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Implementation of Principal Component Analysis-Cluster Analysis on The Extraction of Green Tea Leaf (*Camellia sinensis* (L.) Kuntze)

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Abstract: Several green tea leaf extraction studies only describe the results of the extraction method or the different types of solvents that produce the highest levels of polyphenols or caffeine without further analysis by statistical analysis. In addition, the statistical analysis method still often used is a statistical analysis of variance, which has weaknesses. This study used PCA and CA methods to analyze samples based on the solvent's effect on temperature and pH. The solvents used in extracting green tea leaves were hot distilled water at ±95ºC, distilled water, citrate buffer pH 4.3, and phosphate buffer pH 7.4 without heating (±25ºC). The parameters analyzed were yield, water content, total ash content, acid insoluble ash content, total polyphenol content, and caffeine in green tea leaf extract (*Camellia sinensis* (L.) Kuntze). Classification with PCA results in a 2-dimensional data reduction that represents all data. Therefore, PC1 can extract as much as 68.7% of the information, and PC2 can extract 22.9% of the information. Cumulatively, PC1 and PC2 extracted as much as 91.5% of the information. Classification with CA resulted in 3 clusters. The third cluster, namely numbers 2 and 3, is the cluster that has the closest similarity with the distance between the cluster centroids of 2.08564 and the similarity level of 56.7850%.

Keywords: CA (Cluster Analysis); green tea leaf (*Camellia sinensis* (L.) Kuntze); caffeine; chemometrics; PCA (Principal Component Analysis); polyphenols.

1. Introduction

One of the chemometric approach methods is a PCA (Principal Component Analysis) and CA (Cluster Analysis). PCA is the most frequently used method to process multivariate data with unknown samples or multiple variable analysis methods that aim to simplify the observed variables by reducing (reducing) their dimensions. At the same time, CA is a method for grouping entities (individuals and objects) into separate groups based on the similarities (similarities) between samples [1].

Many studies on green tea leaf extract only describe the results of the extraction method or the different types of solvents that produce the highest levels of polyphenols or caffeine without further analysis by statistical analysis. So that in this study, PCA and CA methods were used to analyze the similarity and correlation between the effect of temperature and pH of the extraction solvent on the yield, water content, total ash content, acid insoluble ash content, total

polyphenol content, and caffeine in green tea leaf extract and grouping the samples into several clusters.

The solvents used in extracting green tea leaves were hot distilled water at ±95°C, distilled water, citrate buffer pH 4.3, and phosphate buffer pH 7.4 without heating (±25°C). The choice of the type of solvent is based on the nature of polyphenols and caffeine. Polyphenolic compounds and caffeine tend to dissolve in polar and semipolar solvents [2,3]. Polyphenol compounds are weak acids, and caffeine compounds are weak bases. The nature of the compound will determine the form of the analyte in a certain atmosphere or environment according to the Henderson–Hasselbalch equation. If you want to obtain an ionized form, the extraction pH used for alkaline compounds is approximately 2 levels below the pKa of the analyte [4].

The analysis results using PCA and CA methods are in the form of screen plots, score plots, loading plots, biplots, and dendrograms. Based on this background, the authors are interested in researching the implementation of the use of PCA and CA methods to analyze the effect of temperature and pH of the solvent with the yield parameters, water content, total ash content, acid insoluble ash content, total polyphenol content and caffeine in the green tea leaf extract (*Camellia sinensis* (L.) Kuntze) by UV-Vis spectrophotometric method.

2. Materials and Methods

2.1. Materials and reagents.

This study used ingredients in the form of green tea leaves (*Camellia sinensis* (L.) Kuntze) obtained in Banjar Mayungan Yet, Baturiti Village, Baturiti District, Tabanan Regency, Bali, Indonesia. Citric acid; sodium citrate (Merck®); NaOH; KH_2PO_4; ethyl acetate (Brataco®); ethanol; aquadest (Brataco®); ash-free sari paper (Whatman TM); Aluminum coating; $FeCl_3$ 1%; HCl 10%; HCl 2N; LP Mayer; Dragendorff LP; LP Wagner; Folin-Ciocalteu reagent (Merck®); Na_2CO_3; gallic acid standard (Sigma); and the caffeine standard (Sigma).

2.2. Sample collection and preparation.

A total of 5 kg of fresh leaves was prepared from the plant (*Camellia sinensis* (L.) Kuntze). Wet sorting is done by selecting the shoots and three leaves below the shoots. The fresh leaf samples that had been sorted were wet and then washed thoroughly with running water. Furthermore, the weathering process was carried out at a temperature of 90°C for 8 minutes. After that, the process of rolling and drying is carried out. There are 2 drying stages; the first stage is at a temperature of 130°C for 30 minutes, followed by the second stage drying process at a temperature of 80°C for 80 minutes until dry leaves are obtained. Furthermore, the dried leaves are powdered with a blender to produce simplicia powder. Finally, the simplicia powder was sieved with a mesh sieve No. 40 to obtain a fine powder with a uniform size.

2.3. Green tea leaf extraction.

Ten grams of dry green tea powder were extracted in 100 mL of hot distilled water at a temperature of ±95°C for 20 minutes using a magnetic stirrer at 200 rpm. The temperature of the distilled water before use was controlled to ±95°C directly on a magnetic stirrer. Next, the extract solution was cooled on ice for 10 minutes and fractioned with 100 mL ethyl acetate.

The solution was shaken for 5 minutes and allowed to stand for 15 minutes until two phases were formed, namely the ethyl acetate and aqueous phases. The phase taken is the ethyl acetate phase. The tea leaf powder was re-extracted in the same way. Extraction was carried out twice. The solvent was evaporated using a water bath at 50^{o}C to obtain a dry extract. Extraction with other solvents is carried out in the same way.

2.4. Determination of extract moisture content.

Determination of water content is done by the gravimetric method. An empty weighing bottle was weighed (W1), the dry extract of green tea leaves was added and weighed 1 gram in a weighing bottle (W2), then placed in the oven at 105°C for 30 minutes, then weighed (W3). Weighing is carried out at a distance of 1 hour until the difference in 2 consecutive weighings is not more than 0.25% [5].

