

Application of factorial design for optimization of microwave-assisted extraction process of theaflavin from black tea (*Camellia sinensis*)

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Abstract

Theaflavin is widely known as a polyphenolic compound found in black tea. Theaflavins were considered to have herbal medicinal potential. It could inhibit RdRp activity by blocking the active site in the catalytic pocket of RdRp in SARS-CoV-2, SARS-CoV and MERS-CoV. Extraction of theaflavins with alcohol and ethyl acetate could produce high theaflavins content, but there were problems with the separation process, and potentially leave toxic solvent residue. The thermolabile nature of theaflavins also caused thermal degradation of thearubigin. Meanwhile, conventional extraction methods require a long time and a large amount of solvent. To overcome this problem, an alternative theaflavin extraction process using microwave-assisted extraction with water solvent was investigated. The effect of operating conditions (microwave power, extraction time, and feed:solvent ratio) on the theaflavin content was observed. Factorial design 2^3 was used to determine the influential factors of microwave-assisted extraction. The results showed that the optimum operating conditions of black tea extraction using microwave-assisted extraction in this study were obtained at 400 W power, 8 min, and feed:solvent ratio of 1:30 g/mL, where theaflavin content yield was 1.61775%. It was also found that the most influential independent variable in theaflavin extraction process was the power variable (W). This was evidenced by the higher power used, the higher the percentage of theaflavin.

1. Introduction

Tea (*Camellia sinensis*) is a functional food plant that has been widely consumed since ancient times and is believed to be rich in antioxidants. One of the antioxidant compounds in tea leaves is theaflavin, a component giving color, taste, and aroma to tea (Feng *et al.*, 2002) and is the result of oxidation of catechin compounds, derivatives of polyphenolic compounds. Theaflavins are considered to have herbal medicinal potential. Several research results state that the antioxidant activity and cancer cell inhibition effect of theaflavins are more potent than catechins (Friedman *et al.*, 2006; Bedran *et al.*, 2015). Theaflavins can be protective agents in various diseases, such as reducing the risk of cardiovascular disease, cancer, diabetes and oral health (Adak and Gabar, 2011; Naveed *et al.*, 2018). Furthermore, theaflavins and their derivatives, such as theaflavin gallate, have shown broad-spectrum antiviral activity against several viruses, including influenza A

and B and hepatitis C viruses (He, 2017). In silico studies, bioinformatics experiments and extensive molecular docking have been carried out on all catechin or theaflavin derivatives from black tea leaves (*Camellia sinensis*) and theaflavin-3'-O-gallate (TFMG), which are proven to block or deactivate proteins in SARS-CoV-2 (Maiti *et al.*, 2022). The highest levels of theaflavins and thearubigins were found in black tea, followed by oolong tea and green tea (Salman *et al.*, 2022). However, the theaflavin content in black tea is quite difficult to isolate because it easily undergoes thermal degradation to form thearubigin compounds, thus requiring an appropriate method to isolate it (Amalia *et al.*, 2020).

The theaflavin extraction process can be done using several methods: Soxhlet extraction, maceration, percolation, infusion, reflux, ultrasound-assisted extraction and microwave-assisted extraction (Suandari, 2016). Conventional extraction methods such as Soxhlet extraction, maceration, percolation and reflux require a

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long time and a large amount of solvent. The ultrasound-assisted extraction method requires a fairly short extraction time of 3-15 min. However, it uses relatively large amounts of solvent, namely 150-300 mL for each addition of 5 g of tea (Dean, 1998). Three parameters, i.e., solvent type, temperature and extraction time, are the important parameters in the experimental studies (Mukhrani, 2014). Theaflavin extraction with organic solvents (alcohol) obtained 100% theaflavin, but it causes difficulties in the downstream separation process between theaflavins and alcohol. High yields of theaflavins extract can be carried out in solvents such as alcohol and ethyl acetate. Still, the separation process of theaflavins from alcohols gives a low overall extraction efficiency (Ganguli *et al.*, 2008). Extraction at high temperatures tends to reduce the levels of theaflavins extracted if it is carried out for a long time (Amalia *et al.*, 2023). This is related to the levels of theaflavins produced; if the temperature is too high and the time used is too long, it will tend to reduce the levels of theaflavins obtained.

