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Analysis of Black Cumin Oil Adulteration Using a Combination of UV-Vis Spectrophotometry and Chemometric Methods

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ABSTRACT: Black cumin oil is a vegetable oil known for its health benefits, but its high price makes it susceptible to adulteration. To address this issue, a combination of UV-vis spectrophotometric and chemometric methods was developed to distinguish black cumin oil from other oils. This research specifically aims to evaluate the effectiveness of UV-vis spectrophotometry and principal component analysis (PCA) and hierarchical cluster analysis (HCA) in differentiating black cumin oil from corn and soybean oil. Samples were analyzed for absorption across a wavelength range of 220-290 nm. The UV-vis spectral data was subsequently processed using PCA and HCA to classify the samples. The results indicate that the PCA model effectively differentiates between black cumin, corn, and soybean oils. Minitab software yielded the most significant PCA results, showing a data variation distribution of 85.7% for Principal Component 1 and 10% for Principal Component 2. Furthermore, the HCA analysis corroborated the PCA findings, revealing similar sample groupings. The dendrogram produced by Minitab showcased the most distinct sample groupings. Overall, the combination of UV-vis spectrophotometry and PCA can effectively differentiate black cumin oil from corn and soybean oil.

Keywords: Chemometrics; Black Cumin Oil; Uv-Vis Spectrophotometry

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INTRODUCTION

Currently, numerous health issues frequently arise, leading to an increasing consideration of vegetable oil as a potential solution due to its effectiveness in addressing various diseases (Calugar, Grozea, and Butnairu, 2024). However, the adulterating of vegetable oil has become a significant problem in recent years (Bian et al., 2022). A common practice in Indonesia involves substituting high-quality vegetable oils with lower-cost alternatives that are more readily available (Zulmi et al., 2022). One such oil that is often adulterated due to its relatively high price is black cumin oil, which can reach Rp1120/ml, making it expensive for just 1 ml. The adulteration of black cumin oil with other vegetable oils can diminish its health benefits. Black cumin oil is known for its various health advantages, including antitumor, anti-inflammatory, central nervous system depressant, analgesic, and hypoglycemic effects. Despite this, the analysis of black cumin oil adulterating has not been extensively studied using UV-Vis spectrophotometry. Previous analyses have been conducted by Rohman & Ariani (2013) utilizing a combination of FTIR spectroscopy and PLS chemometrics methods, as well as using the UHPLC-Q-TOF–MS/MS method (Kotecka-Majchrzak et al., 2020). Therefore, there is a pressing need for a more rapid and sensitive instrumental method to analyze vegetable oil adulteration (Bian et al., 2022).

Vegetable oil adulterating analysis has been the subject of extensive research. One effective approach involves the integration of spectroscopy with chemometric methods. Notably, studies by Ali et al. (2018) explored the combination of Fluorescence Spectroscopy and Principal Component Analysis (PCA). Additionally, research conducted by Han et al. (2020), Dou et al. (2023), and Sufriadi et al. (2023) employed a combination of Fourier Transform Infrared (FTIR) spectroscopy and chemometric techniques, demonstrating successful application in the classification and quantification of adulterate oils. Furthermore, Balbino et al. (2022) utilized Near-Infrared (NIR) spectroscopy alongside chemometric methods, showing that this combination effectively distinguished pure oil from alduterate oil. The analysis of oil adulterating has also incorporated the combination of Gas Chromatography-Mass Spectrometry (GC-MS), phytosterol profile determination, and chemometrics, with findings indicating that these methods are applicable for adulterating analysis (Yang et al., 2024).

Research on the analysis of vegetable oil adulterated has often relied on instruments that are not widely available in all laboratories due to equipment limitations (Andriyani et al., 2019). Therefore, there is a need to develop a more accessible method for analyzing black cumin oil adulterating. One promising approach is the use of UV-Vis spectrophotometry (Jiang et al., 2015). However, when analyzing mixtures of different vegetable oils with UV-Vis spectrophotometry, overlapping spectra can occur because they share the same ultraviolet-active group (Bian et al., 2022). To address this issue, multivariate chemometric analysis is necessary to interpret the UV-Vis spectra accurately. Principal Component Analysis (PCA) and Hierrarchical Cluster Analysis (HCA) is one such chemometric technique that can be effectively employed in the study of vegetable oil adulterating(Jiang et al., 2015 ; Granato et al., 2017).

