Jurnal Riset Kimia

ISSN (print) : 1978-628X
ISSN (online) : 2476-8960

Detection of Clove Essential Oil Purity Using a Combination of Chemometric Models with UV-Vis Spectrophotometry

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Received: July 2024
Accepted: September 2025
Published: March 2026

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Abstract

Clove Essential Oil (CEO) has extensive benefits for health, cosmetics, and food. Low extraction yield (<1%) causes relatively high production costs. This becomes an opportunity for deliberate adulteration to gain greater business profits. This study developed a combination of UV-Vis spectrophotometry with chemometrics to separate pure CEO and adulterated CEO. The adulterants used included Corn Oil (CO) and Olive Oil (OO) at several adulteration levels to see their spectrum and absorption profiles using UV-Vis spectrophotometry. The data were analyzed using PCA and HCA chemometric techniques through R Studio 4.3.3 software. The results of UV-Vis spectrophotometry measurements on CEO adulteration showed similarities in the spectrum profiles of pure CEO and adulterated CEO. Data analysis with PCA can separate pure CEO from adultered CEO with a percentage of cumulative variance of the resulting spectrum of 98.2%. The more similar the composition of a particular oil in a sample, the closer the position of the sample to the oil on the score plot of PCA. HCA analysis can cluster pure CEO separately from adultered CEO. Cluster I in the dendogram consists of samples with large CO concentrations, while samples with large CEO and OO concentrations will be included in Cluster II. The combination of UV-Vis spectrophotometry and chemometrics can be used as a method to separate pure CEO from adultered CEO.

Keywords: *Adulteration; chemometrics; clove essential oil; HCA; PCA; UV-Vis spectrophotometry.*

Introduction

Edible films are commonly used as coatings for food products and can be consumed along with the packaged food [1], [2]. These films are primarily composed of hydrocolloids (proteins, polysaccharides), lipids (waxes, acyl glycerols, fatty acids), or their composites [3]. Starch, a widely used raw material for edible films, offers advantages such as biodegradability, ease of modification, and affordability due to its abundant availability from various sources [4]. Common starch sources for edible film production include cassava, corn [5], taro [6], sago [3], and ganyong [7]. However, since these materials are also staple food commodities, their use in edible film production may lead to competition with food supply chains.

Jackfruit seeds are an alternative starch source that remains underutilized. In Indonesia, they are often discarded after the fruit's flesh is consumed or processed. According to the Central Bureau of Statistics [8]Indonesia produced 906,514 tons of jackfruit in 2021. Jackfruit seeds contribute significantly to the waste percentage, comprising 30–50% of the total fruit mass [9]. They are rich in carbohydrates, containing 36.7 g per 100 g, with starch making up 94.5% of the total carbohydrates [10].

Starch-based edible films typically have poor physical properties and flexibility. These limitations can be addressed by incorporating fillers such as cellulose [11]. However, previous studies have shown that starch-based bioplastics from jackfruit seed starch with added coconut fiber cellulose still exhibit a rough surface and a relatively rigid structure [12]. Reducing cellulose particle size enhances its interaction with starch, improving film elasticity, tensile strength, density, and thickness. Studies have also reported increased tensile strength with higher concentrations of microcrystalline cellulose (MC) [13]. Therefore, this study incorporates MC as a filler in jackfruit seed starch-based edible films.

To date, no studies have incorporated antioxidant materials into jackfruit seed starch-MC edible films. Antioxidants prevent oxidation by donating electrons to highly reactive free radicals [14]. thereby inhibiting spoilage and extending the shelf life of packaged food products. Green tea is a well-known antioxidant source due to its high catechin content, which reaches 6.9%, the highest among all tea varieties [15]. It also exhibits strong antioxidant activity, with a reported value of 58.6 μg/mL [16]. Edible films enriched with green tea extract have demonstrated significant free radical inhibition (52.9%) while enhancing physical and mechanical properties [17]. Therefore, this study aims to develop a jackfruit seed starch-MC edible film incorporating green tea extract as an antioxidant and to evaluate its antioxidant activity, physical and mechanical properties, and overall performance.

Experimental

Equipment

The equipment used in this study included standard laboratory glassware, an analytical balance, an oven, a hot plate, a centrifuge (Hettich Universal), an ultrasonic cleaner (Elmasonic S), a Microtrac II Particle Size Analyzer (PSA), 40- and 100-mesh sieves, a rotary evaporator, a Fourier-transform infrared (FTIR) spectrophotometer (Thermo Scientific), a liquid chromatography-mass spectrometry (LC-MS) system (Acquity UPLC® H-Class System, Waters, USA), UV-Vis spectrophotometer (Shimadzu UV-1780, Japan), vortex (Ohaus, New Jersey, USA), micropipette (Dragonlab, Berlin, US), UV Probe 2.70 software, Design-Expert 13 software, and R Studio 4.3.3 software, glass plates (molds), a digital micrometer screw gauge (Teclock), a tensile strength and elongation tester (Instron), micropipettes (10, 100, 200, and 1000 μL), a 96-well microplate, and a microplate reader (Spectrostar Nano).

Material

The materials used are CEO (PT Syailendra Bumi Investama, Indonesia), CO (Acros Organics, Belgium), OO (PT Syailendra Bumi Investama, Indonesia), and n-Hexane (Merck, Darmstadt, Germany).