2.5. Determination of total ash content of extracts.

The total ash content test was carried out by weighing 1 gram of extract (W1) put in a porcelain dish that had been ignited and weighed beforehand. It is slowly ignited in a kiln (with the temperature being increased gradually to 600 ± 25^{0}C until the charcoal runs out. After that, it is cooled in a desiccator and weighed to a constant weight (W2). The total ash content is calculated [5].

2.6. Determination of acid insoluble ash content of extracts.

The ash obtained to determine the total ash content was boiled with 25 mL of concentrated hydrochloric acid for 5 minutes, collected the acid-insoluble portion separated by filtering using ash-free filter paper, and the residue was rinsed with hot water. The filtered ash and the filter paper were put back in the same silicate crucible. After that, the extract was ignited using a kiln slowly (with the temperature being increased gradually to 600 ± 25°C until the charcoal was used up. Then weighed to a constant weight (W3) [5].

2.7. Determination of total polyphenol content of green tea leaf extract (Camellia sinensis (L.) Kuntze).

Weighed 25 mg of green tea extract and dissolved in 25 mL of citrate buffer pH 4.3 to the limit mark, then diluted again in a 10 mL volumetric flask by pipetting 2.5 mL to obtain a concentration of 250 ppm. The solution with a concentration of 250 ppm was pipetted as much as 0.5 mL and put into a test tube. 5 mL of Folin-Ciocalteu 10% reagent was added, then sonicated for 5 minutes. Leave it for 5 minutes. Added 4 mL of 7.5% Na_2CO_3 and incubated for 46 minutes. The test solution was made triple. The absorbance was measured using a spectrophotometer at a wavelength of 763 nm. The concentration of polyphenols in the test solution was calculated from the calibration plot, and the total polyphenol content was expressed in the mg GAE/gram sample [5].

2.8. Determination of caffeine levels in green tea leaf extract (Camellia sinensis (L.) Kuntze).

The sample assay was carried out by weighing 10 mg of green tea extract and dissolved in 10 mL of phosphate buffer pH 7.4 to the limit mark, then diluted again in a 10 mL volumetric

flask by pipetting 0.4 mL to obtain a concentration of 40 ppm. Then sonicated for 5 minutes. Then the absorbance was measured using a UV-VIS spectrophotometer at 273 nm.

2.9. Data analysis.

Data analysis in this study was carried out with a chemometric approach using the PCA (Principal Component Analysis) and CA (Cluster Analysis) methods. Data collection for each sample was repeated three times. Data are expressed in mean ± standard deviation (SD) using Ms. Excel. Then PCA and CA chemometric analysis was carried out using Minitab software version 17 with the variables that produced the scree plot used to determine how many PCs should be taken to reduce the variable, and the score plot was used to classify and represent the closeness between samples expressed by PC1 and PC2, loading plots were used to provide an evaluation of the correlation between variables based on the angle formed between the variables used, the biplot is a combination of score plots and loading plots, and dendrograms are used to group samples into several clusters.

3. Results and Discussion

3.1. Sample preparation.

The sample used in this study was green tea leaves (*Camellia sinensis* (L.) Kuntze) obtained from Banjar Mayungan Yet, Baturiti Village, Baturiti District, Tabanan Regency, Bali, Indonesia. The dry extract obtained has organoleptic characteristics of blackish green to brownish-green. Then the thick extract that has been obtained is colled and then weighed. The weight of the extract was used to determine the yield produced from 30 g of green tea leaf powder. As is known, the yield can be determined using the following formula:

$$\%Yield = \frac{Extract\ Weight}{Sample\ Weight}\ x\ 100\%$$

From the yield formula above, the yield data obtained are shown in Table 1. The extraction results of tea leaves with hot distilled water at ±95ºC, distilled water, citrate buffer pH 4.3, and phosphate buffer pH 7.4 without heating (±25ºC) resulted. The percentages of different yields are shown in Table 1. The solvent is selected based on the solubility and stability of the target compound. The yield of extraction using hot distilled water at ±95ºC produced the highest percentage compared to other extractions, which was 12.63%. The higher the temperature and the extraction time, the higher the yield of green tea extract will be because the extraction temperature will increase the solution's kinetic energy so that the solvent's diffusion into the tissue cells also increases [6].

Table 1. Percentage of Extract Yield.

Solvents	Weight Powder (g)	Extract Weight (g)	Yield (%w/w)
Hot water temperature ±95ºC	30	3.7789	12.63
Aquades	30	3.1140	10.38
Citrate buffer pH 4.3	30	3.5592	11.86
Phosphate buffer pH 7.4	30	2.9541	9.85

Before the quantitative test was carried out on the dry extract of green tea leaves, sample preparation was first carried out to obtain a sample stock solution that would be used for the

total polyphenol and caffeine test. The total polyphenol test sample was prepared by weighing as much as 25 mg of green tea extract and dissolving in 25 mL of citrate buffer pH 4.3 to the limit mark. Then the solution was sonicated for 5 minutes. Then it was diluted in a 10 mL volumetric flask using a 2.5 mL pipette to obtain a concentration of 250 ppm; each sample was replicated 3 times. At the same time, the preparation of the caffeine test sample was carried out by weighing 10 mg of dry extract and dissolved in 10 mL of phosphate buffer pH 7.4. Then the solution was sonicated for 5 minutes. Then diluted in a 10 mL volumetric flask by pipetting 0.4 mL to obtain a concentration of 40 ppm, each sample was replicated 3 times.

3.2. Standardization of simplicia and extract quality.

Quality standardization means that simplicia or extracts to be used as raw materials must meet the qualification requirements listed in the official monograph [5]. The results of standardization of simplicia and extract quality can be seen in Table 2.