Principal Component Analysis (PCA) is a statistical technique utilized to simplify complex data by grouping samples (Damayanti et al., 2020; Guntarti et al., 2020). This method has been effectively integrated with UV-Vis spectrophotometry to analyze olive oil adulterating, yielding results that successfully differentiate oil mixtures based on the first three principal components (Jiang et al., 2015). Hierarchical Cluster Analysis (HCA) further enhances data interpretation by grouping samples according to their similarities, creating a hierarchical structure illustrated in a dendrogram. This dendrogram elucidates the relationships among samples and the groups formed. HCA can classify oil samples based on their chemical composition, distinguish between different oil types, and identify patterns within oil composition (Granato et al., 2017). In this study, the integration of these methodologies is expected to effectively differentiate between black cumin oil, corn oil, and soybean oil, thereby providing optimal results for identifying instances of black cumin oil adulterating.

METHODS

Sampling

The sampling method employed in this study is consecutive sampling. This approach continues until the required number of samples is achieved. A total of 8 samples of black cumin oil from various brands available in Purwokerto and/or through electronic commerce were collected. Additionally, the standard oils used in this study include black cumin oil, corn oil, and soybean oil, all of which possess a Certificate of Analysis (CoA).

Sample Characteristics Observation

Observation of sample characteristics involves assessing color, odor, texture, and free fatty acid content. Black cumin oil, corn oil, and soybean oil were evaluated organoleptically for their respective color, odor, and texture. The observed characteristics were then compared to reference values for each oil. To determine the acid number, soybean oil samples were analyzed. A 3-gram sample was weighed and placed in a 250 mL Erlenmeyer flask, followed by the addition of 50 mL of 95% ethanol. After mixing, 3 to 5 drops of phenolphthalein (PP) indicator were added. The resulting solution was titrated with a 0.1 N sodium hydroxide standard solution until a pink color appeared. Following the titration, the free fatty acid content was calculated using the formula from Badan Standarisasi Nasional (1998).

$$\text{Free fatty acids (mg KOH/g)} = (V \times T \times 56{,}1)/m$$

Details :
V = volume of NaOH required for imaging, in mL
T = normality of NaOH
m = sample weight, in grams

Absorbance Measurement and Wavelength Spectrum Profile Reading of 100% Oil

Black cumin, corn, and soybean oils, each weighing up to 20 mg, were dissolved in 100 mL of n-Hexane to achieve a concentration of 200 ppm. The resulting solution was then measured for absorbance using UV-Vis spectrophotometry over a wavelength range of 200 to 800 nm (Amereih et al., 2014).

Making Oil Mixtures

The vegetable oil mixture was prepared at concentrations of 20%, 33%, 40%, and 66% (Amereih et al., 2014), using the following formula for comparison:

Table 1. Comparison of Oil Mixtures

No. Sample	Concentration (%)	Black Cumin Oil (mL)	Corn Oil (mL)	Soybean Oil (mL)
1	Black Cumin 66% (A)	10	5	0
2	Black Cumin 66% (B)	10	0	5
3	Corn 66%	5	10	0
4	Corn 40%	10	10	5
5	Soybean 40%	10	5	10
6	Black Cumin 33%	5	5	5
7	Corn 33%	0	5	10
8	Soybean 33%	0	10	5
9	Black Cumin 20%	5	10	10

Wavelength Spectrum Measurement of Oil and Sample Mixtures

Black cumin oil samples collected in Purwokerto were measured at 10 mg and dissolved in 25 mL of n-Hexane, resulting in a solution with a concentration of 400 ppm. Additionally, a Quality Control solution was prepared by mixing the market sample with the previously created oil mixtures. Two Quality Control solutions were developed: Quality Control 1, which contained 8 mL of market sample E and 8 mL of 66% black cumin oil (A), and Quality Control 2, which included 8 mL of market sample E and 8 mL of 40% soybean oil. The sample solution with a concentration of 400 ppm, along with the Quality Control solutions and oil mixtures at concentrations of 20%, 33%, 40%, and 66%, were analyzed for absorbance using UV-Vis spectrophotometry across a wavelength range of 200 - 800 nm (Amereih et al., 2014).