Methods

Organoleptic Analysis

CEO, CO, and OO each as much as 5 ml were put into a test tube and leaned against white paper. The colors of CEO, CO, and OO were observed directly with the eye at an observation distance of 30 cm. In addition, the odor and taste of all three were also observed directly with the sense of odor and taste. The results of the organoleptic analysis will be analyzed to see the profile of similarities and differences between the CEO and adulterants. The color, odor, and taste of CEO, CO, and OO can be linked to possible adulterating motives in CEO. Based on the organoleptic test of oil required in SNI, the panelists used are 3 people or 1 expert. This study uses 3 panelists to conduct an organoleptic analysis of the three oils with a washout period of 1 x 24 hours.

This organoleptic analysis was carried out by panelists who were selected through inclusion and exclusion criteria and had agreed to informed consent. Panelist inclusion criteria are physically and mentally healthy; not color blind; have no history of food allergies; willing to participate in the study by signing an informed consent; do not consume or smell food that has a sharp taste and smell within 6 hours before testing; do not smell anything that smells sharp

 DOI: https://doi.org/10.25077/jrk.v17i1.846

within 6 hours before testing; and do not use cosmetics, such as perfume and lipstick. While panelist exclusion criteria are experiencing hypersensitivity to the odor of the sample during the analysis; taking medications that can affect the function of the senses of sight, taste, and smell; experiencing a sick condition when the organoleptic analysis is to be carried out that can affect the function of the senses of sight, taste, and smell; and leaving the study before completing the complete organoleptic analysis.

Sample Preparation and Adulteration

CEO, CO, and OO were diluted first with n-Hexane before being analyzed using UV-Vis spectrophotometry. The three oils that had been diluted and gave good absorbance were used as CEO stock solutions and adulterant stock solutions for making adulteration samples.

Pure CEO as much as 25 μL was put into a 25 mL measuring flask to be diluted with n-Hexane to the limit mark. Next, 166 μL of this solution was taken to be diluted again with n-Hexane in a 25 mL measuring flask as a CEO stock solution. The total dilution factor of the CEO stock solution was 150.000 times that of pure CEO. Meanwhile, the adulterant stock solution was made by diluting 20 μL of pure CO and pure OO with n-Hexane in a 25 mL measuring flask. This adulterant stock solution is the result of a 1.250 times dilution of pure CO and pure OO.

Sample preparation and adulteration were carried out by mixing the CEO stock solution with the adulterant stock solution. Mixing was carried out homogeneously with adulteration levels ranging from low to high. Low adulteration levels were chosen to represent the detection of possible unintentional adulteration due to misidentification or the taking of materials.

While high adulteration levels would represent intentional adulteration with economic motives [5]. This concentration range was randomized using a factorial design through Design-Expert 13 software to obtain the formula used in adulteration sample preparation, as in Table 1.

Sample analysis using UV-Vis spectrophotometry also requires Quality Control (QC Samples) to determine whether or not there are factors that influence the analysis results that can cause differences in sample measurement results, especially checking the performance of the UV-Vis spectrophotometry used. These QC sample analyses are used as a reference in data normalization during the study. Therefore, the QC samples are made with the formula in Table 2.

Each of these 100 μL mixture was first diluted with n-Hexane to the boundary mark in a 25 mL measuring flask. These solutions were called QC1 and QC2 stock solutions. Furthermore, 33 μL of QC1 and QC2 stock solutions were taken to be diluted again with n-Hexane in a 10 mL measuring flask as QC samples. The absorbance of the QC samples was measured using UV-Vis spectrophotometry. QC samples at certain times during data collection were carried out.

Table 1. Preparation of Adulteration Samples

	Sample Code	Comparison of CEO: CO	Volume to be Taken			Total Volume
			CEO Stock Solution	CO Stock Solution	OO Stock Solution	
CO Adulteration	CO25	75: 25	3.75 ml	1.25 ml	0	5 ml
	CO33	67: 33	3.3 ml	1.7 ml	0	5 ml
	CO50	50: 50	2.5 ml	2.5 ml	0	5 ml
	CO67	33: 67	1.7 ml	3.3 ml	0	5 ml
	CO75	25: 75	1.25 ml	3.75 ml	0	5 ml
OO Adult	Sample Code	Comparison of CEO: OO	Volume to be Taken			
			CEO	CO	OO	Total Volume

			Stock Solution	Stock Solution	Stock Solution	
	OO25	75: 25	3.75 ml	0	1.25 ml	5 ml
	OO33	67: 33	3.3 ml	0	1.7 ml	5 ml
	OO50	50: 50	2.5 ml	0	2.5 ml	5 ml
	OO67	33: 67	1.7 ml	0	3.3 ml	5 ml
	OO75	25: 75	1.25 ml	0	3.75 ml	5 ml
CO-OO Adulteration	**Sample Code**	**Comparison of CEO : CO : OO**	**Volume to be Taken**			**Total Volume**
			CEO Stock Solution	**CO Stock Solution**	**OO Stock Solution**	
	CO16OO16	68 : 16 : 16	3.4 ml	0.8 ml	0.8 ml	5 ml
	CO68OO16	16 : 68 : 16	0.8 ml	3.4 ml	0.8 ml	5 ml
	CO16OO68	16 : 16 : 68	0.8 ml	0.8 ml	3.4 ml	5 ml
	CO33OO33	33 : 33 : 33	1.67 ml	1.67 ml	1.67 ml	5 ml

