraction times (10, 20, and 30 mins). Then, the coarse filter paper was used to filter the aqueous green tea extract.

2.2.2 Decaffeination of green tea extract

Green tea extract decaffeination was done by adapting the blanching method (Liang *et al.*, 2007). Green tea extract was decaffeinated at different temperatures (50, 75, and 100°C) with varying times (1, 3, and 5 mins).

2.2.3 Production of green tea powder-based beverage

Decaffeinated green tea extracts were blended with maltodextrin (ratio of 5% (w/v)), followed by drying using a food dehydrator for 5 hrs at 60°C. The dried decaffeinated green tea was reduced in size using a dry blender and filtered with a sieve of 80 mesh to achieve a green tea powder-based beverage.

2.3 Total flavanoid

Total flavonoid was analyzed by colorimetric method using aluminum chloride reagent (Atanassova *et al.*, 2011). Briefly, a sample of 1 mL was taken and put into a test tube, then 4 mL of distilled water was added. Followed by adding 0.3 mL of 5% $NaNO_2$ solution and vortex to make it homogeneous, then left for 5 mins. Then, 0.3 mL of 10% $AlCl_3$ was added, vortexed, and left for 5 mins. After that, 2 mL of 1 M NaOH was added and adjusted to 10 mL by adding distilled water, vortexed, and incubated for 5 mins. The absorbance mixture solution was measured at a wavelength of 510 nm. Previously, standard quercetin curves were made using the same method as the sample using quercetin concentrations of 0, 20, 40, 60, 80, and 100 mg/L in distilled water. Total flavonoid was calculated based on the standard curve and expressed as quercetin equivalent with Equation 1:

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

Where TF is total flavonoids in mg/g extract in mg GAE/g, C is quercetin concentration obtained from the

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calibration curve in mg/ml, F is dilution factor, V is extracts volume in mL, and M is the weight of sample extracted in grams.

2.4 Antioxidant activity

The Atanassova *et al*. (2011) method was used to measure the free radical activity, and the reagent DPPH (1,1-diphenyl-2-picrylhydrazyl) was used. The green tea extracts were briefly diluted in absolute ethanol at 100, 200, 300, 400, and 500 ppm concentrations. A test tube containing 1 mL of the sample from each dilution was then filled with 4 mL of a 0.004% DPPH solution in ethanol and vortexed until the mixture was homogeneous. The test tube was left to sit at room temperature and in the dark for 1 hr. The aliquot measured the absorbance in triplicate for each sample at 517 nm. By stating the y value as 50 and the x value as IC_{50}, the linear regression formula y = a(x) + b is used to calculate the free radical activity of DPPH in percentage.

2.5 Caffeine content

Ultra High-Performance Liquid Chromatography (UPLC) methods were used to determine the amount of caffeine in the green tea powder-based beverage. An autosampler compartment was set to 16°C with the mobile phase (1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B)) was set to the composition, 0.0-1.5 mins 85% B, 1.5- 4.0 mins 15% B, 4.0-5.0 mins 15% B, 5.5-7.0 mins 85% B. Shortly after, 1 L of the prepared sample was injected into a Hypersil Gold column (150 mm). The MS/MS Triple Quadrupole's operating the positive ionization-moded TSQ Tune software manages conditions. The Electrospray Ionization (ESI) ionization source is used as the ionization source. Selected Reaction Monitoring (SRM) mode was chosen for the target compound analysis method. The capillary temperature is 300°C, the evaporation temperature is 275°C, the nitrogen sheath gas pressure is 40 psi, and the auxiliary gas pressure is ten psi for argon gas. The spray voltage is set at 3.0 kV. The quantification of caffeine content in the samples used the standard curve prepared in advance. Briefly, 0.5, 1, 2, 4, and 8 µg/mL of standard caffeine were dissolved in methanol at concentrations and injected into UPLC to build the standard caffeine curve, with linear regression equation: y = a (x) + b.

