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Simultaneous Caffeine Extraction and Enzymatic Inactivation through Microwave Thermobiochemical Process

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Abstract

Indonesia is known as one of the countries producing tea with high bioactive compounds such as caffeine, phenol, and catechin. This study evaluated the effectiveness of microwave-based thermobiochemical methods to extract the three compounds while simultaneously inactivating enzymes. The extraction process was designed using the Factorial Design method, involving variations in the feed:solvent ratio (1:22 and 1:32), temperature (110 °C and 130 °C), and time (10, 20, and 30 minutes). The results showed that catechin had the highest effect of 5.85 with a probability of 35.87%, followed by phenol (5.18; 59.68%) and caffeine (4.51; 54.47%). The normal probability plot graph indicated a distribution of effects approaching normal with R^2 of 0.9458 (catechin), 0.9395 (phenol), and 0.7946 (caffeine), respectively. Meanwhile, the optimization graph shows a perfect linear relationship (R^2 = 1), with optimal levels of 17.18 mg/L (catechin), 9.36 mg/L (phenol), and 9.77 mg/L (caffeine), respectively. Thus, this method is proven to be efficient and has the potential to be further developed for sustainable natural material processing industry applications. The variables were designed using the Factorial Design method.

Introduction

Tea is a universally consumed beverage after water (Wahyuningsih, 2019) and is the most popular beverage in many countries and in various levels of society, due to its unique taste and health benefits (Corbo et al., 2014; Ashurst, 2016). One of the agricultural commodities in Indonesia that is widely found is produced from tea plant shoots (Camellia sinensis) (Fajar, et al., 2018). This is supported by data on per capita tea consumption in Indonesia of 1,007 pounds or ranked 22nd in the world. Based on these data, it illustrates that Indonesian people like to drink tea.

One of the plants that grows abundantly in the mountains of Asia is tea which is the most popular drink in the world because of its taste, aroma, and health benefits. With 6% of global tea exports, Indonesia is one of the fifth largest tea producing and exporting countries, behind India, China, Sri Lanka, and Kenya (Martono and Martono, 2012). The Camellia sinensis (L.) Kuntze tea plant consists of two types, namely the sinensis and assamica varieties. The sinensis variety is widely planted in China with small leaves, and the assamica variety comes from the young shoots of the plant. This variety is widely used for the production of green tea and is resistant to cold weather. In addition to the sinensis variety, the assamica variety is widely planted in hot India. This variety is suitable for black tea production because it has a tall tree with wide leaves (Ahmed & Step, 2013). Tea (Camellia sinensis (L.) Kuntze) is divided into three main groups based on the processing process, namely green tea, oolong tea,

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and black tea. Green tea (Camellia sinensis (L.) Kuntze) is a tea product that does not undergo fermentation when processed. By inactivating the oxidase and phenolase enzymes in fresh tea leaf shoots, the fermentation process can be prevented. An effective way to do this is by heating the tea leaf shoots using hot steam or by cooking. This can prevent enzymatic oxidation of catechin compounds. In addition, many Asians, especially Chinese and Japanese, consume this type of tea.

Basically, tea is divided into three main groups, namely green tea, oolong tea, and black tea which are distinguished based on their oxidation and fermentation levels (Cionca et al., 2024). More than one quarter of the world's tea is processed into green tea. Green tea is the oldest type of tea whose processing begins with picking green tea leaves and is heated as quickly as possible with steam to deactivate enzymes, then dried. Thus the fermentation process can be prevented. Green tea contains various chemical components, including polyphenols, fluoride, vitamin K, caffeine, and minerals. Green tea contains a lot of polyphenols (Saeed et al. 2017) which are compounds that have antioxidant content (Sundari et al., 2009) including catechins which are included in the flavonoid group.

Antioxidants are essential for maintaining the body's immune system in good condition. Antioxidants are compounds that can absorb or neutralize free radicals, thus preventing degenerative diseases such as cardiovascular, cancer, and other diseases. The body needs antioxidant compounds to neutralize free radicals and prevent damage to normal cells, proteins, and fats.

Catechin is one of the main compounds that determine the quality of tea leaves with a flavan-3-ol framework (Cioanca et al., 2024). Dry tea leaves can contain around 42% polyphenol compounds in the form of catechins (Mastur et al., 2023). Catechin is a colorless compound, gives a bitter taste, is soluble in water, and is astringent. Catechin compounds that are composed of green tea include picatechin (EC), picatechin-3- gallate (ECG), epigallatocatechin (EGC), and epigallocatechin-3 gallate (EGCG) (Zeniusa & Ramadhian, 2017). According to Pratiwi (2017), reported that green tea has the highest total phenol content of 88.3170 mg GAE / g sample compared to black tea and rosella tea, so it has better bioactive abilities. Phenolic compounds have a strong and positive correlation with the antioxidant potential of plants. To obtain efficacious compounds, a pure green tea extraction process is required. The selection of extraction methods and types of solvents is needed to produce maximum active compounds (Gupta et al., 2012; Joana Gil-Chávez et al., 2013).