Table 2. Preparation of QC Stock Solution

Sample Code	Volume to be Taken			Total Volume
	Pure CEO	Pure CO	Pure OO	
QC1	50 µL	50 µL	0	100 µL
QC2	50 µL	0	50 µL	100 µL

When QC samples at different measurement times show grouping at the same or adjacent points on the score plot of PCA, it can be concluded that the measurement results are not significantly different. If the score plot of the QC samples shows significant separation, then normalization of the measurement data can be done by referring to the shift in absorbance measurements that occur in the QC samples.

Sample Analysis by UV-Vis Spectrophotometry

UV-Vis spectrophotometry is used to obtain UV spectrum data of samples. Before the sample measurement is carried out, the maximum wavelength is determined first to increase accuracy in the measurement. This maximum wavelength will be used in the analysis of the spectrum profile of each sample. Next, each sample is scanned for the UV spectrum profile by overlay, so that it can show a similar UV spectrum profile.

Scanning with UV-Vis spectrophotometry was carried out in the wavelength range of 200-400 nm with a wavelength interval of 1 nm using a quartz cuvette. The blank used in the UV-Vis spectrophotometry analysis in this study was n-Hexane with a UV cutoff at 210 nm. Each sample solution was replicated 3 times to be included in the PCA and HCA chemometric analysis.

Data Analysis

The results of the CEO's organoleptic analysis will be compared with the results of the adulterant organoleptic analysis in the form of CO and OO. This serves as information that describes the differences or similarities between the three oils organoleptically. The information obtained can be linked to the possibility of counterfeiting motives in the CEO.

The spectrum profile data generated from each sample was processed through UV Probe software and then exported in .xlsx format. Data processing was continued with R Studio 4.3.3 software. Observations were made on pure CEO and adulterated CEO through the separation image on the score plot and clustering on the

 DOI: https://doi.org/10.25077/jrk.v17i1.846

HCA dendogram. The results of this chemometric analysis are expected to be used as a method for detecting adulterants in CEO. The successful method was demonstrated through a clear separation between pure CEO samples and the CEO with adulterants added

Results and Discussion

Organoleptic Analysis

Organoleptic analysis of CEO, CO, and OO was conducted by observing the color, odor, and taste of the three oils. The results of this analysis were compared with the Certificate of Analysis (CoA) and quality requirements stated in the SNI for each oil to ensure the truth of the identity and quality of the oil used in the study (Table 3). The color comparison between the three oils can be seen in Figure 1.



Figure 1. a) Clove essential oil, b) Corn oil, and c) Olive oil

Table 3. Organoleptic comparison between panelist observation results with CoA and SNI

	Organoleptic of CEO		
	Panelist Observation Results	**SNI:06-2387-1996**	**Certificate of Analysis**
Color	Light brown	Yellow – dark brown	Yellow – light brown
Odor	Strong (typically of clove)	Typically of clove oil	Spicy (typically of eugenol)
Taste	Spicy-bitter	-	-
	Organoleptic of CO		
	Panelist Observation Results	**SNI 01-3394-1994**	**Certificate of Analysis**
Color	Yellowish	Yellow	Bright yellow
Odor	Tasteless	Normal	-
Taste	Tasteless	Normal	-
	Organoleptic of OO		
	Panelist Observation Results	**SNI 01-4474-1998**	**Certificate of Analysis**
Color	Pale yellow	Normal	Pale yellow – greenish yellow
Odor	Weak (typically of olive)	Normal	Tasteless
Taste	Tasteless	Normal	Tasteless

ISSN (print) : 1978-628X
ISSN (online) : 2476-8960

CEO is an essential oil obtained through the distillation of clove flowers. This causes the organoleptic properties of CEO to tend to be colored, tasted, and odored like cloves. Clove flowers have a blackish brown color, a distinctive clove odor, and a spicy and bitter taste. The distinctive taste and odor of CEO come from its essential oil content, mainly eugenol [15]. All three panelists said the same results. The color and odor of the CEO are in accordance with CoA and SNI quality requirements.

CO is the result of utilizing corn plants (Zea mays L.), especially the seeds. This oil contains polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids, ubiquinone, and tocopherol [16]. The yellowish color of CO is obtained from the extraction process of corn seeds, which are also yellowish. The organoleptic analysis results of the three panelists were in line with the CoA and SNI quality requirements of CO.

OO is a fatty oil processed from olives (Olea europaea L.), which contains fatty acids and polyphenols [17]. Its organoleptic properties include pale yellow or bright greenish yellow, a weak, distinctive odor, and a slightly spicy aftertaste [18]. The results carried out by three panelists are in accordance with the CoA and quality requirements in SNI.

The organoleptic analysis of CO and OO as adulterants of CEO shows organoleptic properties that are relatively weak compared to CEO. The color, odor, and taste that are not too sharp make it often used as a mixture in CEO counterfeiting. Therefore, an analysis instrument is needed that can detect the addition of adulterants in CEO.