2.6 Research design, statistical analysis, and verification of optimum condition

The Design Expert 13.0 software (trial version) was used to optimize the extraction time, decaffeination time, and decaffeination temperature during the simultaneous extraction and decaffeination processes to produce green tea powder using the Response Surface Methodology (RSM) and a Box Behnken design (BBD). Based on preliminary research, Table 1 shows the study's upper, middle, and lower limits for extraction time, decaffeination time, and decaffeination temperature. A second-order polynomial equation fitted the responses to the green tea powder-based beverages, and an empirical model based on the independent variables was built using multiple data regression. Equation 2 represents the general second-order polynomial equation. The precision of the polynomial model was evaluated using Design-Expert software to determine correlation R^2, adjusted-R^2, adequate precision, and lack of fit for total flavonoid content, IC_{50}, and caffeine content responses. The verification in the laboratory was conducted three times based on the optimum prediction from the software. Minitab 17 used a paired t-test to assess the software's prediction and verification results (trial version).

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

Where Y is the predicted responses (total flavonoid, IC_{50}, and caffeine content); 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.

Table 1. The range of independent factors in the production of green tea powder-based beverage.

Independent factors	Unit	Lower limit (-1)	Center (0)	Upper limit (+1)
Extraction time	Min	10	20	30
Decaffeination time	Min	1	3	5
Decaffeination temperature	°C	50	75	100

3. Results and discussion

3.1 Mathematical model selection and fitting

This experiment evaluated the effect of extraction time, decaffeination temperature, and decaffeination time on the total flavonoid, antioxidant activity, and caffeine content of green tea powder-based beverages using response surface methodology. The Box-Behnken Design experimental data of green tea powder-based beverages, the mathematical model selection for each response of green tea powder, and the significant model of the second-order polynomial for green tea powder are displayed in Tables 2, 3, and 4 in sequence. Table 3 presents the mathematical model selection for total flavonoid, antioxidant activity (IC_{50}), and caffeine content of green tea powder was influenced by the different extraction time, decaffeination time, and decaffeination temperatures. The quadratic model followed for the response of total flavonoid and antioxidant activity, but the linear model was followed by the caffeine content of green tea powder, with a

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Table 2. The green tea powder-based beverage responses by BBD-RSM.

Std	Independent factor			Response		
	Extraction time (min)	Decaffeination time (min)	Decaffeination temperature (°C)	Total flavonoid (mg QE/g)	IC_{50} (ppm)	Caffeine content (mg/g)
1	10	1	75	77.00	35.59	1.56
2	30	1	75	84.56	42.55	1.79
3	10	5	75	51.13	36.04	1.28
4	30	5	75	69.64	56.12	1.17
5	10	3	50	80.44	43.14	2.34
6	30	3	50	98.94	26.09	1.90
7	10	3	100	54.50	59.76	1.58
8	30	3	100	72.51	58.47	1.11
9	20	1	50	108.38	41.47	2.61
10	20	5	50	105.69	34.14	1.52
11	20	1	100	74.10	50.96	1.74
12	20	5	100	61.91	60.01	0.86
13	20	3	75	89.73	46.16	1.51
14	20	3	75	74.71	53.28	1.65
15	20	3	75	74.64	42.82	1.50
16	20	3	75	84.52	44.76	1.61
17	20	3	75	80.02	47.47	1.58
Prediction	29.17	5.00	50.00	100.18±0.00[a]	32.64±0.00[b]	1.54±0.00[c]
Verification	29.17	5.00	50.00	96.79±6.37[a]	30.91[b]	1.42±0.14[c]

Table 3. The model selection for each response of green tea powder.

Parameters	Total flavonoid	IC_{50}	Caffeine content
Mathematical model	Quadratic	Quadratic	Linear
Significance model	0.0057[s]	0.1216[ns]	<0.0001[s]
Lack of fit	0.3611[ns]	0.0529[ns]	0.0134[s]
R^2	0.913	0.7616	0.8128
Adjusted-R^2	0.8012	0.4551	0.7696
Adequate precision	10.301	5.3981	15.2132

Table 4. Significance responses of the green tea powder-based beverage.