Microwave-assisted extraction (MAE) is an extraction technique that provides a lower extraction period and less energy consumption (Serdar *et al.*, 2016). In addition, the microwave produces a high-temperature area that facilitates interference with the cell wall. As a result, the active compound is extracted more quickly into the solvent. Pan *et al.* (2003) stated that water can be used as a solvent in MAE because it has the largest dielectric constant compared to other solvents, so that the absorption rate of microwave energy in water is more optimal. This study will investigate the microwave-assisted extraction (MAE) of black tea using water as a solvent. The optimum operating conditions in the microwave-assisted extraction (MAE) process with the influence of temperature, time, and feed: solvent ratio on theaflavin levels were studied using factorial design 2^3 .

2. Materials and methods

2.1 Materials

The main material used in this research was black tea CTC Pagar Alam from Dempo Mountain, Sumatra, Indonesia and distilled water as a solvent. Other chemicals for analysis include ethyl acetate p.a. FULLTIME 6801-04 and methanol p.a. FULLTIME 6508-04. The main equipment used includes a microwave extraction tool and a Genesys 20 Thermo Scientific UV-Vis spectrophotometer with a wavelength of 380 nm.

2.2 Experimental design using factorial design

The experimental design was carried out to obtain the data used to determine the most influential process

variables and the optimum process conditions modelled in the empirical equation. Table 1 shows the variables used in the experimental design: power, extraction time, and feed-to-solvent ratio, each evaluated at low and high points. While the MAE experiments based on a factorial design 2^3 are summarized in Table 2.

Table 1. Experimental design variable.

No	Variable	Low Point	High Point
1	Power (W)	200	400
2	Time (mins)	4	8
3	Feed:Solvent (g/mL)	1:30	1:40

Table 2. MAE experimental design with factorial design.

Running	Power (W)	Time (Mins)	Feed: Solvent Ratio
1	-	-	-
2	+	-	-
3	-	+	-
4	+	+	-
5	-	-	+
6	+	-	+
7	-	+	+
8	+	+	+

Power: (-) 200 W and (+) 400 W, Time: (-) 4 mins and (+) 8 mins, Feed:Solvent ratio: (-) 1:30 and (+) 1:40

2.2 Theaflavin extraction

Dried black tea was weighed, then put into a two-neck round-bottom flask and added with distilled water at a ratio of feed:solvent of 1:30 and 1:40 (g/mL). The two-necked round-bottom flask was then placed in a microwave oven at 200 W and 400 W for 4 and 8 min. After the extraction process, the filtrate was separated using filter paper and subjected to spectrophotometric analysis for theaflavin.

2.3 Theaflavin analysis

Each black tea sample was extracted to determine the theaflavin (Akuli *et al.*, 2016). The solution blank was prepared by diluting 4 mL of ethyl acetate with 25 mL of methanol. To determine theaflavin, 50 mL of the cool, well-shaken, and filtered standard tea extract was mixed with 50 mL of ethyl acetate, then separated in separator funnel. UV-Vis spectrophotometer with a wavelength of 380 nm was used to analyzed theaflavin content (Ullah, 2019).

$$\% \text{ TF} = 2.25 \times E_l \quad (1)$$

Where E_l was the absorbance of solution A at a wavelength of 380 nm after being standardized with a blank.

3. Results and discussion

3.1 Determination of optimal operating conditions

Extraction of Theaflavin from CTC black tea used a factorial design experimental method with three variables of change: power, operating time, and feed:solvent ratio. This method was used because it only required a small number of experiments to determine the effects on experimental variables. In addition, the optimum operating conditions obtained were more precise because they included interaction factors. The factorial design method would obtain the most influential variables in the theaflavin extraction experiment from black tea. The extraction process was carried out using the Microwave-assisted Extraction (MAE) method to extract the theaflavin compounds produced from CTC black tea. The data obtained were then analyzed by a spectrophotometer to determine the percentage content of theaflavins formed during the extraction process.