Table 2. Results of Quality Standardization of Simplicia Powder and Extract

Samples	Water Content (%w/w)	Total Ash Content (%w/w)	Acid Insoluble Ash Content (%w/w)
Simplicia Powder	2.99 ± 0.209*	-	-
Hot water temperature ±95ºC	2.21 ± 0.054*	6.32 ± 0.048*	0.67 ± 0.094*
Aquadest	6.83 ± 0.155*	6.21 ± 0.143*	0.75 ± 0.019*
Citrate buffer pH 4.3	7.47 ± 0.417*	6.42 ± 0.079*	0.65 ± 0.029*
Phosphate buffer pH 7.4	6.76 ± 0.096*	7.02 ± 0.114*	0.88 ± 0.021*

The results of testing the water content of simplicia powder and green tea leaf extract using hot distilled water at ±95ºC, distilled water, citrate buffer pH 4.3, and phosphate buffer pH 7.4 were 2.99 ± 0.209% w/w, respectively; 2.21 ± 0.054%w/w; 6.83 ± 0.155%w/w; 7.47 ± 0.417%w/w; and 6.76 ± 0.096%w/w. By the requirements of SNI 3945-2016 [7] regarding green tea, the good water content of green tea is a maximum of 8%, so the water content in simplicia powder and green tea leaf extract meets the standard.

The results of testing the total ash content of green tea leaf extract using hot distilled water at ±95ºC, distilled water, citrate buffer pH 4.3 and phosphate buffer pH 7.4 were 6.32 ± 0.048% w/w; 6.21 ± 0.1435%w/w; 6.42 ± 0.079%w/w; and 7.02 ± 0.114% w/w and the acid insoluble ash content of the extract was 0.67 ± 0.094% w/w, respectively; 0.75 ± 0.019%w/w; 0.65 ± 0.029%w/w; and 0.88 ± 0.021% w/w. By the requirements of SNI 3945-2016 [7] regarding green tea, a good total ash content of green tea is 4-8%, while the requirement for acid-insoluble ash content is a maximum of 1%, so that the total ash content and acid insoluble ash content in green tea leaf extract using different extraction solvents have met the standard.

3.3. Determination of total polyphenol content.

The determination of total polyphenol content in this study used the Folin-Ciocalteu method concerning several modifications. This method is the most frequently used method to determine the phenolic content [8,9]. TPC is expressed in mg gallic acid equivalent/g sample (mg GAE/g), the number of mg gallic acid equivalents in a 1-gram sample. In this study, the standard curve of the relationship between the standard concentration of gallic acid (x-axis) and the absorbance of gallic acid was obtained after being reacted with the Folin-Ciocalteu

reagent (y-axis) with the equation y = 0.0077x + 0.2355 and the value of the coefficient of determination (r2) = 0.9962 as shown in Figure 1.

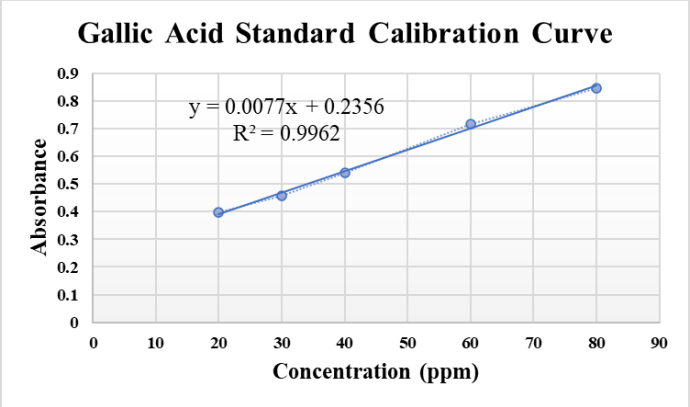

Figure 1. Gallic acid standard calibration curve.

The sample which reacts with the Folin Ciocalteau reagent produces a yellow color which indicates that it contains phenolics. After that, it is added to the Na_2CO_3 solution as an alkaline agent. During the reaction, the hydroxyl groups of the phenolic compounds react with Folin Ciocalteau, forming a blue molybdenum-tungsten complex that a spectrophotometer can detect. The blue color formed will be more concentrated, equivalent to the concentration of phenolic ions formed [10]. The results of the measurement of total polyphenol levels can be seen in Table 3.

Table 3. Results of determination of total polyphenol content of green tea leaf extract.

Solvents	Replication	Total Polyphenol Content (mg GAE/g)	Average Value of Total Polyphenol Content (mg GAE/g) ± SD
Hot water temperature ±95°C	1	257.97	250.33 ± 6.73
	2	247.79	
	3	245.24	
Aquadest	1	213.19	221.35 ± 7.06
	2	225.35	
	3	225.50	
Citrate buffer pH 4.3	1	235.06	239.75 ± 4.36
	2	240.51	
	3	243.68	
Phosphate buffer pH 7.4	1	218.23	220.36 ± 1.84
	2	221.29	
	3	221.55	

Extraction using hot distilled water at a temperature of ±95°C resulted in the highest total polyphenol content of 250.33 mg GAE/g. The results obtained are comparable to previous studies conducted by Balci and Ozdemir [11] on the extraction of green tea leaves by the brewing method using hot distilled water at a temperature of ±95°C for 20 minutes showing the highest total phenol content gain of 131.3 mg GAE/g compared to the treatment other. The extraction results using citrate buffer pH 4.3 resulted in a higher total polyphenol content value compared to phosphate buffer pH 7.4, which was 239.75 mg GAE/g. The research of Vuong *et al.* [12] stated that to minimize the degradation of polyphenols and maximize the extraction yield, the pH in the extraction process was maintained at an acidic condition, in the pH range

of 3.0-5.3. This is because the higher the pH of a solution, the greater the percentage of degraded polyphenols [13].

3.4. Determination of caffeine content.

In this study, the caffeine content test used a caffeine standard, and a standard curve of the relationship between the concentration of caffeine standard (x-axis) and caffeine absorbance (y-axis) was obtained with the equation y = 0.039 + 0.1332, and the coefficient of determination (r2) = 0.9853 as in Figure 2. Based on the research that has been done shows that the acquisition of caffeine content from each sample from different extraction solvents produces different levels. The data listed in Table 4 shows that there are differences in the caffeine content of each sample. Extraction using hot distilled water at a temperature of ±95ºC resulted in the highest caffeine content of 168.69 mg/g.