Coefficient	Total flavonoid	IC_{50}	Caffeine content
Intercept	80.73	46.90	1.61
X_1-Extraction time	7.82*	1.09[ns]	-0.0980[ns]
X_2-Decaffeination time	-6.96*	1.97[ns]	-0.3570***
X_3- Decaffeination temperature	-16.30***	10.55*	-0.3858***
$X_1 X_2$	2.74[ns]	3.28[ns]	
$X_1 X_3$	-0.1230[ns]	3.94[ns]	
$X_2 X_3$	-2.37[ns]	4.10[ns]	
X_1^2	-10.53*	-2.05[ns]	
X_2^2	0.3894[ns]	-2.27[ns]	
X_3^2	6.41[ns]	2.02[ns]	

Significance remarks: [ns]P>0.05; *0.01<P<0.05; **0.001<P<0.01; ***P<0.001

Remarks: [s]: significant, and [ns]: not significant

significant model range of <0.0013 to 0.1216, as shown in Table 4. The lowest p-value of the significance model indicated it was highly significant (Bahar *et al.*, 2013). The total flavonoid, antioxidant activity (IC_{50}), and caffeine content of green tea powder had an R^2 of 0.9130, 0.7616, and 0.8128, respectively. Furthermore, the adjusted R^2 of total flavonoid, antioxidant activity (IC_{50}), and caffeine content of green tea powder was 0.8012, 0.4551, and 0.7696 in sequence. Additionally, the green tea powder had adequate precision of 10.3010 for total flavonoid, 5.3981 for antioxidant activity (IC_{50}), and 15.2132 for caffeine content. The high R^2 and adjusted R^2 indicate that the quadratic model can explain all response variations (Witoyo *et al.*, 2022). In addition, the signal-to-noise ratio is less than 4 for all responses, which indicates sufficient precision (Rahmawati *et al.*, 2020). Except for caffeine content response, the lack of fit in this study is more than 0.05, indicating that the selected model was highly accurate and precise to pure error (Bahar *et al.*, 2013).

3.2 Total flavonoid

The total flavonoid in green tea powder was 51.13 to 108.38 mg QE/g, as in Table 2. The results were higher than the total flavonoid content (TPC) of decaffeinated green tea processed using hot water (10.78-14.87 mg QE/g) or ethyl acetate (7.59-10.62 mg QE/g) (Das *et al.*, 2019). The flavonoid in this study was affected by extraction time, decaffeination time, decaffeination temperature in linear terms, and extraction time in quadratic terms, as presented in Table 4. Figures 1A and 1B revealed the increasing extraction time to increase the total flavonoid content of the green tea powder, both in linear and quadratic terms. Balci and Özdemir (2016) reported the positive correlation between extraction time and the total flavonoid in Turkish green tea extracts. Komes *et al.* (2010) also reported that the total flavonoids in green tea leaf extract increased with increasing extraction time until the optimal time, and then the total flavonoid content decreased when the time was increased, which might be the prolonged extraction time at high temperatures can cause degradation of catechins. Moreover, the total flavonoid linearly decreased significantly with the increasing decaffeinated time, as revealed in Figures 1A and 1C. The total flavonoids linearly decreased significantly with increasing decaffeination temperature, as in Figures 1B and 1C. Liang *et al.* (2007) reported that the decaffeinated process of fresh green tea leaves using hot water decreased the flavonoid content, which is the catechin, the main flavonoid component in green tea leaves. Perva-Uzunalic *et al.* (2006) also reported that prolonging decaffeination time at higher temperatures (85 to 90°C) will decrease catechin content in green tea extract. Vuong *et al.* (2012, 2013) also reported that the prolonged decaffeination time at a high temperature could degrade the old tea leaves' catechin content and affect the total flavonoid content in the old tea leaves extract.

Moreover, compared with the raw materials, the total flavonoid content of the green tea-based powder decreased by 13.96 to 59.41%. It is suspected that this process uses simultaneous conventional extraction and decaffeination methods, which involve heat treatment resulting in a degradation of the chemical structure of the extracted green coffee flavonoids, which can decrease bioactivity, thus impacting the levels when tested (Chávez-González *et al.,* 2020). Degradation of flavonoids at 100°C into more polar compounds, namely quercetine-3-O-β-D-glucopyranoside (e.g., isoquercitrin), is suspected as a possible cause of loss of flavonoids during the simultaneous extraction and decaffeination process, causing these new compounds to be challenging to dissolve with increasing water temperature (Das *et al.,* 2019).