Extraction is one of the chemical separation techniques to separate compounds from a sample using an appropriate solvent (Abubakar & Haque, 2020). The purpose of natural material extraction is to extract chemical components contained in natural materials. Active ingredients such as antimicrobial compounds and antioxidants found in plants are generally extracted with solvents. Various extraction methods can be used, one of which is the conventional method, but the MAE method has many advantages compared to other extraction methods, namely shorter extraction time, producing greater yields, lower energy consumption and more cost-effective due to reduced solvent usage. MAE (Microwave Assisted Extraction) is an extraction method assisted by high-frequency electromagnetic waves with a frequency range of 0.3 to 300 GHz. The MAE method is different from conventional extraction methods. In conventional extraction methods, heat penetrates slowly from the outside into a material, while in the MAE method, heating occurs right at the core of the material being heated and heat spreads from the inside to the outside of the material (Lopez-Avila, 2000).

The principle of the MAE method is based on the direct effect of microwaves on the molecules of the material. The transformation of electromagnetic energy into heat energy occurs by two mechanisms, namely ionic conduction and dipole rotation, both in the solvent and the sample.

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In many applications, these two mechanisms occur simultaneously because they effectively convert microwave energy into heat energy (Michel et al., 2013).

In a study conducted by Saleem et al. (2024) regarding the extraction of caffeine and catechin compounds using the Microwave Assisted Extraction (MAE) and Ultrasonic Assisted Extraction (UAE) methods from green tea leaves. The analysis used to calculate the levels of phenol and catechin compounds was HPLC, TP. From this study, phenol was produced as much as 56 mg/g and the best catechin yield was 90% at optimal conditions of 7.8 minutes, temperature 65oC, power 180 Watts, and solvent ratio of 1:40. In addition, Fujioka et al. (2022) have also conducted research on the optimization of catechin extraction from green tea leaves using the Microwave Assisted Extraction (MAE) and Ultrasonic Assisted Extraction (UAE) methods. The solvents used were nine combinations of green solvents and analysis of compound results using HPLC-UV and GraphPad Prism. The results of the study showed that the catechin compound content was 54% at an optimal temperature of 60-80oC, extraction time of 5 minutes and a wavelength of 275 nm.

Furthermore, based on the research of Sökmen et al. (2018) regarding the optimization of caffeine and catechin extraction from green tea biomass using the optimized Supercritical Fluid Extraction (SFE) method, 2.90% catechin compound was obtained at an optimum temperature of 60oC, a pressure of 250 MPa for 3 hours using ethanol solvent with a flow rate of 0.5 mL/min. Although the yield of catechin extract is low, this method seems to be quite selective for catechin.

Therefore, in the research that I will do, namely the extraction of catechins and phenols from caffeine-free green tea raw materials using the Microwave Assisted Extraction (MAE) process. The research variables will be designed using the factorial design method then the results of catechins and phenols will be analyzed with a UV-Vis spectrophotometer where in previous studies this analysis has not been done. The extraction process with microwaves Microwave Assisted Extraction (MAE) is one of the best alternatives with higher recovery and quality to replace the conventional extraction process because it is more efficient. With this research innovation, it is expected to determine the content of catechin and phenol compounds in green tea from the factors of temperature, time, solvent ratio: solid material to the extraction method used.

Methods

The implementation time for applied research is carried out from the date of issuance of the research permit within a period of approximately 1 month for data collection and data processing. The tools used in applied research include a set of Microwave Assisted Extraction (MAE) tools, separating funnels, containers/trays, test tubes, beakers, Erlenmeyers, digital scales, scissors, measuring cups, extraction flasks, suction balls, volume pipettes, droppers, cuvettes, and UV-Vis spectrophotometers. The main ingredients used are green tea leaves (Camelia sinensis L.) from PT. Berbagai Rumpun Sari Medini (Indonesia) as much as 120 grams, Folin Ciocalteu, Sodium carbonate, Iron (III) chloride, Aquades, Chloroform, Hydrochloric Acid, Lead and Sulfuric Acid obtained from e-commerce and Indrasari Chemical Store, Semarang, Central Java.

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the microwave, connecting it to the three-neck flask and condenser then connecting it to the cooling water condition on at a time variable of 4 minutes and 8 minutes. Setting the temperature at certain conditions (70 ° C and 80 ° C) to achieve the desired temperature requires a heating time of several minutes and a power of 300 Watts.When the temperature reaches the desired condition, the extraction process begins at (t=0). After the extraction process is complete, put the extract filtrate into the Erlenmeyer to separate the filtrate and residue. The sample produced from this separation process will be tested using UV-Vis spectrophotometry. After the experiment is complete, turn off the microwave, clean the equipment used, and put the equipment back in its place. The filtrate from the separation was analyzed using a UV-Vis Spectrophotometer. Take 1 mL of extract then add 0.5 mL of Folin Ciocalteu to the test tube and homogenize. Let it stand for 5 minutes then add 1.5 mL of Na2CO3.