Interpretation of UV-Vis Spectrum

Detection of adulteration of a compound can be done by many methods, such as FTIR, Raman spectroscopy, HPLC, GC-MS, including UV-Vis Spectrophotometry. This study utilized the use of UV-Vis spectrophotometry to analyze the addition of adulterants to CEO. The advantages of this method are low cost and simple sample preparation. However, this method has lower selectivity and sensitivity compared to other analytical methods. Therefore, chemometric techniques are often combined with UV-Vis spectrophotometry as a tool to analyze in-depth information contained in the UV-Vis spectrum. Several studies have used this combination for the analysis of vegetable oils, including colza oil, corn oil, sunflower oil, soybean oil, and olive oil [19]. Malavi et al.'s (2023) study compared the results of the classification and quantification performance of olive oil adulteration using a combination of chemometrics (PLS-DA) with HSI, FTIR, UV-Vis, Raman, and GC-MS, producing precise classification accuracies of 100%, 99.8%, 99.6%, 96.6%, and 93.7%, respectively [20]. The results provided by the combination of chemometrics with UV-Vis Spectrophotometry are quite high, so this study applies it to the analysis of clove essential oil.

CEO, CO, and OO stock solutions are measured for absorption by UV-Vis spectrophotometry to obtain the UV-Vis spectrum. The selection of the wavelength range is based on the wavelength range that is able to provide the spectral pattern information of the analyte contained in the sample. In addition, single-spectrum information can be used as a reference in predicting the mixed-spectrum pattern of the CEO and adulterant that will be produced [21]. The UV spectrum of the CEO, CO, and OO stock solution can be seen in Figure 2.

The results of scanning the CEO stock and adulterant solutions in this study are listed in Table 4. In the wavelength range of 200-400 nm, CEO, CO, and OO stock solutions provide good absorption. The absorbance of each peak is in the range of 0.2 – 0.8. This is in accordance with the Farmakope Indonesia Edition VI, which states that a good absorbance range is $0,2 \leq A \leq 0,8$ in the area of UV-Vis light [18]. Therefore, the sample scanning in the study was carried out in the wavelength range of 200-400 nm.

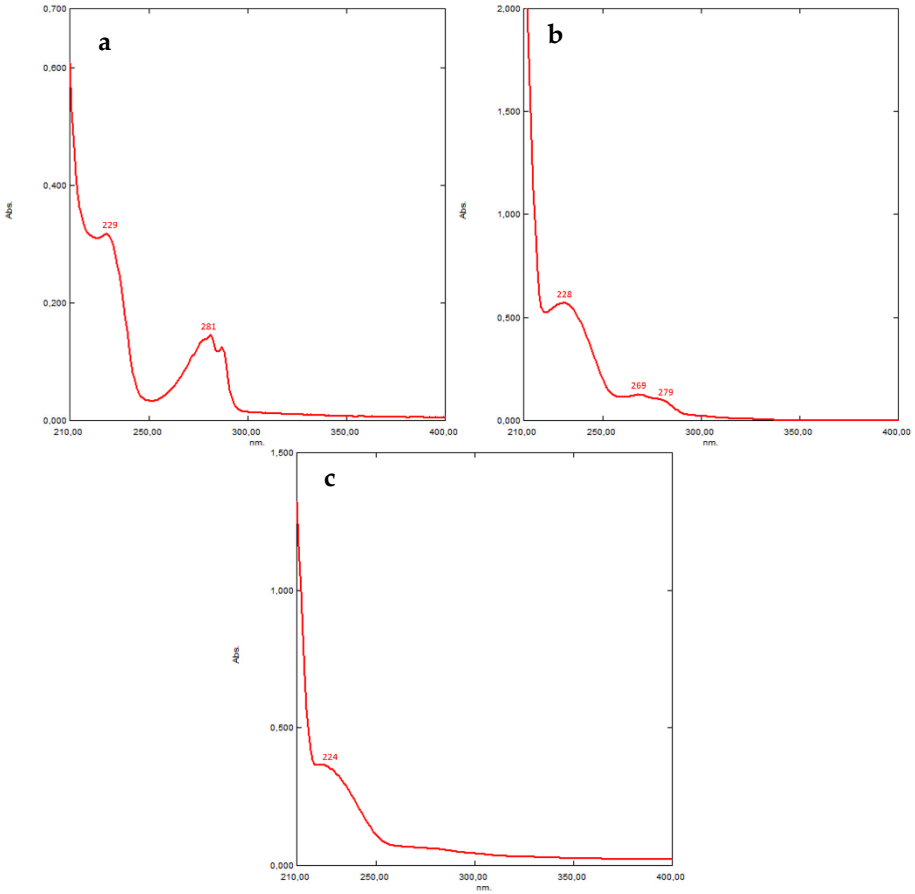

Figure 2. UV Spectrum of a) CEO stock solution, b) CO stock solution, and c) OO stock solution

Table 4. Scanning results of CEO, CO, and OO stock solution samples

Sample	Peak Absorption	Absorption Area
CEO Stock Solution	229 nm and 281 nm	200 – 400 nm
CO Stock Solution	228 nm, 269 nm, and 279 nm	200 – 400 nm
OO Stock Solution	224 nm	200 – 400 nm