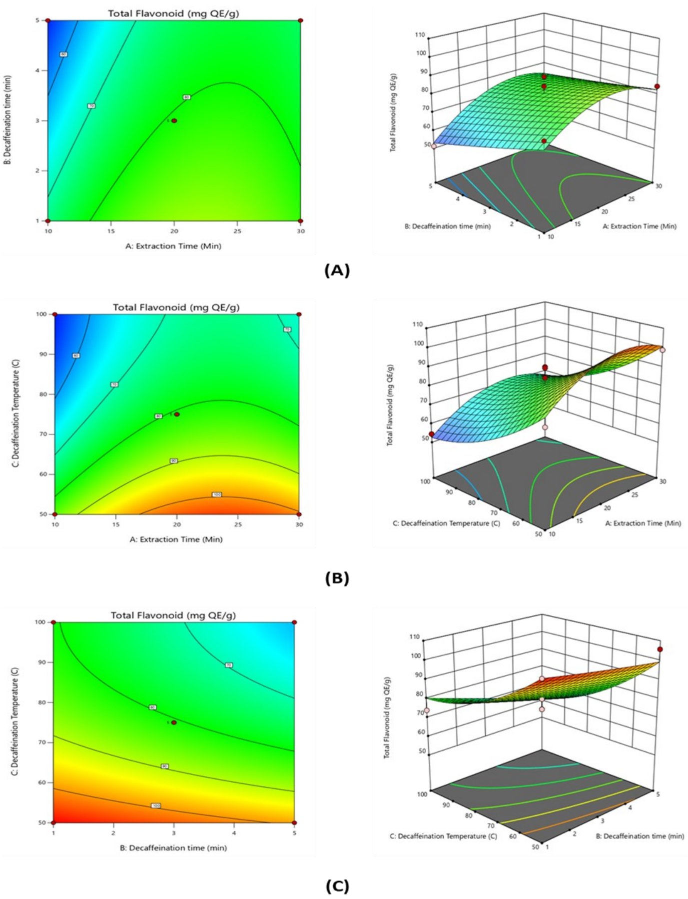

Figure 1. Contour plot and response surface plot of the effect of an independent factor on total flavonoid of green tea-based powder.

3.3 Antioxidant activity

The IC_{50} of green tea powder was 26.09 to 60.01 ppm, as shown in Table 2. The result was higher than the IC_{50} of green tea leaves extract from Ciwedey, West Java, Indonesia (0.54 ppm) (Novilla *et al.*, 2022). The IC_{50} was only affected by the decaffeination temperature found in this study. Figure 2A reveals that the IC_{50} of green tea powder tends to increase with decreasing extraction time and increasing decaffeination time. Furthermore, increasing the extraction time decreased IC_{50} insignificantly, and in contrast, the increasing decaffeinated temperature significantly increased the IC_{50}, as revealed in Figure 2B. In addition, Figure 2C depicts the increasing decaffeinated time decreasing insignificantly of IC_{50}, and decaffeinated temperature significantly increased the IC_{50} in the green tea powder. The increasing IC_{50} affected by decaffeination temperature was related to the decreasing total flavonoid and the caffeine content of green tea powder. Zhang *et al.* (2013) reported that the higher IC_{50} in green tea samples demonstrated lower antioxidant capacity. In this study, the IC_{50} in green tea powder-based beverages decreased by 24.37 to 118.62% than the raw material, except for treatment with an extraction time of 30 mins, decaffeination time of 3 mins, and decaffeination time of 50°C, as increasing IC_{50} value by 4.95% (Std 6, in Table 2). It might be due to the more prolonged extraction and decaffeination as well as the higher decaffeination temperature able to change and degrade the bioactive compounds contained in the green tea, like epigallocatechin gallate (EGCG) and catechin, so affected in the increasing of IC_{50} in the green-tea powder -based beverages.