After standing for 30 minutes, measure the phenol absorption value using a UV-Vis spectrophotometer at a maximum absorption wavelength of 765 nm (Paramita V, 2020). Take 4 mL of the tea solution sample using a measuring pipette. Place 4 mL of the tea sample into a 150 mL separating funnel. Pipette 2 mL of chloroform then put it in a beaker. Put chloroform into a separating funnel to wash the tea solution sample (shake 3x) until homogeneous. Separate the bottom layer into an Erlenmeyer flask. The separation process is carried out 4 times to remove caffeine pigments and other polar impurities. Take a sample of tea that has been washed using chloroform and then put it in a test tube. Then the sample goes into the incubator to see the absorbance value. The sample solution and standard catechin solution will be scanned at a wavelength of 280 nm using a 1 cm cuvette by UV-Vis spectrophotometry. Then the sample is compared with the standard wavelength (Nur Syamsu, 2020). The initial step is to calculate the factorial design on the effect of time and temperature on the content of phenol, catechin and caffeine. The second step is to conduct an assessment with a comparison graph of experimental data on phenol, catechin and caffeine levels. And the final step is to calculate the predicted value of the optimum phenol, catechin and caffeine content at the critical value of temperature and time. If there is the best sample, further testing can be carried out with High-pressure liquid chromatography.

Results and Discussion

UV-Vis Spectrophotometry Analysis

After the extraction process is complete, the sample is tested using UV-Vis Spectrophotometry to determine the levels of phenol, caffeine and catechin compounds contained in it. Before the test is carried out, the absorbance and maximum wavelength of the standard solution of phenol, caffeine and catechin need to be measured first. This data will be used as a comparison in determining the levels of phenol, caffeine and catechin in the extracted sample.

According to research conducted by Nugraheni & Mahdi (2022) the maximum wavelength in determining phenol content is 765 nm. According to research conducted by Lestary & Amaria (2023) the maximum wavelength in determining caffeine content is 272 nm and according to research conducted by Bronner & Beecher (1998) the maximum wavelength in determining catechin content is 280 nm. Then from the results of absorbance measurements from the maximum wavelength of standard solutions of phenol, caffeine and catechin, the two research results made a calibration curve of phenol, caffeine and catechin to obtain a linear regression equation $y = bx \pm a$ which is used to determine the concentration of phenol, caffeine and catechin solutions (x) tested. By using a wavelength of 765 nm for phenol, 272 nm for caffeine and 280 nm for catechin, the coefficient of determination value (R^2) of 0.9827 (phenol), 0.9968 (caffeine) and 0.9954 (catechin). In this study, wavelengths of 765 nm, 272 nm and 280 nm were used to determine the levels of phenol, caffeine and catechin from the extraction

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results because at these wavelengthsR^2which is produced closer to 1. Then there is a calibration curve used as a comparison in measuring the levels of phenol, caffeine and catechin compounds in green tea leaves. The calibration curves of the standard solutions of phenol, caffeine and catechin are shown in Figure 1, figure 2 and figure 3.

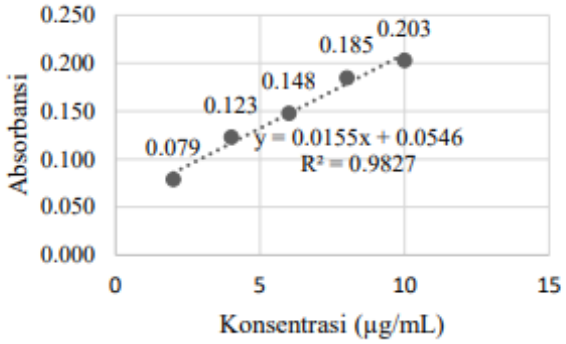

Figure 1. Phenol Standard Calibration Curve (Nugraheni et al., 2022)

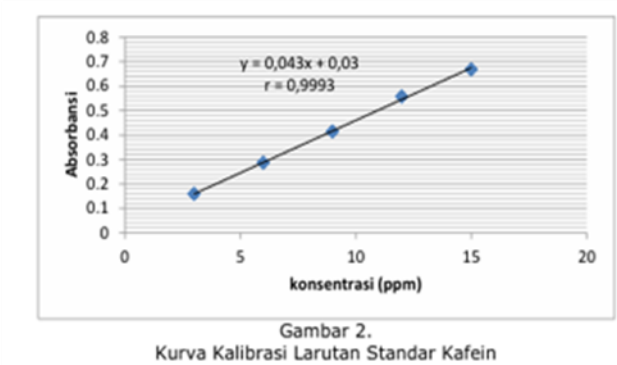

Figure 2. Caffeine Standard Calibration Curve (Lestary et al., 2023)

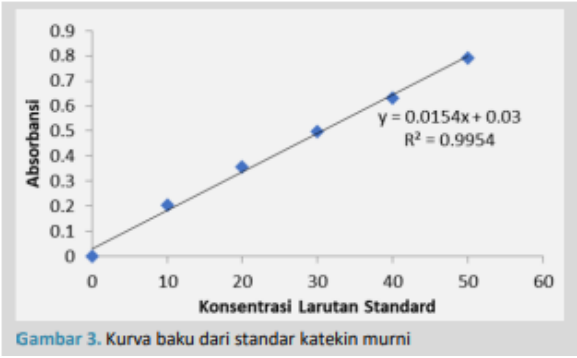

Figure 3. Standard Calibration Curve of Catechin (Nur Syamsu et al., 2020)