Table 3 shows that the largest theaflavin levels were in the 4th experiment, namely 1.61775% when the power was 400W, the time was 8 mins and the ratio:feed ratio was 1:30. Meanwhile, the smallest theaflavin levels were found in the 5th experiment at 0.783% when the power was 200 W, the time was 4 mins and ratio:feed ratio was 1:40.

Table 3. Results of research design.

Running	Power (W)	Time (Mins)	Feed: Solvent Ratio	Theaflavin (%w)
1	200	4	1:30	0.90225
2	400	4	1:30	1.548
3	200	8	1:30	1.13175
4	400	8	1:30	1.61775
5	200	4	1:40	0.783
6	400	4	1:40	1.2825
7	200	8	1:40	0.96525
8	400	8	1:40	1.4175

Higher power could produce products with higher theaflavin extract content. This was due to the high solubility of the extract in the solvent, so that the solvent could carry away more theaflavin extract. In addition, according to Hu *et al.* (2008), microwave power acted as a driving force to break the membrane structure of black tea cells so that theaflavin compounds could diffuse out and dissolve in the solvent. With the addition of microwave power used in general, it would increase the % theaflavin and speed up the extraction time. This was because the greater the power used, the higher the operating temperature could rise above the solvent's boiling point. Furthermore, Table 3 shows that microwave power increased % of theaflavin levels. Microwave heating occurred in all parts of the sample and solvent. This involved the absorption of energy

directly and was extracted without involving the container to produce a perfect extraction (Kusuma and Mahfud, 2015).

In addition, there was a significant effect on the time variable on the percentage of theaflavin levels. It was known that increasing the time used to produce % theaflavin levels increased optimally. The longer the extraction time, would cause the bioactive compounds are damaged, while the shorter the extraction time, the of the active compounds are extracted from the material. Over time, theaflavins would change from theaflavin compounds to thearubigin compounds, so that when solvents penetrated theaflavins for a long time, theaflavins were thermally degraded into more complex compounds, namely thearubigin compounds. Therefore, proper operating conditions were needed to ensure optimal theaflavin content (Amalia *et al.*, 2020)

In addition to the power and time variables that affected % theaflavin levels, other variables that had effects include the feed: solvent ratio used in the extraction of CTC black tea. This ratio factor was related to how dense (amount) the condition of the raw materials was included in the distillation flask (distiller), so that the extraction and evaporation process of theaflavin compounds could run perfectly. The density of the material was closely related to the size of the space between the materials. The material density that was too high and uneven can lead to the formation of "rat holes" which could reduce the theaflavin % and black tea quality (Guenther, 1987). In addition, the greater the density of the material, the slower the evaporation rate of black tea due to the obstruction of the movement space to evaporate upwards. This caused the yield and evaporation efficiency to decrease. Table 3 showed that the feed: solvent ratio variable of 1:30 produced a higher percentage of theaflavin content compared to the feed:solvent ratio variable of 1:40. With the optimization of the operating conditions used, it was expected to produce the maximum % theaflavin content.

3.2 Calculation of variable effects on the percentage of theaflavin levels

The main and interaction effects on the theaflavin extraction process from CTC black tea on the percentage of theaflavin levels could be seen in Table 4.

Figure 1 shows that the power variable was the farthest point, i.e., 0.5208. Thus, the power variable was the most influential variable on the percentage of theaflavin. In this study, the highest theaflavin value was obtained in the 4th experiment with a percentage of 1.61775% at 400 W power treatment, 8 mins, and a feed:solvent ratio of 1:30.

Table 4. Calculation of variable effects on the percentage of theaflavin levels.