Chemometric Analysis

The absorption measurement data from all samples, obtained through UV-Vis spectrophotometry at wavelengths ranging from 200 to 300 nm, were converted into CSV format. This CSV file was subsequently utilized for multivariate analysis employing Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) with Minitab 22 Trial, R Studio 4.3.3, and Metaboanalyst 6.0 software. The incorporation of three different software platforms in this study aims to evaluate the similarities and differences in the analysis results across these tools.

RESULT AND DISCUSSION

Sample Characteristics

The results of observations of sample characteristics can be seen in Table 2. Black cumin oil is yellowish-brown, has a distinctive fresh cumin aroma, and features a slightly thick texture. These observations regarding color, odor, and texture align with findings from Azzahra et al. (2018). The results are consistent with those reported by both Azzahra et al. (2018) and Farhan et al. (2021). However, there are variations in the acid number: Azzahra et al. (2018) report it as 15.69 mg KOH/g, while Farhan et al. (2021) state it as 14.3 mg KOH/g. The acid number of black cumin oil, according to the Certificate of Analysis, is 14.18 mg KOH/g.

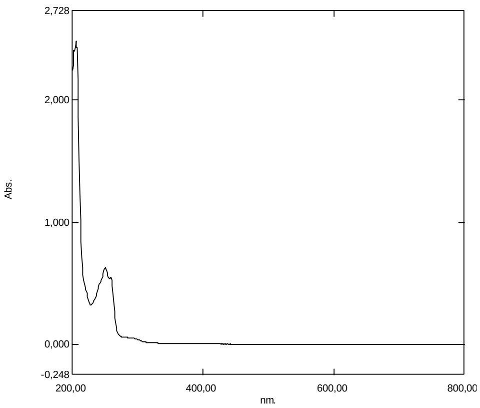
Table 2. Sample Characteristics

No	Sample	Characteristic			
		Color	Smell	Texture	Acid Number (mg KOH/g)
1	Black Cumin Oil	Tawny	Fresh cumin flavor	A little thick	14.18
2	Corn Oil	Yellow	Typical weak corn	A little thick	0.16
3	Soybean Oil	Clear yellowish	Soybean specialty	Liquid	2.46

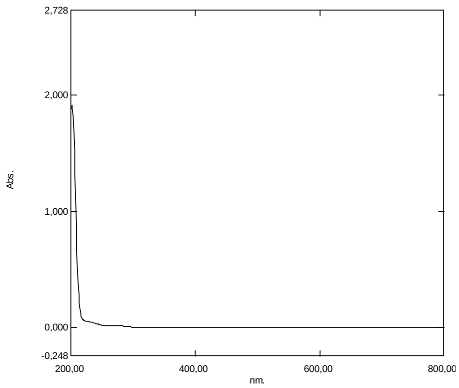
Corn oil is characterized by a light yellow color, a faint corn aroma, and a slightly thick texture, consistent with SNI 01-3394-1998 standards. According to the Certificate of Analysis, the acid number of corn oil is 0.16 mg KOH/g. In contrast, soybean oil is yellow, has a distinct soybean scent, and a liquid texture, with an acid number of 2.46 mg KOH/g. These findings align with Puspita (2016), who noted that the maximum acceptable acid number is 3 mg KOH/g.

Black Cumin, Corn and Soybean Oil Spectrum Profile Analysis 100%

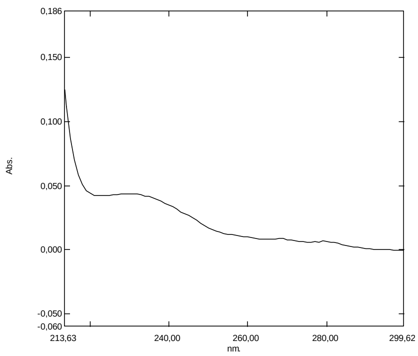
Black cumin oil exhibits two characteristic peaks at wavelengths of 250 and 258 nm, as shown in Figure 1 (a). In contrast, corn oil displays two peaks at wavelengths of 268 and 279 nm, depicted in Figure 1 (b). Additionally, soybean oil has a single characteristic peak at a wavelength of 230 nm, illustrated in Figure 1 (c).