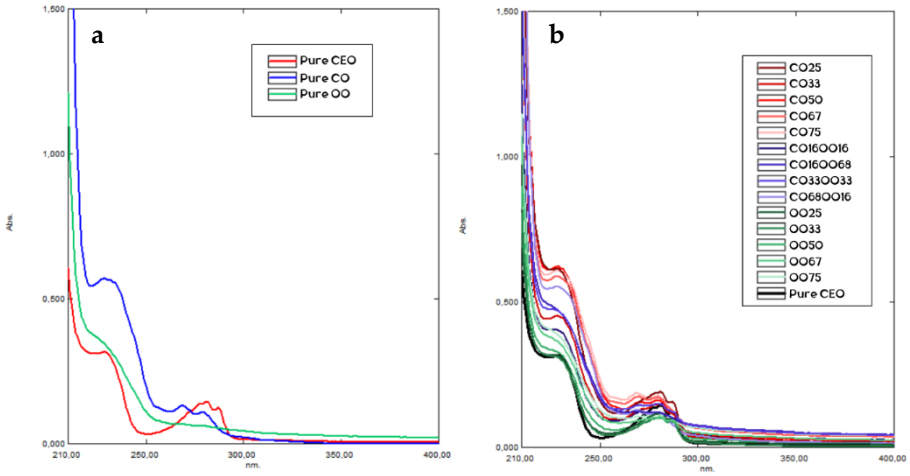

Figure 3. a) Spectrum UV-Vis overlay of CEO, CO, and OO stock solution , and b) UV spectrum overlay of CEO stock solution with samples of CO adulteration, OO adulteration, and CO-OO adulteration

CEO stock solutions produce peak absorption at 229 nm and 281 nm wavelengths. This is in accordance with the fact that eugenol in CEO provides UV absorption at 225-230 nm for the –OH group and 270-290 nm for the –CO group [11]. CO provides absorption at wavelengths of 220–240 nm and 250–285 nm [12]. The results of the absorption measurement of the CO stock solution are in accordance with the study, with the absorption peaks shown at wavelengths of 228, 269, and 279 nm. Meanwhile, the OO stock solution provides a peak absorption at a wavelength of 224 nm. Despite having a similar spectral shape, these results are less consistent with studies that state that OO provides typical absorption in the wavelength range of 276-500 nm [13]. This difference can be due to the type of solvent used in the analysis, where the study used cyclohexane as the OO solvent, while this study used n-hexane in the analysis. Despite having similar chemical composition and hydrophobicity, the different structural shapes allow for different solvation-free energy differences [22].

Adulteration of the CEO

CEO adulteration is made by mixing CO and OO as adulterants in CEO with a randomized 1-100% adulteration rate range. The CEO has distinctive and strong characteristics in the organoleptic side, especially in color and odor. Meanwhile, adulterants in the form of CO and OO have a color that tends to be clear and a taste bland. When adulterated with CEO, the distinctive color and odor of cloves tend to remain. The organoleptic similarity between pure CEO and adulterated CEO makes CEO adulteration difficult to detect, requiring additional detection methods, such as UV-Vis spectrophotometry.

The results of the absorption measurements of CEO, CO, and OO, which were deliberately overlapped with the overlay feature in the UV Probe 2.70 software in Figure 3, show the similarity in shape between the three in general. Each oil has its own distinctive spectrum profile as a distinguishing feature. When CO and OO are mixed into the CEO, the UV spectrum formed is quite similar to the UV spectrum of CEO, as seen in Figure 3.

Chemometric Analysis with Principal Component Analysis (PCA)

Wavelength selection

Sample scanning was carried out in the wavelength range of 200-400 nm with a scanning interval of 1 nm, so that absorbance data in the wavelength range were obtained. Data analysis with chemometric methods, especially those aimed at discriminating a sample, requires the selection of the right wavelength range. The wavelengths chosen to build the model in

 DOI: https://doi.org/10.25077/jrk.v17i1.846

chemometric analysis should be able to provide the typical spectrum information on each sample, thus being able to produce good grouping and separation according to the characteristics of the sample spectrum [23].

The results of scanning samples in the wavelength range of 200-400 nm were observed, and the wavelength was selected selectively to produce the best chemometric model. The wavelength range of 200-210 nm was not included in the modeling because the UV cutoff of the n-Hexane solvent used was in that range. The spectrum profile of the n-Hexane solvent is shown in Appendix 9. The wavelength range of 200-210 nm was not included in the modeling to minimize the influence of solvents in the data analysis. After several models, the sample provided a typical spectrum and absorption in the wavelength range of 228 – 282 nm. Wavelengths outside this range were not included in the modeling because they did not provide significant information from the analyzed sample.

Scree Plot Analysis

Scree plots in PCA chemometric analysis are used to determine a number of principal components (PCs) that explain the total variance in the data set being analyzed. The determination of a PC can be done with the Kaiser criteria, namely by choosing a PC that has an eigenvalue of more than 1, which means "significant". Based on Kaiser's criteria, the first two PCs, PC1 and PC2, are the most commonly used PCs in PCA analysis. The large ability of PC1 and PC2 compared to other PCs in explaining variance in data will provide the best analysis results [24],[25]. Chemometric analysis of PCA from the sample data yielded a scree plot as shown in Figure 4.