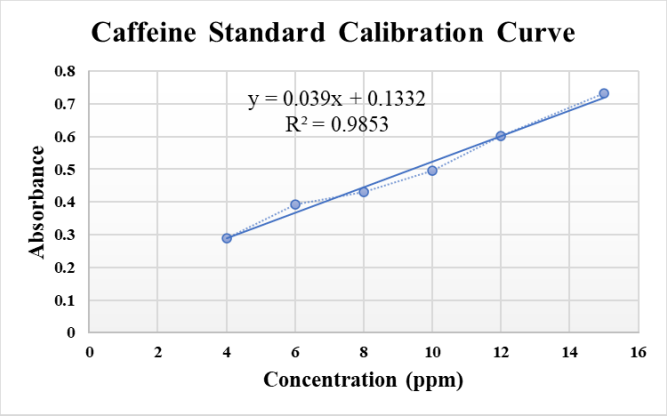

Figure 2. Caffeine standard calibration curve.

Table 4. Results of determination of caffeine contents in the green tea leaf extract.

Solvents	Replication	Caffeine Contents (mg/g)	Average Value of Caffeine Contents (mg/g) ± SD
Hot water temperature ±95ºC	1	169.62	168.69 ± 0.96
	2	167.69	
	3	168.78	
Aquades	1	110.32	113.09 ± 3.33
	2	116.79	
	3	112.18	
Citrate buffer pH 4.3	1	103.14	102.86 ± 0.26
	2	102.82	
	3	102.63	
Phosphate buffer pH 7.4	1	114.29	116.54 ± 2.35
	2	116.35	
	3	118.97	

The extraction results using phosphate buffer pH 7.4 resulted in a higher value of caffeine content compared to citrate buffer solvent pH 4.3, which was 116.54 mg/g. This is comparable to research conducted by Bekhterev [14] and Vuong *et al.* [12]. The results of Bekhterev's research [14] on caffeine extraction with several solvent pH treatments resulted in a higher % caffeine recovery in the pH range of 6-7, which was 87%. While the research results of Vuong *et al.* [12], the caffeine content extracted at pH values of 1 to 9 ranged from 25.1 to 27.6 mg/g. In addition, previous research by Kim *et al.* [15] found that the caffeine concentration increased when the pH of the extraction solution was increased from 4 to 7. Thus,

in general, the caffeine content increased when the pH of the solution was alkaline, i.e., above pH 7.

3.5. Chemometric analysis.

3.5.1. Principal component analysis.

At this stage, the yield data, water content, total ash content, acid insoluble ash content, total polyphenol content, and caffeine dry extract of green tea leaves were analyzed using chemometric principal component analysis (PCA) and cluster analysis (CA) techniques with Minitab software (Windows). PCA is a multivariate data reduction technique when the variables are correlated with each other. Samples with almost the same PC (Principle Component) will have almost the same physical and chemical properties so that PCA can be used for grouping [16-18]. One way to determine how many PCs to take to reduce a variable is to use a scree plot [19]. The scree plot is a plot of eigenvalue data obtained from PCA data analysis shown in Table 5. The scree plot is shown in Figure 3. From Figure 3 it can be seen that two principal components have eigenvalues of more than one, so it can be concluded that as many as two principal components can already be used to analyze the data.

Table 5. Eigen analysis of correlation matrix.

No	Eigen Value	Proportion	Cumulative
1	4.1194	0.687	0.687
2	1.3734	0.229	0.915
3	0.5072	0.085	1.000
4	0.0000	0.000	1.000
5	0.0000	0.000	1.000
6	-0.0000	-0.000	1.000

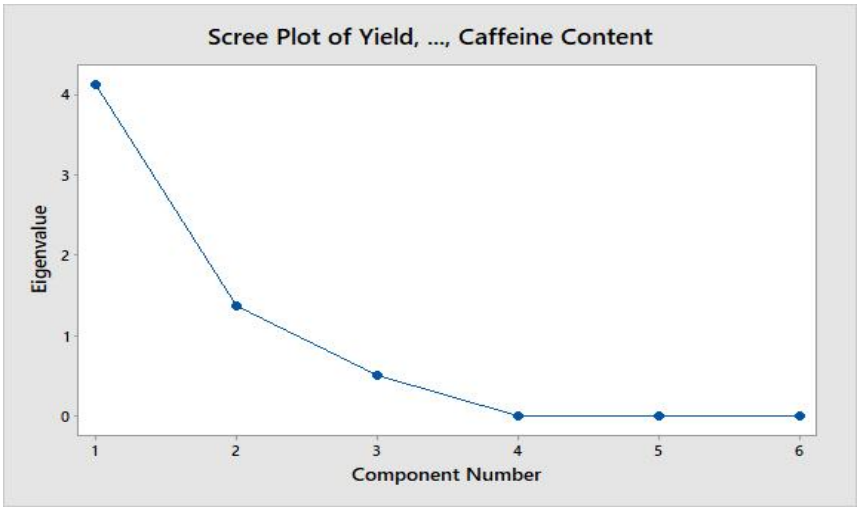

Figure 3. Scree plot of the relationship of each PC with eigenvalues.

Eigenvalue shows the value of the contribution given to the diversity of the data. The eigenvalues obtained (Table 5) show that PC1 and PC2 contributed 68.7% and 22.9% of the variance of the variables, respectively. In this study, PC1 and PC2 were used, which represented 91.5% of the variables.

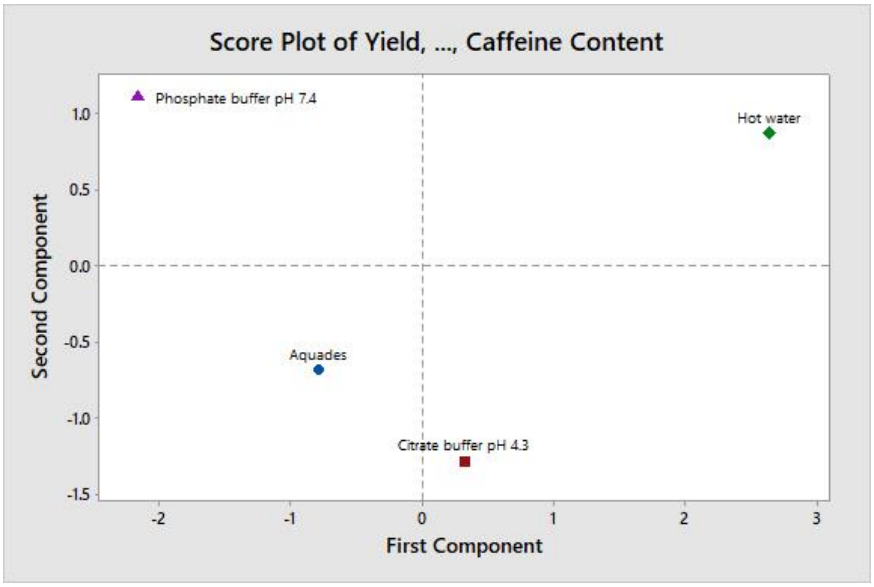

Figure 4. Score Plot PCA.