(a)



(b)



(c)

Figure 1. Spectrum Profile of 100% Oil (a) Black Cumin Oil (b) Corn Oil (c) Soybean Oil

Oil Mixture Spectrum Profile Analysis

Almost all samples exhibit two typical peaks in the wavelength range of 250-260 nm, similar to the absorption peaks of 100% black cumin oil, except for the 33% corn and soybean oils. This discrepancy arises because these two concentration series do not contain 100% black cumin oil. For the other oil concentration series, the peaks primarily differ in intensity. At wavelengths of 220-240 nm, both the 33% corn and soybean oils show typical peaks characteristic of 100% soybean oil. The spectrum profile of the oil mixture does not reveal any typical peaks associated with 100% corn oil at 265-270 nm. However, a peak resembling that of 100% corn oil appears in the 250-260 nm range. This variation in the typical peak is likely due to the oil mixture containing not only 100% corn oil but also 100% black cumin and soybean oils, consistent with the formulated mixture (Uncu & Ozen, 2019). The spectrum profile of the oil mixture is illustrated in Figure 2.

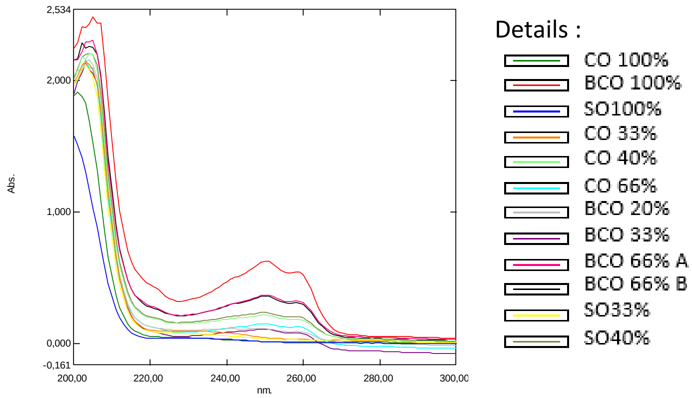


Figure 2. Oil Mixture Spectrum Profile

Analysis of Black Cumin Oil Spectrum Profile in the Market

The black cumin oil samples available on the market, as well as those used for quality control (QC), exhibit a diverse range of spectral shapes and intensities in the wavelength range of 200-800 nm, as shown in Figure 3. Market samples A, B, C, and D demonstrate peak absorption between 220-310 nm, while market sample E peaks at 240-280 nm. Market samples F, G, and H show peak absorption at 230-300 nm. In contrast, QC samples 1 and 2 have peak absorption at 250-260 nm. Variations in peak positions and the potential absence of a discernible absorption peak may result from differences in quality, variety, and geographic origin of the samples analyzed (Uncu & Ozen, 2019).

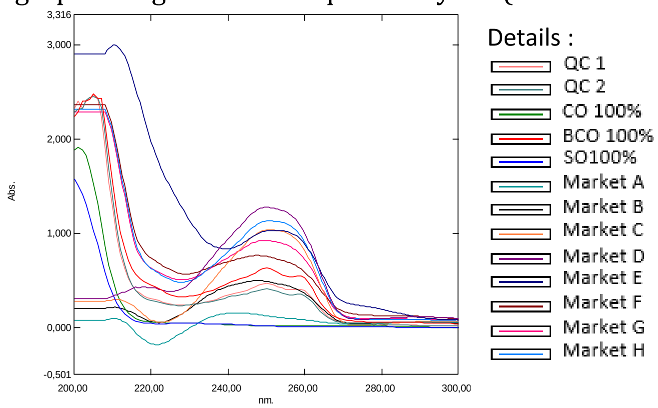


Figure 3. Spectrum Profile of Black Cumin Oil in the Market

Chemometric Analysis
Wavelength Selection

The optimal wavelength selection process should consider the 100% oil profile (Figure 1) and the solvent's UV cut-off (Figure 4). To ensure accurate data, absorbance values recorded between 200 and 210 nm must be excluded (Raja and Barron 2024). The selected wavelengths indicate that absorbance values in the 220-290 nm range can yield effective grouping data.