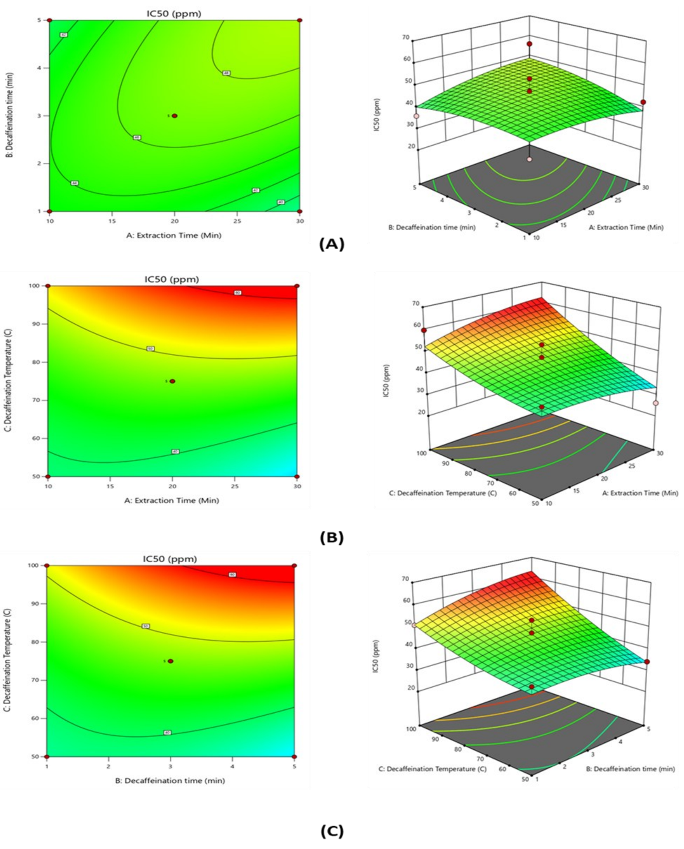

Figure 2. Contour plot and response surface plot of the effect of an independent factor on IC_{50} of green tea powder-based beverages.

3.3 Caffeine content

The caffeine content of green tea powder was 0.86 to 2.34 mg/g, as presented in Table 2. The results were lower than decaffeinated green tea powders from old tea leaves (7.3 mg/g) (Vuong *et al.*, 2013) and decaffeinated green tea powders produced using other decaffeination methods such as organic solvents (37.89 mg/g) (Ye *et al.*, 2007), activated charcoal (7.81 mg/g) (Dong *et al.*, 2011), and microwave-enhanced vacuum ice water (5.48 mg/g) (Lou *et al.*, 2012). In this study, the decaffeination time and temperatures influenced the caffeine content of green tea powder, but the extraction time had an insignificant effect. The single factor of extraction time in linear terms revealed that the increasing extraction time insignificantly increased the caffeine content of the green tea powder (Figures 3A and 3B). Moreover, in linear terms, a similar trend in both the decaffeination time and decaffeination temperature affects the caffeine content of green tea powder. The increase in decaffeination time (Figures 3A and 3C) and decaffeination temperature (Figures 3B and 3C) decreased the caffeine content in the green tea powder. Vuong *et al.* (2012, 2013) reported that the prolonged decaffeination time at a high temperature (90 to 100°C) simultaneously degrades the catechin and caffeine content of the old tea leaves. It caused caffeine's solubility, and the diffusion coefficient was higher released at high temperatures (90 to 100°C) than at lower temperatures during the decaffeination process of tea (Sharif *et al.*, 2014). Compared with the raw materials, the caffeine content in green tea-based powder decreased by 91.39 to 97.16%. It indicated that the simultaneous extraction and decaffeination process by hot water effectively decreased green tea's caffeine content. This finding was in line with Vuong *et al.* (2013), which also reported that blanching with hot water for 4 mins