Calibration curves of standard phenol solutions (Figure 1), standard caffeine solutions (Figure 2) and standard catechin solutions (Figure 3) show that the relationship between solution concentration (ppm) and the absorbance obtained is in accordance with Lambert Beer's law. Based on Lambert Beer's law, absorbance is a measure of how much light can be absorbed by the sample being tested. Thus, if the sample concentration is higher, it will cause very little light to pass through the sample due to the density of the ion content in the sample, which will cause the absorbance value to be greater. Likewise, when the sample concentration is lower, it will cause light to pass through all parts of the sample and cause the absorbance value to be small. Based on the calibration curves of standard phenol solutions (Figure 4.1), catechin (Figure 4.2) and caffeine (Figure 4.3), a linear regression equation is obtainedy = 0.0155x + 0.0546 with the valueR^2=0.9827, y = 0.0154x + 0.03 with a value ofR^2= 0.9954 and y =

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49.668x – 16.536 with the valueR^2= 0.9968. This equation was used in determining the levels of phenol, catechin and caffeine from green tea leaves in this study.The calculation of phenol, catechin and caffeine levels from the extraction of ginger dregs containing treatment before extraction, namely the addition of UVB light, is shown in Table 1, Table 2 and Table 3.

Table 1. Calculation of Phenol Content of Green Tea Extract Results

Run	Solvent : Feed Ratio (v/b)	Temperature (°C)	Time (minutes)	Absorbance	Phenol Content (ppm)
1	1:10	70	4	0.095	2.60
2	1:15	70	4	0.120	4.19
3	1:10	80	4	0.130	4.84
4	1:15	80	4	0.110	3.55
5	1:10	70	8	0.175	7.74
6	1:15	70	8	0.160	6.77
7	1:10	80	8	0.205	9.69
8	1:15	80	8	0.190	8.71

Based on the data obtained, the phenol content produced in the extraction is influenced by the solvent to feed ratio, temperature, and extraction time. In general, the results show that increasing temperature and extraction time tend to increase the extracted phenol content, although not always linearly. From the data, it can be seen that increasing the solvent:feed ratio from 1:10 to 1:15 with the same extraction time at 70°C and 80°C for 4 minutes does not always result in a significant increase in phenol content. For example, at conditions of 1:10, 70°C, 4 minutes, the phenol content produced was 2.60 ppm, while at conditions of 1:15, 70°C, 4 minutes, the phenol content increased to 4.19 ppm. However, at a temperature of 80°C for 4 minutes, the phenol content at a ratio of 1:10 was 4.84 ppm, while at a ratio of 1:15 it actually decreased to 3.55 ppm. This suggests that although higher solvent ratios can increase phenol solubility, there may be dilution effects or partition imbalances that cause the phenol content in the solvent not to increase proportionally.

When the extraction time was extended to 8 minutes, the increase in phenol content was more significant. At temperatures of 70°C and 80°C with a solvent:feed ratio of 1:15, the phenol content increased to 8.71 ppm compared to a ratio of 1:10 which produced the highest phenol content of 9.69 ppm. This shows that longer extraction times allow more phenol to dissolve in the solvent, although increasing the solvent ratio does not always provide optimal results. The longer the contact time between the solvent and the feed, the greater the possibility of phenol moving from the solid matrix into the solution, in accordance with the Fick diffusion theory which states that mass transfer increases with increasing contact time and diffusion surface area. However, if the extraction time is too long, solvent saturation can occur which causes the extraction efficiency to decrease.

From the results obtained, the optimal conditions for phenol extraction are at a solvent:feed ratio of 1:10, a temperature of 80°C, and an extraction time of 8 minutes, with the highest phenol content of 9.69 ppm. These results are consistent with the extraction theory which states that increasing temperature can increase the solubility of phenol in solvents and accelerate the diffusion rate (Rahman et al., 2020). In addition, longer extraction times provide optimal results if accompanied by a solvent ratio that is not too large to avoid the dilution effect. The temperature factor also has a significant effect on extraction, because higher temperatures can accelerate the release of phenol from the material through the mechanism of increasing the kinetic energy of the molecules. However, at temperatures that are too high

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or too long, there is a possibility of degradation of phenolic compounds which can reduce extraction results.

In theory, the extraction of phenol from a solution is influenced by the principle of partition equilibrium, where the greater the solvent to feed ratio, the more phenol can be transferred into the solvent phase. However, if the solvent ratio is too large, the concentration of phenol in the solvent becomes more dilute, which can reduce the extraction efficiency. In addition, Fick's diffusion theory states that the extraction rate is influenced by the concentration gradient between the feed and solvent, which increases with increasing solvent ratio up to a certain point before saturation occurs. Therefore, optimization of the solvent ratio, temperature, and extraction time is very important in obtaining the best results without wasting materials and energy.

Table 2. Calculation of Caffeine Content of Green Tea Extract Results

Run	Solvent: Feed Ratio (v/b)	Temperature (°C)	Time (minutes)	Absorbance	Caffeine Content (ppm)
1	1:10	70	4	0.120	2.09
2	1:15	70	4	0.160	3.02
3	1:10	80	4	0.180	3.49
4	1:15	80	4	0.140	2.56
5	1:10	70	8	0.310	6.51
6	1:15	70	8	0.275	5.70
7	1:10	80	8	0.420	9.07
8	1:15	80	8	0.450	9.77

The results showed that caffeine levels did not always increase linearly, which could be due to various factors such as dissolution efficiency and the solubility limit of caffeine in the solvent used. In general, increasing temperature and extraction time tended to increase caffeine levels, but there were some conditions that showed less than optimal results.