No	D	t	R	% Theaflavin	-1	-2	-3	Divisor	Effect	Result
1	-	-	-	0.90225	2.45025	5.19975	9.648	8	Average	1.206
2	+	-	-	1.548	2.7495	4.44825	2.0835	4	D	0.5208
3	-	+	-	1.13175	2.0655	1.13175	0.6165	4	t	0.1541
4	+	+	-	1.61775	2.38275	0.95175	-0.207	4	Dt	-0.0517
5	-	-	+	0.783	0.64575	0.29925	-0.7515	4	R	-0.1878
6	+	-	+	1.2825	0.486	0.31725	-0.18	4	DR	-0.045
7	-	+	+	0.96525	0.4995	-0.15975	0.018	4	tR	0.0045
8	+	+	+	1.4175	0.45225	-0.04725	0.1125	4	DtR	0.02812

Figure 2 shows a Pareto diagram that was created to help identify the significant factors that affected the % of theaflavin produced.

3.3 Effects between variables on the percentage of theaflavin levels

Furthermore, to analyze the relationship between variables, contour plots could be used to analyze the effect of power and time variables on the percentage of theaflavin (Figure 3), the power variable and feed ratio:solvent to percentage of theaflavin (Figure 4) and the effect of time variable and feed ratio:solvent to % theaflavins (Figure 5).

In the contour plot section (Figure 3), it could be seen that the part that had the most influence on the optimum condition (producing the largest % theaflavin) was marked with a dark green color at 400 W of power and 8 min. The greater the power, the higher the levels of theaflavins produced. Likewise, with power, if the time was longer, it would cause higher theaflavin levels. However, it must be reconsidered if the time used is too long, it would cause theaflavins to be thermally degraded into thearubigin. This was because theaflavins were thermolabile, so when a solvent penetrated black tea for a long time, the theaflavin compounds would be degraded.

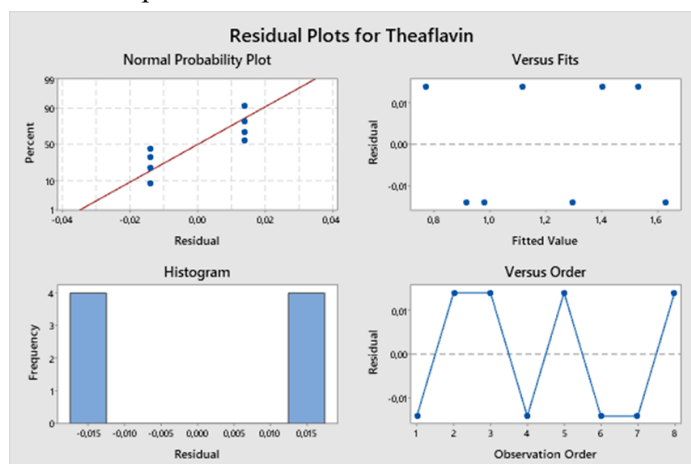


Figure 1. P relationship with effect value.

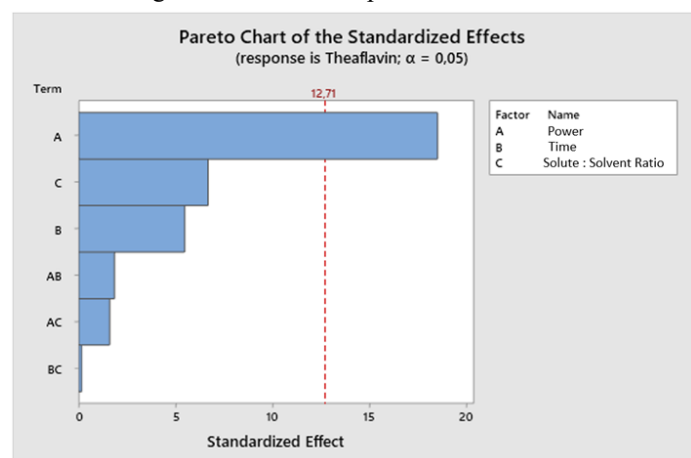


Figure 2. Pareto chart of the standardized effect of theaflavin.