The score plot is used to classify and represent the closeness between samples as stated by the First Principle Component (PC1), which calculates the largest variation of all variables, and the Second Principle Component (PC2), which calculates the second largest variation of all variables [20-24]. Based on the score plot (Figure 4), it can be seen that the extraction results with these solvent variations do not have physical and chemical similarities because each sample is not in one quadrant. The loading plot shows how strongly each variable affects the PC by depicting it as a vector. The two variables are positively correlated if the two vectors form an angle less than 90o. If it forms an angle of about 90º, the two variables are unlikely to be correlated. Meanwhile, if it forms a wider angle (more than 90º) or around 180º, the two variables show a negative correlation [25-27].

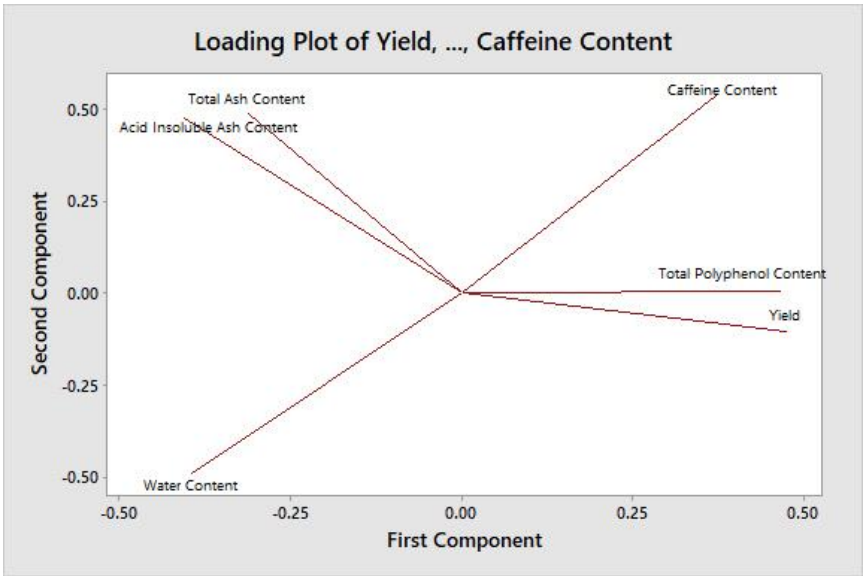

Figure 5. Loading Plot PCA.

Based on the loading plot as shown in Figure 5, the extract yield is positively correlated (high correlation) with the total polyphenol content because it has an angle of less than 90° which is supported by the correlation coefficient (r) in the scatter plot as shown in Figure 6 with a value of r = 0.9888 and r^2 = 0.9778, where the yield contributed 97.78% to the total polyphenol content obtained. While the yield does not correlate significantly (medium

correlation, not too low or high) to the caffeine content because it has an angle close to 90°, which is supported by the correlation coefficient (r) on the scatter plot as shown in Figure 7 with r = 0.6061 and r2. = 0.3673, the yield contributed 36.73% to the caffeine content.

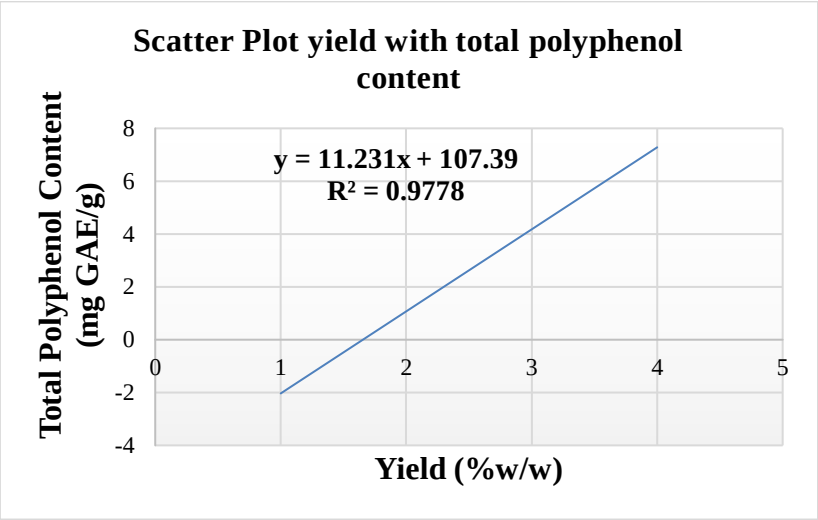

Figure 6. Scatter plot between yield and total polyphenol content.

Likewise, the correlation between caffeine content and total polyphenol content is not significantly correlated (medium correlation, not too low or high) because it has an angle close to 90°, which is supported by the correlation coefficient value (r) on the scatter plot as shown in Figure 8 with a value of r = 0.6593 and r^2 = 0.4347.