Figure 4 is a scree plot of sample data consisting of pure samples (CEO, CO, and OO), adulterated samples, and QC samples with eigenvalues on PC1, PC2, PC3, PC4, and PC5, respectively, of 47.8, 6,2, 0,9, 0,1, and 0. The eigenvalue of the first two PCs, namely PC1 and PC2, is more than 1, so PC1 and PC2 are significant for further use in the analysis of the score plot in PCA. PC1 explains a spectrum variance of 87%, while PC2 accounts for a spectrum variance of 11.2%. The percentage of cumulative variance of the spectrum produced by these two PCs is 98.2%. In addition, PC2 and PC3 are also analyzed in PCA models. The PC2 spectrum variance of 11.2% and PC3 of 1.7% resulted in a percentage of cumulative variance of 12.9%. The results of plot scores PC1 vs PC2 with PC2 vs PC3 will be compared to get the plot score with the best separation.

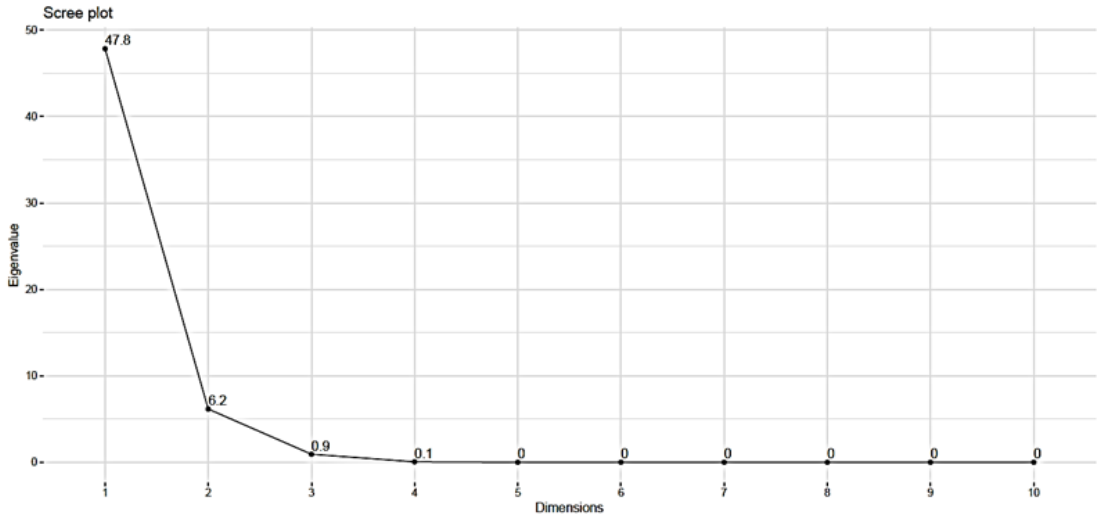

Figure 4. Scree plot of PCA

Scree Plot Analysis

After conducting the scree plot analysis, the two selected PCs will be used to display the clustering of the data by grouping samples that have similar scores in adjacent positions. This clustering is displayed in the form of a score plot. Adjacent samples in the score plot visualization can be assumed to be samples that have similar characteristics [24]. In this study, the two PCs selected to be analyzed in the PCA plot score model were PC1 vs PC2 and PC2 vs PC3. The results of the PC1 vs PC2 plot score analysis are shown in Figure 6 and used as the selected model in the study. Meanwhile, the plot score with PC2 vs PC3 (Figure 5) was not chosen because it showed a separation that was not better than PC1 vs PC2. An eigenvalue above 1 indicates that the evaluated principal component weight values are reliable and more informative [26]. The eigenvalues of PC1 and PC2 are above 1, while those of PC3 are below 1. This may be the cause of the poor separation in the PC2 vs PC3 score plot compared to the PC1 vs PC2 score plot.