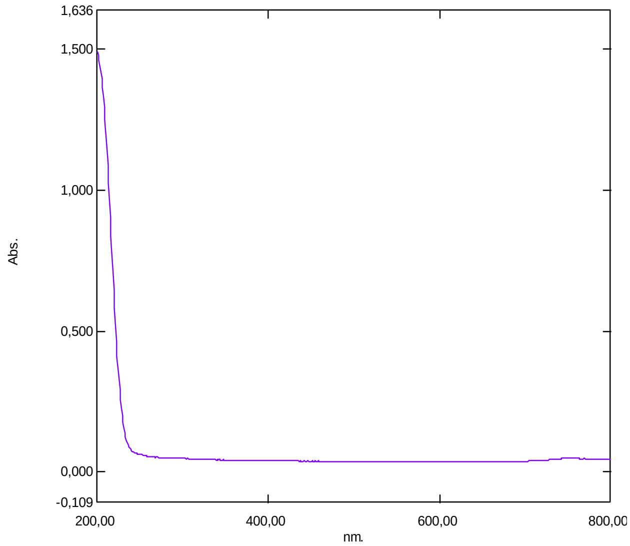
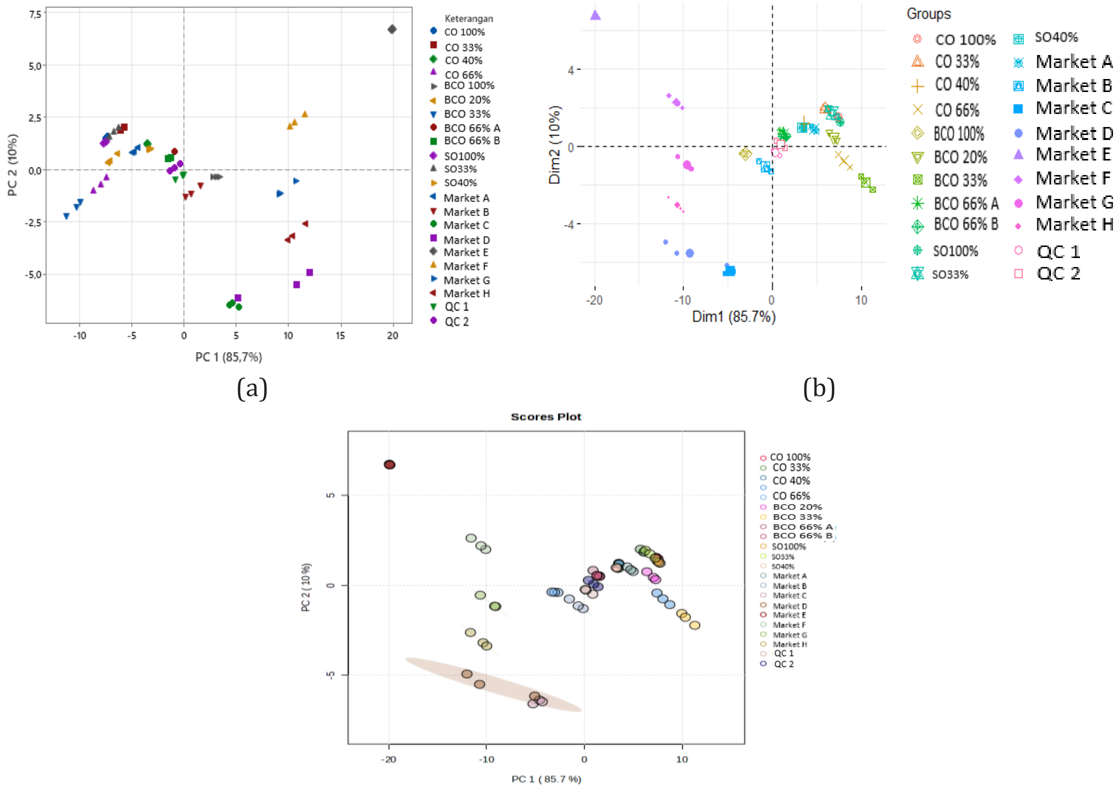


Figure 4. UV-Cut off N-hexane

Principal Component Analysis

Principal Component Analysis (PCA) is an effective method for reducing data dimensionality by identifying principal components that encapsulate the greatest variability of the original dataset. This PCA procedure employs an operation matrix to transform the data into a series of new components that are mutually orthogonal. The outcomes of the PCA are represented in a score plot, which delineates the similarities and differences among samples, and a loading plot, which elucidates the variables that drive the observed differences. In the context of oil mixture analysis, PCA can be utilized to identify patterns in oil composition, differentiate between various oil types, and evaluate other parameters within the oil composition (Arina, Shiyan, and Suprayetno, 2022; Andriansyah et al., 2022; Beattie and Esmonde-White, 2021).