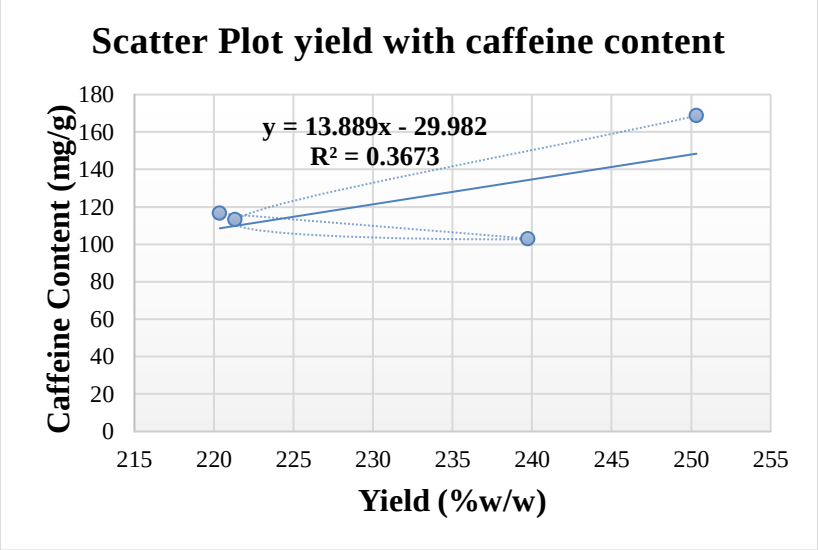

Figure 7. Scatter plot between yield and caffeine content.

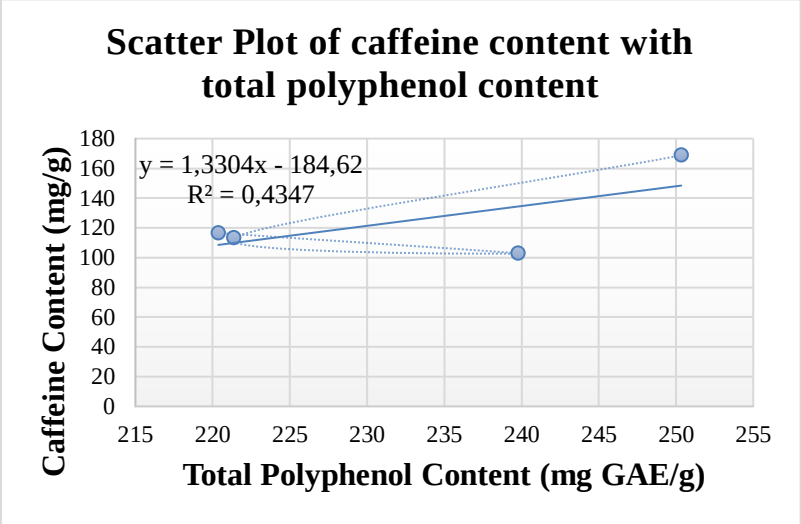

Figure 8. Scatter plot between total polyphenol content and caffeine content.

The correlation between the total ash content and the acid insoluble ash content of the extract based on the loading plot is positively correlated because it has an angle of less than 90° which is supported by the correlation coefficient (r) on the scatter plot as shown in Figure 9 with a value of r = 0.7908 and r^2 = 0.6254, where the total ash content contributed 62.54% to the acid insoluble ash content of the extract. In contrast, the correlation between total ash content or acid insoluble ash content and caffeine content does not show a correlation because the angle formed is close to 90°.

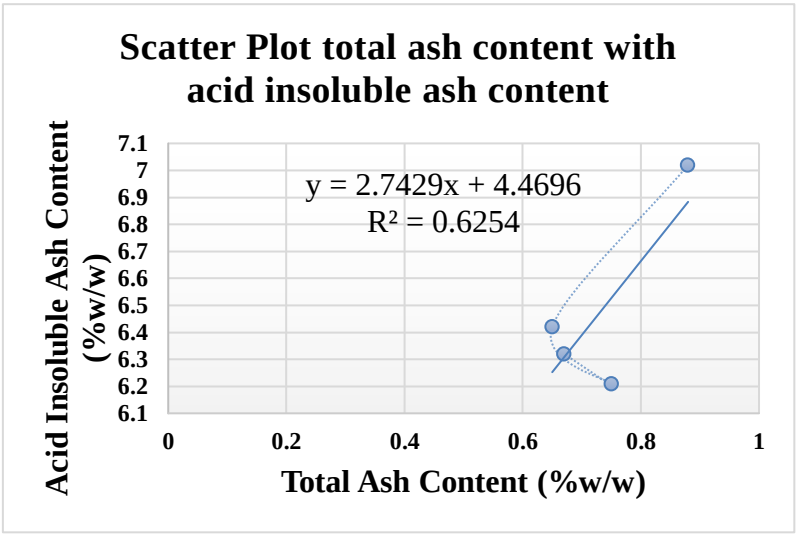

Figure 9. Scatter plot between total ash content and acid insoluble ash content.

In addition, the negative correlation shown in the loading plot is between yield and other variables such as water content, total ash content, and acid insoluble ash content because the angle formed between these variables is more than 90° or close to 180°. Likewise, between water content and variables such as caffeine content, total polyphenol content is negatively correlated because it forms an angle close to 180°. This means that the higher the value of caffeine content and total polyphenol content, the lower the value of the water content of the extract produced. To see which variables contribute negative or positive values for the first and second principal components can be seen in the biplot curve generated by the PCA process with Minitab in Figure 10.

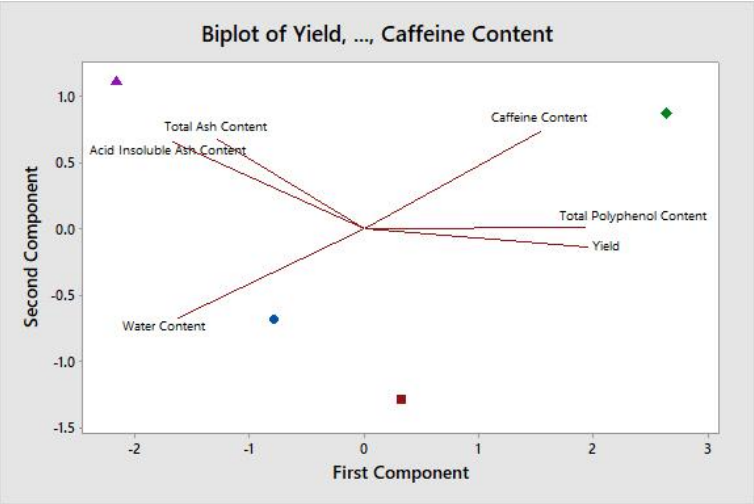

Figure 10. PCA biplot curve.