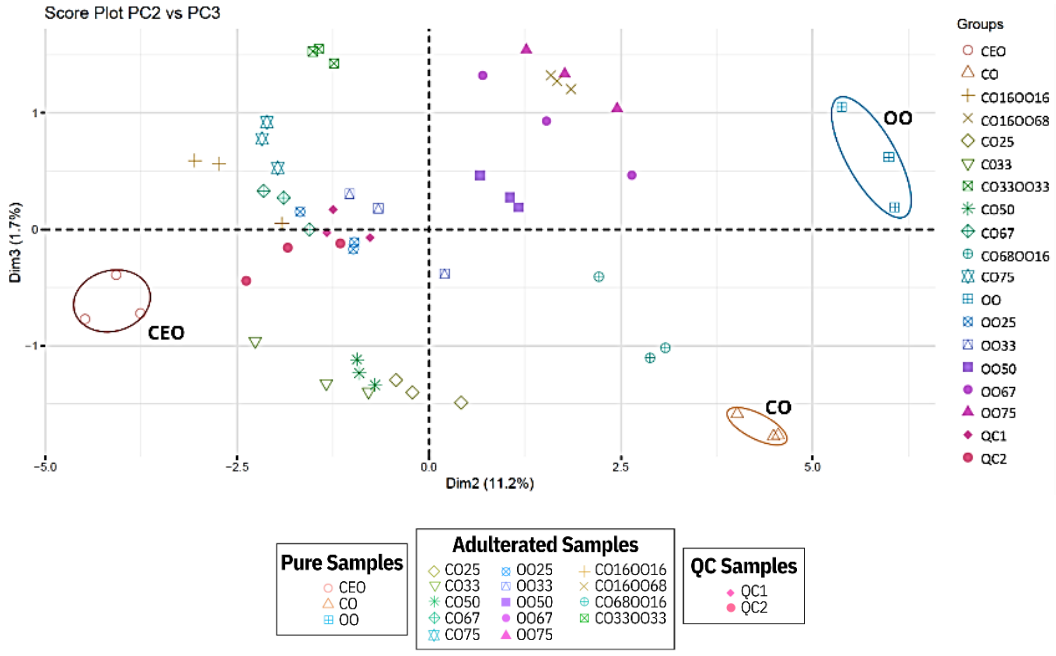

Figure 5. Score plot of PCA with PC2 vs PC3

 DOI: https://doi.org/10.25077/jrk.v17i1.846

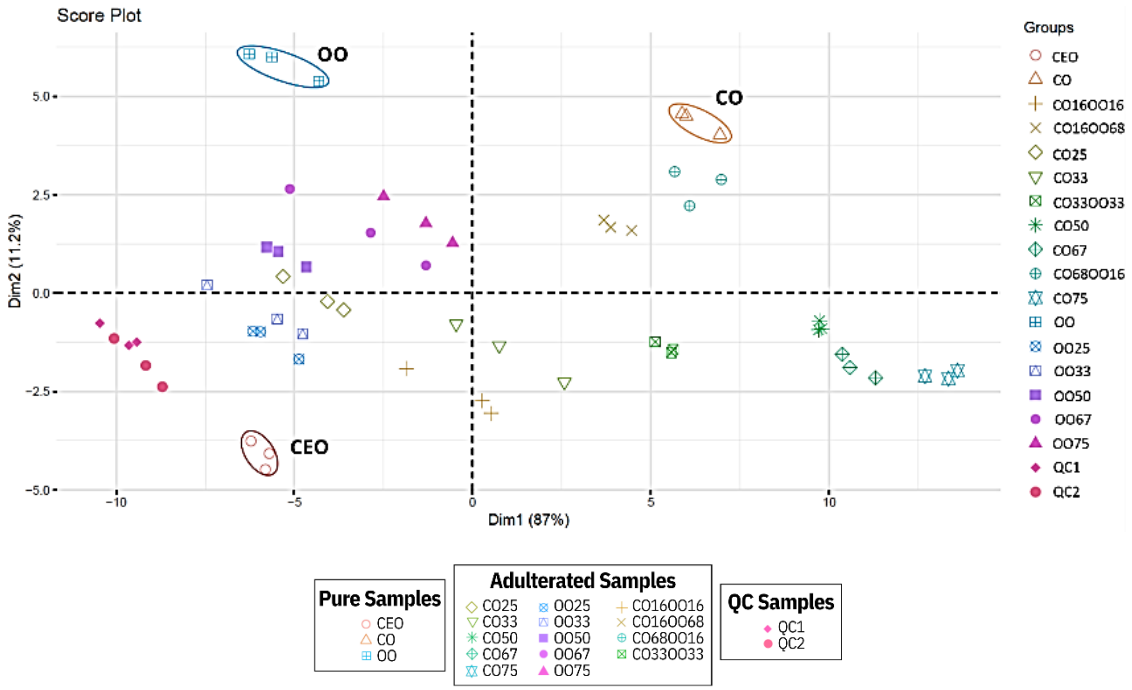

Figure 6. Score plot of PCA with PC1 vs PC2

Figure 6 is a score plot that uses PC1 and PC2 in its clustering. This plot score shows the separation between pure CEO and adulterated CEO with CO and OO. CEO, CO, OO, and CEO adulterated with both CO and OO show a fairly clear separation. CEO, CO, and OO are in positions that are far away from each other. CO adulterated samples (CO25, CO33, CO50, CO67, and CO75) were dispersed between pure CEO and pure CO by considerable distances. Similarly, the OO adulterated samples (OO25, OO33, OO50, OO67, and OO75) were spread between pure CEO and pure OO. Meanwhile, the CO-OO adulterated samples (CO16, OO16, CO16OO68, CO33OO33, and CO68OO16) were spread between the CO adulterated samples and the OO adulterated samples.

The comparison of the concentrations of CEO, CO, and OO in a sample affects its location on the score plot. The more similar the composition of a particular oil in a sample, the closer the position of the sample is to that oil. CO adulterated samples, including CO25, CO33, CO50, CO67, and CO75, clustered in the same region. CO25 has a greater CEO concentration than CO75, so CO25 tends to be closer to pure CEO while still showing a clear separation. This also applies to OO adulterated samples, namely OO25, OO33, OO50, OO67, and OO75. All five OO adulterated samples tended to gather in the same area. Pure CEO has a higher affinity to OO25 than to OO75. Meanwhile, the CO-OO adulterated samples, which include CO16OO16, CO16OO68, CO33OO33, and CO68OO16, are located in adjacent positions while still separating from the largest constituent oil components. CO16OO16 is located relatively close to the CEO, CO16OO68 is adjacent to OO, CO33OO33 is located relatively centrally between the three oils, and CO68OO16 is adjacent to CO.