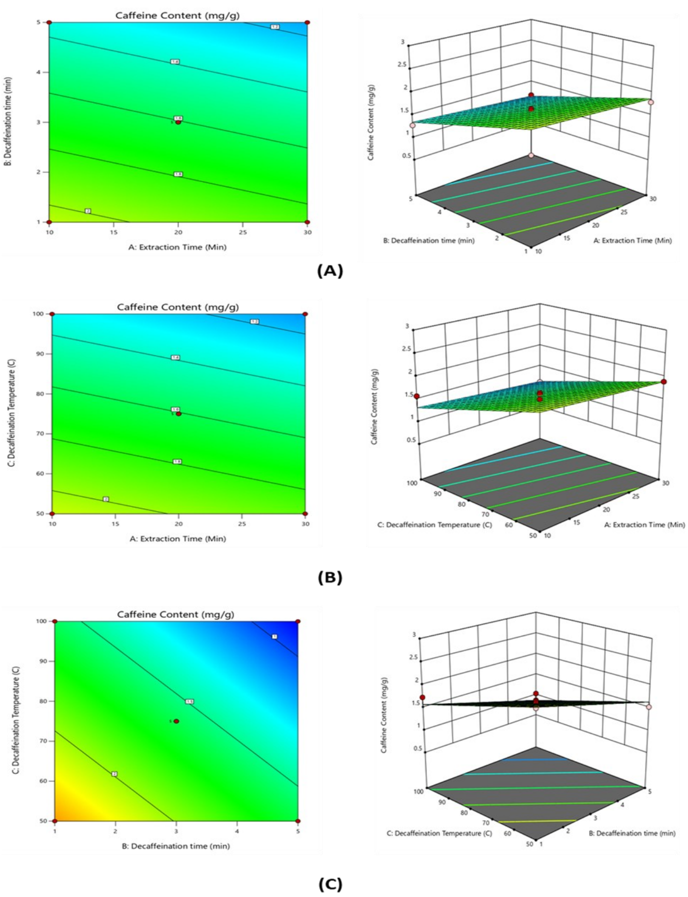

Figure 3. Contour plot and response surface plot of the effect of an independent factor on the caffeine content of green tea powder-based beverages.

effectively to decreased the caffeine content in dried green tea leaves by 83% and maintained the catechin content of 94%. Our method in this study has a high percentage in removing caffeine content from green tea leaves compared to the supercritical CO_2 extraction method and the ultrasonic-enhanced supercritical liquid method, which are only able to reduce caffeine by 70.2% and 82.28%, respectively (Sun *et al*., 2010; Tang *et al*., 2010). However, this study's percentage removal of caffeine content was lower than the supercritical CO_2-based decaffeination (SCD), which removed the caffeine to 98.2% (Wang *et al*., 2022).

3.4 Verification of the optimum conditions

This study expects to produce green tea powder-based beverages with the lowest caffeine and IC_{50} and high total flavonoid. The independent factors were set in the range, the total flavonoid was set to maximize, and IC_{50} and caffeine content were set to minimize. The predicted optimum conditions for the extraction and decaffeination process of green tea-based beverage were an extraction time of 29.17 mins, a decaffeination time of 5 hrs, and a decaffeination temperature of 50°C, with the following results: total flavonoid of 100.18 mg QE/g, IC_{50} of 32.64 ppm, and caffeine content of 1.54 mg/g, with a desirability value of 0.774. Predicting the optimum point has a better level of validity when the desirability value is close to 1 (Solano *et al*., 2022). Three replications confirmed the best condition, yielding a total flavonoid content of 96.796.37 mg QE/g, IC_{50} of 30.91 ppm, and caffeine content of 1.420.14 mg/g. In ideal conditions, the experimental values corresponded well with the predicted values, as shown in Table 2, and a paired t-test revealed no significant variance at P>0.05.

4. Conclusion

An experimental design was created to investigate the impact of simultaneous extraction and decaffeination processes on the quality of green tea-based beverages. The mathematical model selection revealed that the total flavonoid and IC_{50} followed the quadratic model, but the caffeine content followed the linear model. The total flavonoid, IC_{50}, and caffeine content of green tea powder -based beverages are affected by the extraction and decaffeination parameters. The optimal conditions were attained in 29.17 mins of extraction time, 5 hrs of decaffeination time, and 50°C of decaffeination temperature, with a total flavonoid of 96.79 mg QE/g, IC_{50} of 30.91 ppm, and caffeine content of 1.42 mg/g. The estimated response value coincides quite well with the experimental results. This research is likely to aid in developing green tea-based functional beverages with low caffeine concentrations and high antioxidant properties.

Conflict of interest

The authors declare no conflict of interest.

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