Variables that contribute to the formation of the large values of PC1 and PC2 can be seen from the biplot curve in Figure 10. Based on the biplot curve, the variables that contribute positively to the formation of the first principal component (PC1) are yield (%),total polyphenol content (mg GAE/g), and caffeine content (mg/g), and variables that contributed negatively were total ash content (%w/w), acid insoluble ash content (%w/w), and water content (%w/w). While the variables that contributed positively to the formation of the second principal component (PC2) were total ash content (%w/w), acid insoluble ash content (%w/w), total polyphenol content (mg GAE/g), and caffeine content (mg/g) and the variables that contributed negatively were the yield (%) and water content (%w/w).

3.5.2. Cluster analysis.

Figure 11 shows the dendrogram of the Cluster Analysis results from the sample (1) extracted using hot distilled water at a temperature of ±95ºC, (2) the results of the extraction using distilled water, (3) the extraction results using citrate buffer pH 4.3, and (4) the results extraction using phosphate buffer pH 7.4. In the dendrogram, it can be seen that on the x-axis is the number of objects (observations) and on the y-axis is the degree of similarity (similarity) between clusters in percent (%).

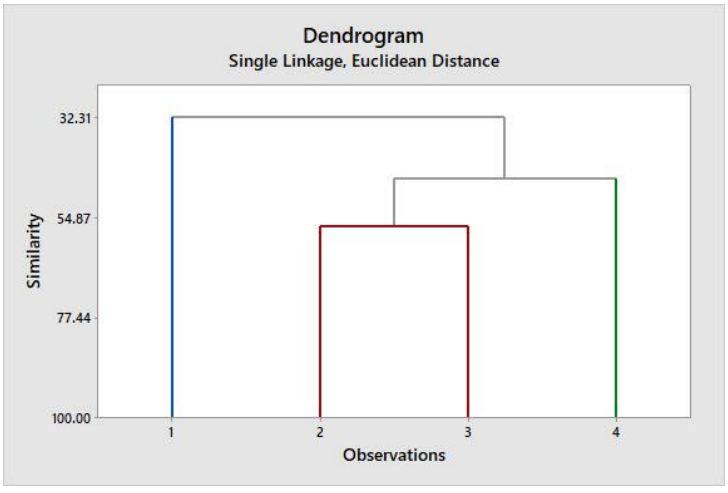

Figure 11. Dendrogram CA.

The cluster grouping shown in Figure 11 shows that there are 3 groups. The first cluster is cluster numbers 1, 2, 3, and 4, with a similarity level of 32,3120%. The second cluster

numbers 2, 3, and 4, with a similarity level of 46.1773%. Furthermore, the third cluster is numbers 2 and 3 with a similarity level of 56.7850% and is the cluster has the closest resemblance to the distance between the cluster centroids of 2.08564.

4. Conclusions

This study concludes that the solvent's effect on the solvent's temperature and pH in the extraction of green tea leaves (*Camellia sinensis* (L.) Kuntze) can be classified using PCA and CA multivariate analysis methods. The results obtained by the PCA analysis have formed 4 groups that have different physical and chemical properties. Of the 4 groups, the extraction results using distilled water and citrate buffer pH 4.3 had the closest distance compared to the extraction results with other solvents. The results of the PCA analysis are also supported by the analysis using CA, namely, the results of extraction using distilled water and citrate buffer pH 4.3 into one cluster with a distance between cluster centroids of 2.08564 and a similarity level of 56.7850%. Thus, the extraction result using distilled water is equivalent to or the same as the extraction result using citrate buffer pH 4.3.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Shaum Shiyan, Z.; Galih Pratiwi. Chemometric Approach to Assess Response Correlation and its Classification in simplex centroid design for Pre-Optimization stage of Catechin-SNEDDS. *Res J Pharm and Tech* **2021**, *14*, 5863-5870, https://doi.org/10.52711/0974-360X.2021.01020.
2. Mohammad Salamatullah, A.; Hayat, K.; Mabood Husain, F.; Asif Ahmed, M.; Arzoo, S.; Musaad Althbiti, M.; Alzahrani, A.; Al-Zaied, B.A.M.; Kahlil Alyahya, H.; Albader, N. Effects of Different Solvents Extractions on Total Polyphenol Content, HPLC Analysis, Antioxidant Capacity, and Antimicrobial Properties of Peppers (Red, Yellow, and Green (*Capsicum annum* L.)). *J Evid-Based Complement Alternat Med* **2022**, *2022*, https://doi.org/10.1155/2022/7372101.
3. Setiawati, Y.; Ramadhani, M.; Bobsaid, J.; Hulwah, D.O.Z. MIC and MBC Levels of Combination *Camellia sinensis* and *Mentha piperita* Extract Mouthwash Against *Streptococcus mutans*. *Nusant Med Sci J* **2022**, *7*, 39-49, https://doi.org/10.20956/nmsj.v7i1.19203.
4. Zhu, S.-C.; Shi, M.-Z.; Yu, Y.-L.; Cao, J. Simultaneous extraction and enrichment of alkaloids from lotus leaf by in-situ cloud point-reinforced ionic liquid assisted mechanochemical extraction technology. *J Indust Crops and Products* **2022**, *183*, 114968, https://doi.org/10.1016/j.indcrop.2022.114968.
5. Directorate General of POM. General Standard Parameters of Medicinal Plant Extracts, First Printing, Ministry of Health of the Republic of Indonesia; Jakarta, Indonesia, 2000.
6. Shamansoori, M.T.; Karimi, E.; Oskoueian, E. Rheum ribes extract loaded nanoliposome as a novel phytogenic antibiotic alternative in mice challenged by *Escherichia coli* (O157: H7). *J Biotech Appl Biochem* **2022**, https://doi.org/10.1002/bab.2303.
7. National Standardization Organization. SNI 3945:2016 Green tea, National Standardization Agency, Jakarta, Indonesia **2016**.