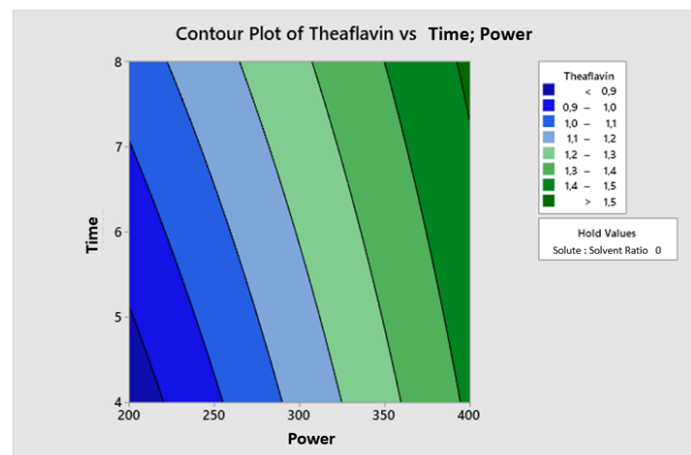


Figure 3. Analysis of the effect of power and time on the percentage of theaflavin.

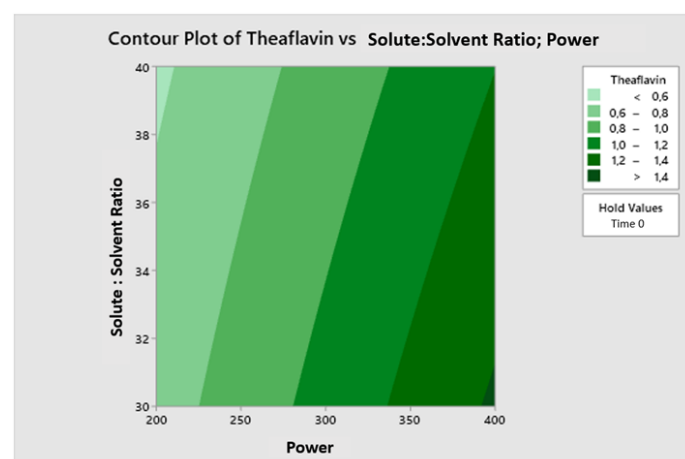


Figure 4. Analysis of the effect of feed:solvent ratio and power on the percentage of theaflavin.

Figure 4 shows that the most optimum condition was marked in dark green color (at 400 W and the feed:solvent ratio of 1:30). The smaller the power, the % theaflavin in the resultant yield would be smaller, and vice versa. For the feed:solvent ratio, the smaller the

ratio, the higher the % theaflavin produced.

Figure 5 shows that the most optimum part (producing the largest percentage theaflavins) was marked in dark green and was presented at 8 mins with a feed:solvent ratio of 1:30. The longer the time used, the higher the % theaflavin. Meanwhile, the feed:solvent ratio had no significant effect.

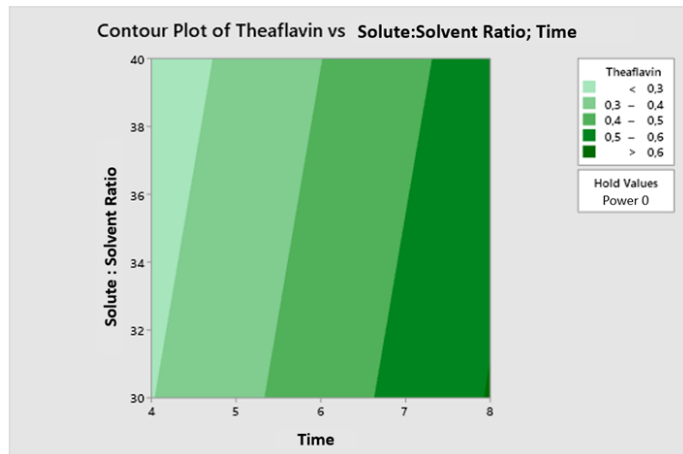


Figure 5. Analysis of the effect of time on the ratio of feed:solvent to % theaflavin.