For example, sample 4 (1:15, 80°C, 4 minutes) has a lower caffeine content (2.56 ppm) compared to sample 3 (1:10, 80°C, 4 minutes) which reaches 3.49 ppm. This could be due to the dilution effect due to the larger solvent ratio, which reduces the concentration of caffeine in the solution. On the other hand, at longer extraction times, such as sample 7 (1:10, 80°C, 8 minutes) and sample 8 (1:15, 80°C, 8 minutes), the caffeine content is higher, reaching 9.07 ppm And 9.77 ppm, indicating that high temperatures and longer extraction times allow for maximum caffeine release.

Based on the data, the optimal condition was obtained in sample 8 (1:15, 80°C, 8 minutes) with the highest caffeine content of 9.77 ppm. This shows that increasing temperature and extraction time can increase caffeine release. Extraction theory states that higher temperatures increase solute diffusion, while longer extraction times allow more caffeine to be released from the sample matrix. However, if the extraction time is too long or the temperature is too high, caffeine degradation can occur, so it is important to find the optimal balance.

In conclusion, optimization of extraction parameters is very important to get the best results. If the main goal is to get high caffeine content, then the conditions 1:15, 80°C, 8 minutes is the most optimal, while still taking into account the possibility of compound degradation at too high a temperature.

Table 3. Calculation of Catechin Content from Green Tea Leaf Extraction

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Run	Solvent : Feed Ratio (v/b)	Temperature (°C)	Time (minutes)	Absorbance	Catechin Content (ppm)
1	1:10	70	4	0.125	6.16
2	1:15	70	4	0.185	10.06
3	1:10	80	4	0.155	8.12
4	1:15	80	4	0.220	12.34
5	1:10	70	8	0.250	14.29
6	1:15	70	8	0.200	11.04
7	1:10	80	8	0.315	18.51
8	1:15	80	8	0.280	16.23

Based on the data obtained, the levels of catechins produced in the extraction were influenced by the ratio of solvent to feed, temperature, and extraction time. Variations in extraction parameters resulted in different levels of catechins, indicating that certain extraction conditions could improve the results obtained.

In terms of solvent:feed ratio, increasing from 1:10 to 1:15 did not always result in higher catechin levels. At 70°C for 4 minutes, catechin levels increased from 6.16 ppm (1:10 ratio) to 10.06 ppm (1:15 ratio). However, at 80°C for 8 minutes, catechin levels were actually higher at a 1:10 ratio (18.51 ppm) compared to a 1:15 ratio (16.23 ppm). This suggests that a higher solvent ratio can increase catechin solubility under certain conditions, but if there is too much solvent, a dilution effect can occur which causes a decrease in the concentration of catechin in the extract solution.

Extraction temperature also plays a role in determining the amount of catechin extracted. At a ratio of 1:10 and an extraction time of 4 minutes, increasing the temperature from 70°C to 80°C causes the catechin content to increase from 6.16 ppm to 8.12 ppm. The same thing also happens at a ratio of 1:15, where the catechin content increases from 10.06 ppm to 12.34 ppm. Increasing the temperature can increase the solubility of catechin in the solvent and accelerate the mass transfer from the sample matrix into the extract solution.

In addition, the extraction time has a significant effect on the levels of catechin obtained. At a temperature of 70°C with a ratio of 1:10, an extension of time from 4 minutes to 8 minutes increases the levels of catechin from 6.16 ppm to 14.29 ppm. An increase in catechin levels also occurs in other conditions when the extraction time is extended, although in some conditions, such as a ratio of 1:15 and a temperature of 70°C, the levels of catechin actually decrease slightly after 8 minutes. This may occur due to catechin degradation that may occur if the extraction time is too long.

Based on the results obtained, the extraction conditions that produced the highest catechin content were at a solvent:feed ratio of 1:10, a temperature of 80°C, and an extraction time of 8 minutes, with a catechin content of 18.51 ppm. This condition shows that although increasing temperature and extraction time tends to increase catechin content, a larger solvent ratio does not always provide the best results because it can cause a dilution effect or degradation of the active compound. Therefore, in catechin extraction, a balance is needed between the solvent ratio, temperature, and extraction time to obtain optimal results without causing a decrease in catechin content due to excessive extraction conditions.

Data analysis

In this study, data analysis was processed using the factorial design method.

Table 4. Results of Calculation of Main Effects and Interactions on Phenol Levels

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Effect	Effect Value
A	-0.50
B	1.00
C	5.18
AB	-0.72
air conditioning	0.26
BC	-0.83
A B C	-0.19

Table 5. Results of Calculation of Main Effects and Interactions on Caffeine Levels

Effect	Effect Value
A	0.18
B	1.18
C	4.51
AB	-0.40
air conditioning	0.64
BC	1.35
A B C	-0.02

Table 6. Results of Calculation of Main Effects and Interactions on Catechin Levels

Effect	Effect Value
A	-0.86
B	3.16
C	5.85
AB	-2.32
air conditioning	-0.62
BC	-1.88
A B C	1.62

Based on the results of the analysis using the factorial method, it was obtained that each variable of the solvent to feed ratio, extraction temperature, and extraction time had a different effect on the levels of phenol, caffeine, and catechin produced.