8. Halim, M.R.A.; Tan, M.S.M.Z.; Ismail, S.; Mahmud, R. Standardization and phytochemical studies of *Curcuma xanthorrhiza* Roxb. *Int J Pharm and Pharmaceutical Sci* **2012**, *4*, 606–610.
9. Nurcholis, W.; Priosoeryanto, B.P.; Purwakusumah, E.D.; Katayama, T.; Suzuki, T. Antioxidant, Cytotoxic Activities and Total Phenolic Content of Four Indonesian Medicinal Plants. *J Chem Valensi* **2012**, *2*, 501–510, https://doi.org/10.15408/jkv.v2i4.267.
10. Nonglang, F.P.; Khale, A.; Bhan, S. Phytochemical characterization of the ethanolic extract of *Kaempferia galanga* rhizome for anti-oxidant activities by HPTLC and GCMS. *J Future of Pharmaceutical Sci* **2022**, *8*, 1-12, https://doi.org/10.1186/s43094-021-00394-1.
11. Balci, F.; Özdemir, F. Influence of shooting period and extraction conditions on bioactive compounds in Turkish green tea. *Food Sci and Tech (Brazil)* **2016**, *36*, 737–743, https://doi.org/10.1590/1678-457X.17016.
12. Vuong, Q. V.; Golding, J. B.; Stathopoulos, C. E.; Roach, P. D. Effects of aqueous brewing solution pH on the extraction of the major green tea constituents. *Food Res Int* **2013**, *53*, 713–719, https://doi.org/10.1016/j.foodres.2012.09.017.
13. Zeng, L.; Ma, M.; Li, C.; Luo, L. Stability of tea polyphenols solution with different pH at different temperatures. *Int J Food Properties* **2017**, *20*, 1–18, https://doi.org/10.1080/10942912.2014.983605.
14. Bekhterev, V. N. *Extractive Freezing-out of Caffeine from Aqueous Solutions in a Centrifugal Force Field. J Anal Chem* **2020**, *75*, 1103–1107, https://doi.org/10.1134/s1061934820090051.
15. Kim, S.; Park, J.; Lee, L.; Han, D. Effect of pH on the green tea extraction. *Korean J Food Sci and Tech* **1999**, *31*, 1024–1028, https://www.semanticscholar.org/paper/Effect-of-pH-on-the-Green-Tea-Extraction-Kim-Park/946c2732ead34a3ac68cc59a8b22bfb73fc12d5e.
16. Pratiwi, G.; Ramadhiani, A.R.; Shiyan, S. Understanding the combination of fractional factorial design and chemometrics analysis for screening super-saturable quercetin-self nano emulsifying components. *Pharmacia* **2022**, *69*, 273-284, https://doi.org/10.3897/pharmacia.69.e80594.
17. Widyastuti, I.; Luthfah, H.Z.; Hartono, Y.I.; Islamadina, R.; Can, A.T.; Rohman, A. Antioxidant activity of temulawak (*Curcuma xanthorrhiza* Roxb.) and its classification with chemometrics. *Indonesian J Chem Pharm Anal* **2021**, *1*, 28-41, https://doi.org/10.22146/ijcpa.507.
18. Setyawan, E.I.; Rohman, A.; Setyowati, E.P.; Nugroho, A.K. The combination of simplex lattice design and chemometrics in the formulation of green tea leaves as transdermal matrix patch. *Pharmacia* **2021**, *68*, 275–282, https://doi.org/10.3897/pharmacia.68.e61734.
19. Arifah, M.F.; Rohman, A. A Chemometric Approach to Chromatography for Authentication Milk Product. *Indonesian J Chem Pharm Anal* **2021**, *1*, 121-132, https://doi.org/10.22146/ijcpa.3696.
20. Saeed, N.; Khan, M. R.; Shabbir, M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement and Alternat Med* **2012**, *12*, 1–12, https://doi.org/10.1186/1472-6882-12-221.
21. Windarsih, A.; Rohman, A.; Riyanto, S. The Combination of Vibrational Spectroscopy and Chemometrics for Analysis of Milk Products Adulteration. *Int J Food Sci* **2021**, *2021*, 1-15, https://doi.org/10.1155/2021/8853358.
22. Rohman, A.; Ikhtiarini, A.N.; Setyaningsih, W.; Rafi, M.; Aminah, N.S.; Insanu, M.; Irnawati, I.; Santosa, D. The Use of Chemometrics for Classification of Sidaguri (*Sida rhombifolia*) Based on FTIR Spectra and Antiradical Activities. *Indonesia J Chem* **2021**, *21*, 1568 - 1576, https://doi.org/10.22146/ijc.64360.
23. Arifah, M.F.; Nisa, K.; Windarsih, A.; Rohman, A. The Application of FTIR Spectroscopy and Chemometrics for the Authentication Analysis of Horse Milk. *Int J Food Sci* **2022**, *2022*, 1-9, https://doi.org/10.1155/2022/7643959.
24. Putri, D.C.A.; Gani, M.R.; Octa, F.D. Chemometrics-Assisted UV Spectrophotometric Method for Simultaneous Determination of Paracetamol and Tramadol in Divided Powder Dosage Form. *Int J Pharm Res* **2021**, *13*, 1901-1907, https://doi.org/10.31838/ijpr/2021.13.01.075.
25. Riswanto, F.D.O.; Rohman, A.; Pramono, S.; Martono, S. The employment of UV-Vis spectroscopy and chemometrics techniques for analyzing the combination of genistein and curcumin. *J Appl Pharm Sci* **2021**, *11*, 154-161, https://doi.org/10.7324/JAPS.2021.110318.
26. Nurani, L.H.; Rohman, A.; Windarsih, A.; Guntarti, A.; Riswanto, F.D.O.; Lukitaningsih, E.; Fadzillah, N.A.; Rafi, M. Metabolite Fingerprinting Using 1H-NMR Spectroscopy and Chemometrics for Classification of Three Curcuma Species from Different Origins. *Molecules* **2021**, *26*, 7626, https://doi.org/10.3390/molecules26247626.
27. Inyinbor, A.A.; Adekola, F.A.; Bello, O.S.; Bankole, D.T.; Oreofe, T.A.; Lukman, A.F.; Olatunji, G.A. Surface functionalized plant residue in Cu^{2+} scavenging: Chemometrics of operational parameters for process economy validation. *J South African Chem Engineering* **2022**, *40*, 144-153, https://doi.org/10.1016/j.sajce.2022.03.001.