In this study, Principal Components 1 and 2 were selected, accounting for 95.7% of the data variability. The results illustrated in Figure 5 depict the sample replication points. The score plot indicates distinct groupings based on the concentration of the oil mixture and its components. Notably, the 100% black cumin oil sample is distinguishably separated from the oil mixture group, as well as from the 100% corn and soybean oil samples. Conversely, the 100% corn and soybean oils occupy overlapping areas within the plot, making visual differentiation challenging. This difficulty is likely attributable to the similar physical and chemical properties of corn and soybean oils (Rohman et al., 2021). A significant factor influencing the results of the PCA analysis is the concentration of free fatty acids. The findings indicate that the free fatty acid values of corn and soybean oils are closely aligned, resulting in their proximity on the score plot. In contrast, black cumin oil is positioned distinctly away from the other two. These results underscore the considerable impact of chemical characteristics, such as free fatty acids, on chemometric analysis (Baskara, Lelono, and Widodo, 2016).



(a) (b)

(c)

Figure 5. Score Plot of PCA Analysis on Software (a) Minitab (b) R Studio (c) Metaboanalyst

In the oil mixture sample, the location of each sample plot corresponds closely to the dominant component of each mixture. The plot for the 66% black cumin oil sample is situated near the 100% black cumin oil point, as 100% black cumin oil is the predominant component in its formulation. Conversely, the 33% soybean and corn oil plot is positioned near the 100% soybean and corn oil point, reflecting its composition, which is primarily influenced by 100% soybean or corn oil. The plots for the 40% corn and soybean oil, 66% corn, and 33% black cumin oil are located between the groups of black cumin, corn, and 100% soybean oil, indicating that none of these compositions are significantly dominant.

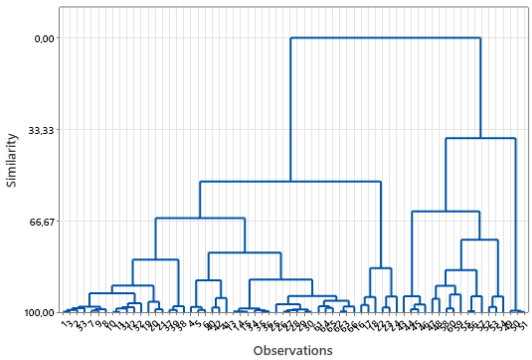
The market samples exhibited no discernible grouping. Market sample B is identified as the closest to 100% black cumin oil, based on its similar physical and chemical properties. In contrast, market sample A belongs to the oil mixture group, positioned adjacent to the sample containing 40% corn and soybean oil. It is estimated that market sample A shares comparable physical and chemical properties with the 40% corn and soybean oil. Meanwhile, market samples C, D, E, F, G, and H are located in regions that are not adjacent to either the oil mixture group or the 100% black cumin oil. Consequently, the oil content of samples other than A and B remains unknown, as indicated by the score plot, which does not position them near the oil mixture group, 100% black cumin oil, or 100% corn and soybean oil. In the quality control (QC) sample, a grouping is observed with 66% black cumin oil samples A and B, attributed to the QC sample's composition of 66% black cumin oil, resulting in similarities in their physical and chemical properties (Rafi et al., 2018; Batubara et al., 2022; Rohman et al., 2021). Therefore, the developed PCA model can effectively differentiate between groups of oil mixtures, including black cumin oil, corn, and 100% soybean oil.

The findings of the study indicated that there were no significant differences in the PCA analysis results among the three software programs utilized. Variations were observed primarily in the presentation of the score plot data. In Minitab, the separation between samples is readily discernible visually, aided by the inclusion of distinct symbols for each sample, which facilitates result interpretation. Conversely, in R Studio, the symbols for each sample are less visually accessible, making analysis more challenging. In Metaboanalyst, the color coding of samples does not exhibit substantial differences, potentially leading to inaccuracies in visual analysis of the PCA results.

Hierarchial Clustering Analysis

Hierarchical Cluster Analysis (HCA) is a statistical method that groups samples according to their similarity, resulting in a hierarchical structure represented by a dendrogram. This dendrogram illustrates the relationships among samples and the resulting groups. HCA can be employed to classify oil samples based on their chemical composition, differentiate various types of oil, or identify patterns in oil composition (Granato et al., 2017).