OnTable 4 The most influential factors in the phenol extraction process are extraction time (C) with the largest effect value of 5.18, followed by extraction temperature (B) with an effect value of 1.00. This shows that the longer the extraction time and the higher the temperature used, the more phenol is extracted. Meanwhile, the solvent to feed ratio (A) has a negative effect with an effect value of -0.50, indicating that increasing the solvent ratio does not significantly increase the phenol content obtained. According to Gil-Martín et al. (202), temperature and extraction time play an important role in increasing phenol release from natural materials because higher temperatures can increase phenol solubility and accelerate the diffusion rate.

In Table 5, the most influential factor on caffeine content is extraction time (C) with an effect value of 4.51, followed by extraction temperature (B) which has an effect value of 1.18. This shows that longer extraction duration and higher temperature allow more caffeine to dissolve in the solvent. The solvent to feed ratio factor (A) has a smaller effect of 0.18, indicating that changes in the solvent ratio in this range do not significantly affect caffeine content. Memon & Idress (2024) explained that higher temperatures can reduce solvent viscosity and increase

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mass transfer rates, thereby accelerating caffeine extraction. However, temperatures that are too high can also cause degradation of active compounds, so a balance is needed in determining optimal extraction parameters.

In Table 6, extraction time (C) is again the most dominant factor in increasing catechin levels with an effect value of 5.85, followed by extraction temperature (B) with an effect value of 3.16. These results indicate that the longer the extraction process, the higher the catechin levels obtained, and increasing temperature also helps accelerate the release of catechins into the solvent. However, the solvent to feed ratio (A) has a negative effect with an effect value of -0.86, which means that increasing the solvent ratio does not always increase the levels of extracted catechins. According to Cioanca et al. (2024), catechins tend to be more soluble in solvents at high temperatures due to the increased kinetic energy of the molecules which accelerates the rate of mass transfer. However, temperatures that are too high can also cause oxidation of catechins, which can reduce extraction yields (Li et al., 2011).

Overall, extraction time and extraction temperature have a greater influence than the solvent to feed ratio in the extraction process of phenol, caffeine, and catechin. This can be seen from the calculation of the main effects which show that these two variables produce more significant changes in compound levels compared to changes in the solvent ratio. Increasing the optimal extraction temperature and time can increase the extraction yield, but it should be noted that extreme extraction conditions can cause degradation of the target compound.

Table 7. Determination of Variables Influencing Phenolic Compounds

Effect	P (%)
0.19	2.19%
0.26	2.99%
0.50	5.76%
0.72	8.29%
0.83	9.56%
1.00	11.52%
5.18	59.68%

Table 8. Determination of Variables Influencing Caffeine Compounds

Effect	P (%)
0.02	0.24%
0.18	2.17%
0.40	4.83%
0.64	7.73%
1.18	14.25%
1.35	16.31%
4.51	54.47%

Table 9. Determination of Variables Influencing Catechin Compounds

Effect	P (%)
0.62	3.80%
0.86	5.27%
1.62	9.93%
1.88	11.53%
2.32	14.22%

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3.16	19.37%
5.85	35.87%

Based on the results of the analysis using the factorial method, each compound has the most influential variable in its extraction process. Normal Probability Plot (P vs Effect) graph to identify which point gives the greatest "effect". The optimal point for the results is determined based on the highest Effect value, not how far the point is from the regression line.

In Table 7, the variable that most influences phenol content is extraction time, with an effect value of 5.18 and a P percentage of 59.68%. This shows that the longer the extraction time, the higher the phenol content obtained. Increasing the extraction temperature also contributes to phenol extraction, although in a smaller percentage compared to time. This is in accordance with the research of Li et al. (2020) which states that longer extraction times allow more phenol to be released from the cell matrix due to increased interactions between the solvent and phenol compounds. In addition, higher temperatures can accelerate the diffusion rate and increase the solubility of phenol in the solvent.

In Table 8, the variable that has the greatest influence on caffeine content is extraction time, with an effect value of 4.51 and a P percentage of 54.47%. This shows that the longer the extraction time, the more caffeine can be extracted into the solvent. Other factors such as temperature also have an influence, but not as much as extraction time. According to Memon & Idress (2024), longer extraction times provide more opportunities for caffeine to diffuse from the material into the solvent. However, too long extraction can cause caffeine degradation due to excessive heat exposure, so a balance is needed in determining the optimal extraction time.

In Table 9, the most influential factor on catechin levels is extraction time, with an effect value of 5.85 and a P percentage of 35.87%. Another factor that is quite influential is extraction temperature, with an effect value of 3.16 and a P percentage of 19.37%. This shows Cioanca catechins are more easily extracted in longer times and higher temperatures. According to Lee et al. (2024), catechin compounds tend to be more soluble in solvents at high temperatures, due to the increased kinetic energy of the molecules which accelerates mass transfer. However, temperatures that are too high can also cause catechin degradation due to oxidation or hydrolysis, so a balance is needed between temperature and extraction time to obtain optimal results (Li et al., 2011).

Overall, the extraction time variable has a dominant influence on the levels of the three compounds, especially in increasing the release of active compounds from the material into the solvent. Extraction temperature also makes a significant contribution, especially in increasing the solubility of the compound. Meanwhile, the solvent to feed ratio has a smaller influence than the other two variables. Therefore, optimization is important to obtain maximum extraction results.