The stability of the sample with UV-Vis spectrophotometry is seen for stability by observing the results of QC samples measurements at each time of sampling data. The QC samples, both QC1 and QC2, are in close proximity. This shows that the measurement results carried out have stable results, even though they were taken at several different times.

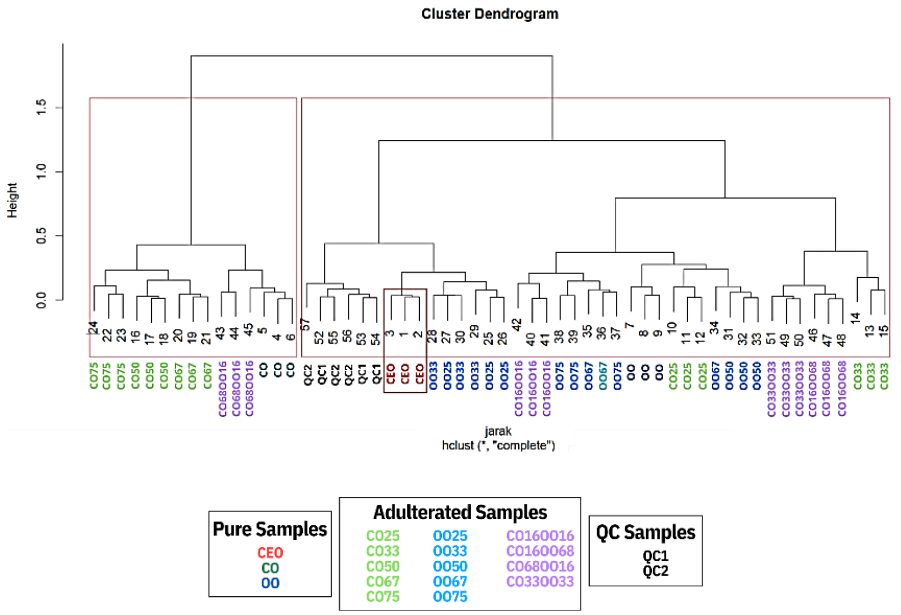

Figure 7. Dendogram HCA

Chemometric Analyis with Hierarchical Clustering Analysis (HCA)

HCA is a hierarchy-based clustering method in research that uses a large amount of data. This method will place samples in clusters that have similarities. The results of the HCA analysis will be displayed in the form of a dendogram. The horizontal part shows the relationships between clusters. While the vertical part shows the magnitude of similarities between interconnected clusters. Similarity is calculated by Euclidean squared spacing and the Single-Linkage method to consider the total amount of deviation of each sample in relation to the average of the data set of the analyzed sample [27].

Figure 7 is an overview of the HCA dendogram from the sample used in this study. This dendogram is in line with the resulting PCA plot score. There are two large clusters, namely Cluster I (left arm) and Cluster II (right arm). Cluster I consists of CO, CO68OO16, CO50, CO67, and CO75. Cluster II consists of CEO, CO25, CO33, OO, OO25, OO33, OO50, OO67, OO75, CO16OO16, CO16OO68, CO33OO33, QC1, and QC2. These two clusters are formed based on the similarity of the properties of the sample. The large concentration of CO in the sample causes the sample to be included in Cluster I. While samples that have a large concentration of CEO and OO will be included in Cluster II. The closer the location of the sample number indicates the similarity of characteristics between the samples, and vice versa. The magnitude of the similarity will be shown from the low difference in height between samples. Although CEO and OO are in the same cluster (Cluster II), they remain on different arms within the cluster. This shows that CEO is still distinguishable from OO.

Conclusions

CEO adulteration with the addition of CO and OO can be detected by a combination of UV-Vis spectrophotometry and chemometric methods. The score plot PCA using PC1 vs PC2 model with a percentage of cumulative variance of 98,2% successfully separated pure CEO from adulterated CEO. The HCA dendogram categorized samples into distinct clusters based on the composition of the CEO, CO, and OO mixtures they contain. These findings provide insight into detecting adulteration in CEO.

DOI: https://doi.org/10.25077/jrk.v17i1.846

Acknowledgment

We are grateful to Jenderal Soedirman University's Institute for Research and Community Service (LPPM), which funded this study under the UNSOED 2024 Institutional Research grant no. 26.712 /UN23.35.5/PT.01/II/2024.

Declaration of Generative AI and AI-Assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

R.: Methodology and investigation. I.N.D.: Data collection and formal analysis. N.E.E.: Data curation, visualization, and writing —original draft preparation. M.W.S..: Methodology, validation, and writing —review and editing. T.H.W.: Conceptualization, corresponding author responsibilitiesand, and supervision. Both authors have read and approved the final version of the manuscript.

Funding

This research was funded by Jenderal Soedirman University's Institute for Research and Community Service (LPPM), which funded this study under the UNSOED 2024 Institutional Research grant no. 26.712 /UN23.35.5/PT.01/II/2024.

Declaration of Conflicting Interests

The authors declare no conflict of interest.

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 DOI: https://doi.org/10.25077/jrk.v17i1.846