3.4 Analysis of variance on the percentage of theaflavin levels

Factorial design on the Minitab-19 application can be used to analyze the experimental results between the percentages of theaflavins with independent variables. The summary model of theaflavins could be seen in Table 5.

Table 5. Summary of factorial regression designs for theaflavin.

S	R-sq	R sq(adj)	R-sq(pred)
0.0397748	99.76%	98.35%	84.93%

Table 5 indicates that data interpretation should be made by looking at the value of R-square adjusted (R-sq (adj)). In this case, its values tended to be constant and sensitive to changes in independent variables, while R-square would tend to increase if other variables were

added. The more independent variables used, the more “noise” in the model; this could not be explained by R-square and made the weakness of R-square itself. Furthermore, the R-square adjusted value was 98.35% which implies that 98.35% of the value of the dependent variable is influenced by the independent variables contained in the study (power, time, and feed:solvent ratio). While other factors influenced the rest. Furthermore, an analysis of variance was carried out, and the results can be seen in Table 6

From the analysis, it could be seen in the linear part that the P-value on power was 0.034, where this value was smaller than ($\alpha = 0.05$), which means the power variable had a significant effect on the value of % theaflavin produced. Furthermore, the time and feed:solvent ratio variables showed a P-value of 0.115 and 0.095, respectively, where these values were greater than ($\alpha = 0.05$), which means that time and feed:solvent ratio did not significantly affect the value of % theaflavins produced. Finally, the overall model's P-value was 0.091, where the P-value was > 0.05 . This means that the factorial design regression test was also considered not to be able to represent all of the existing data.

Then, the regression equation without units can be written as follow:

$$\% \text{ Theaflavin} = 1.2060 + 0.2604 \text{ Power} + 0.0771 \text{ Time} - 0.0939 \text{ Feed:Solvent Ratio} - 0.0259 \text{ Power*time} - 0.0225 \text{ Power*Feed:Solvent Ratio} + 0.0023 \text{ Time*Feed:Solvent Ratio}.$$

4. Conclusion

This study discussed the optimization process on microwave-assisted extraction (MAE) of theaflavin from black tea with the effect of power, time, and feed solvent ratio. It can be concluded that the value of the theaflavin extract under operating conditions at 400 W, 8 mins, and feed:solvent ratio of 1:30 resulted in the highest theaflavin value of 1.61775% with the farthest point of

Table 6. Analysis of variance from regression factorial design of % theaflavin.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	0.670171	0.111695	70.60	0.091
Linear	3	0.660725	0.220242	139.21	0.062
Power	1	0.542622	0.542622	342.99	0.034
Time	1	0.047509	0.047509	30.03	0.115
Feed: Solvent Ratio	1	0.070594	0.070594	44.62	0.095
2-Way Interactions	3	0.009447	0.003149	1.99	0.470
Power*Time	1	0.005356	0.005356	3.39	0.317
Power*Feed:Solvent Ratio	1	0.004050	0.004050	2.56	0.356
Time*Feed:Solvent Ratio	1	0.000041	0.000041	0.03	0.899
Error	1	0.001582	0.001582		
Total	7	0.671753			

0.5208. The most influential independent variable in the theaflavin extraction process was the power of the microwave. Power acted as a driving force to break down the structure of the black tea cell membrane so the theaflavin compounds could diffuse out and dissolve in the solution. This proved that the higher the power used, the higher the yield of theaflavin obtained. R-square adjust value was 98.35% which implies that 98.35% of the value of the dependent variable was influenced by the independent variables contained in the study (power, time, and feed:solvent).

Conflict of interest

The authors state no conflicts of interest and have received no payment in preparation of this manuscript.

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