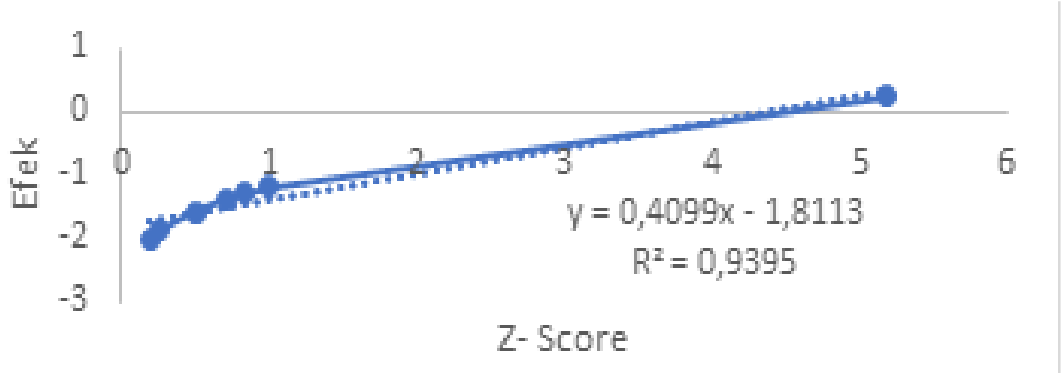

Figure 4. Normal Probability Plot (P vs Effect) For Phenol Compounds

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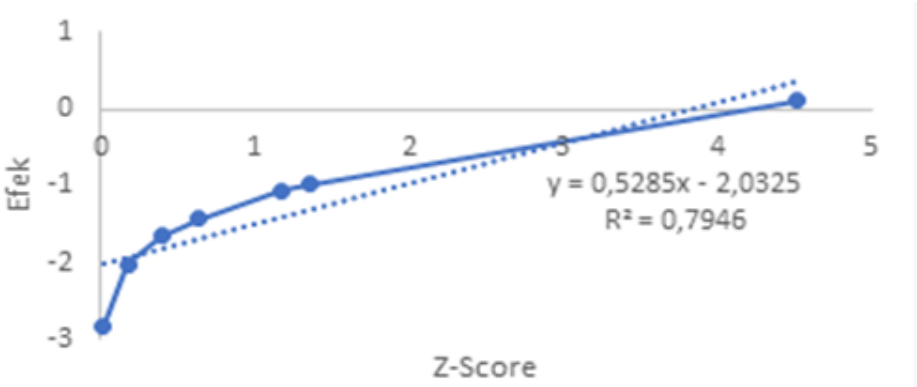

Figure 5. Normal Probability Plot (P vs Effect) For Caffeine Compound

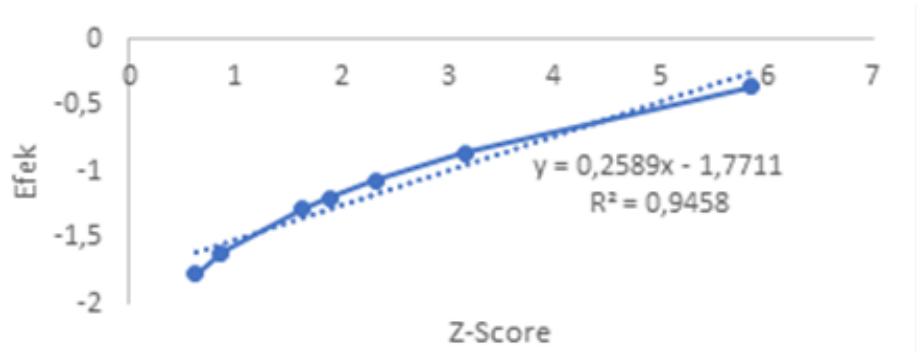

Figure 6. Normal Probability Plot (P vs Effect) For Catechin Compounds

Normal Probability Plot(P vs Effect) for phenolic compounds in a series of factorial design experiments. This graph plots the treatment effect values against a normal probability distribution (Z-score). The linear regression line on the plot shows the tendency of the data to follow a normal distribution. In the context of this analysis, treatments whose points are close to or along the regression line are the ones that have the most effect or are significant to the response (following a normal distribution). Conversely, points that are far from the line are considered to have small or statistically insignificant effects.

Based on Figure 4, it can be seen that most of the data points follow the dotted regression line pattern which represents the normal distribution. The regression line has the equation$y$= 0.4099x− 1.8113 with a coefficient of determinationR^2=0.9395, indicating that 93.95% of the variation in the effect can be explained by the normal distribution model. The point that is considered the most optimal for the normal distribution is the point that is closest to the regression line, because it shows that the effect value at that point has a distribution that is closest to normal. Based on the data and graphs, the point with an effect of 0.83 (P = 9.56%) or 1.00 (P = 11.52%) is the most optimal point because its position is in the middle of the distribution and very close to the regression line. In contrast, the point with an effect of 5.18 which has a P of 59.68% looks quite far from the line, and is likely an outlier that does not follow a normal distribution pattern.

According to Knief & Forstmeier (2021), Normal Probability Plot (P vs Effect) is useful in analyzing the accuracy of the model and detecting data that deviates from the general pattern. In addition, this effect analysis is very important in factorial design to assess the significance of the treatment. Therefore, the treatment with an effect of 0.83 (P = 9.56%) in Figure 5 can be used as a reference in optimizing the phenol compound extraction process, because it is proven to provide the greatest contribution compared to other treatments.

Meanwhile, Figure 4.5 shows the Normal Probability Plot (P vs Effect) for the caffeine compound. The equation of the regression line formed is y = 0.5285x−2.0325 with a coefficient of determination value ofR^2= 0.7946, which means that about 79.46% of the

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variation in the effect data can be explained by the normal distribution model. In the graph, the points closest to the dotted line (regression line) indicate the data that best fits the normal distribution pattern. The most optimal point for the normal distribution is the point with an effect of 1.18 and a P of 14.25%, because its position is closest to the regression line. In contrast, the point with an effect of 4.51 (P = 54.47%) appears furthest from the line, indicating that it deviates from the normal distribution and can be categorized as an outlier. Therefore, the point with an effect of 1.18 is the best representation in the normal distribution of caffeine compounds.

In Figure 6, based on the graph, the Normal Probability Plot (P vs Effect) is shown between the effect value and P (%) for the catechin compound. Based on the graph, the regression equation is obtained y = 0.2589x − 1.7711 with a coefficient of determination value ofR^2= 0.9458. The value is shows that the normal distribution model explains about 94.58% of the variation in the data, so it can be said that the data has a very good level of conformity to the normal distribution.

The point that has the highest proximity to the regression line is the data with an effect value of 2.32 and P of 14.22%, which indicates that at that point the catechin compound shows the most optimal and statistically consistent effect. Meanwhile, the point with the highest effect value, which is 5.85 (P = 35.87%), seems to deviate quite far from the line, which means that its effect tends not to represent the general pattern of normal distribution. This indicates that the highest effectiveness is not necessarily the most stable or consistent in terms of data distribution.

The accuracy of a research model can be seen from the coefficient of determination (R^2) value, which indicates the extent to which data variation can be explained by the regression model created. In this study, the R^2 value for phenol compounds was 0.9395, which means the model explains 93.95% of the total data variation. For caffeine compounds, the R^2 was 0.7946, indicating that the model explains 79.46% of the data variation. While for catechins, the R^2 was 0.9468, indicating that the model can explain 94.68% of the data variation. According to Novianty et al. (2023), a good regression model has an R^2 of at least 0.8 in order to be said to be able to predict responses with a high proportion of variables. Of the three compounds, the regression model for catechin extraction has the highest level of accuracy, while the model for caffeine has the lowest level of prediction.

Conclusion

Based on the research on Caffeine Extraction and Enzymatic Inactivation Simultaneously Through Microwave Thermobiochemical Process, it can be concluded that the extraction method used has high effectiveness in obtaining caffeine and catechin compounds from samples. The results of the analysis show that there is a linear relationship between compound levels and absorbance, as shown in the optimization graph of each compound.

Analysis of the effect and probability (P) values of the three compounds tested, namely phenol, caffeine, and catechin, obtained variations that reflect the extent to which each compound influences the extraction. Phenolic compounds showed effect values between 0.19 and 5.18 with P ranging from 2.19% to 59.68%, while caffeine had an effect range from 0.02 to 4.51 with P of 0.24% to 54.47%. Meanwhile, catechin recorded the highest effect of the three, with a range of 0.62 to 5.85 and the highest probability of 35.87%. In general, the greater the effect value indicates the stronger the effect of the treatment on the compound, and a higher P (%) indicates a tendency for the effect to be significant.

Through the Normal Probability Plot (P vs Effect) graph, the distribution of effects against the assumption of normality can be evaluated. Phenolic compounds in a distribution that is quite close to normal with an R^2 of 0.9395, where the highest effect point of 5.18 appears as

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the largest deviation from the regression line, indicating that certain treatments have a dominant effect on phenol levels. Caffeine shows a more widespread distribution pattern with an R^2 value of 0.7946, indicating a more significant deviation from the normal distribution. The optimal point of caffeine is at an effect of 4.51 with a P of 54.47%. Meanwhile, catechin shows the closest effect distribution fit to the regression line, with an R^2 of 0.9458, and the highest effect point reaches 5.85, making it the most influential point on distribution deviations and at the same time indicating the effectiveness of treatment on this compound.

The optimization results of each compound are shown through a linear relationship graph between absorbance and content. For phenol compounds, the graph produces a regression equation with a perfect determination coefficient $R^2 = 1$. The maximum content value is obtained at an absorbance of 0.20 which is equivalent to a content of around 9.36 mg/L. Caffeine also shows a strong linear relationship through the equation, with an R^2 value = 1, and the optimal content is achieved at an absorbance of 0.45 which gives a content of around 9.77 mg/L. Meanwhile, catechin, with an equation and $R^2 = 1$, shows the highest extraction results among the three at an absorbance of 0.31, with a content approaching 17.18 mg/L.

Overall, catechin showed the best performance in all three parameters analyzed. This compound not only produced the highest effect value, but also approached the normal distribution statistically, and provided the highest compound content from the optimization results.

Thus, the microwave thermobiochemical method applied in this study proved effective for simultaneous extraction of caffeine and enzyme inactivation. This technique allows for increased extraction efficiency with shorter time and optimal bioactive compound recovery. Further development can be done to increase the production scale and optimize the extraction process of other bioactive compounds with similar methods.

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