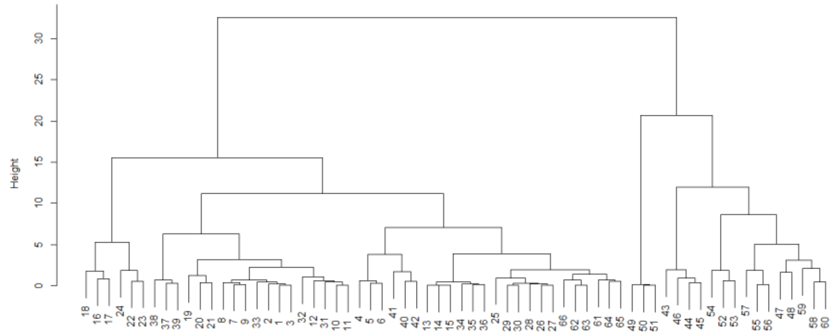
The HCA clustering results presented in Figure 6 effectively distinguished between the oil mixture cluster and the 100% oil category. Among the eight market samples analyzed, this method identified that market sample B exhibited a higher similarity to 100% black cumin oil, while market sample A showed a notable similarity to the 40% corn and soybean oil mixture. One of the chemical components influencing the HCA analysis is the concentration of free fatty acids. The results of this study indicate that the free fatty acid values of corn and soybean oils are relatively similar, which is reflected in their close positioning on the dendrogram. In contrast, black cumin oil is positioned significantly further away from these two oils. These findings suggest that chemical characteristics, such as free fatty acids, have a considerable impact on chemometric analysis (Baskara, Lelono, and Widodo, 2016).



Details :

1 – 3 = CO 100%	34 – 36 = SO40%
4 – 6 = BCO 100%	37 – 39 = Market A
7 – 9 = SO100%	40 – 42 = Market B
10 – 12 = CO 33%	43 – 45 = Market C
13 – 15 = CO 40%	46 – 48 = Market D
16 – 18 = CO 66%	49 – 51 = Market E
19 – 21 = BCO 20%	52 – 54 = Market F
22 – 24 = BCO 33%	55 – 57 = Market G
25 – 27 = BCO 66% A	58 – 60 = Market H
28 – 30 = BCO 66% B	61 – 63 = QC 1
31 – 33 = SO33%	64 – 66 = QC 2

(a)



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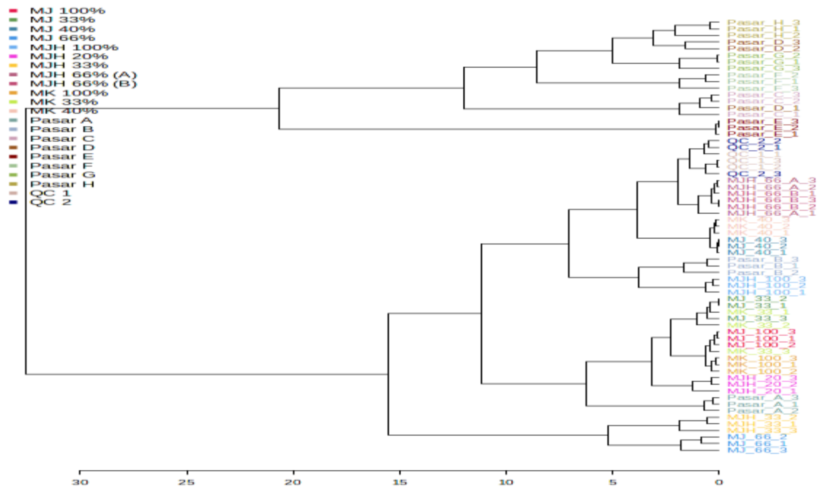

(c)

Figure 6. HCA Analysis Dendogram in Software (a) Minitab (b) R Studio (c) Metaboanalyst

The results of the HCA analysis demonstrated significant differences among the three software programs utilized. Specifically, there were instances of multiple replications in samples that were not grouped. This was particularly evident in the Metaboanalyst software, which revealed four samples where the three replications remained ungrouped. In contrast, the Minitab and R Studio software did not exhibit similar findings.

This study was conducted with qualitative chemometric analysis using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) to see the grouping of oil mixtures between black cumin oil, corn oil and soybean oil. Chemometric analysis using PCA and HCA is appropriate, but other chemometric analysis can be used to complement the results of this study. Other chemometric analysis include LDA (Linear Discriminant Analysis), SVM (Support Vector Machines and SIMCA (Soft Independent Modeling of Class Analyze).

CONCLUSION

The analysis in this study concluded that combining UV-vis spectrophotometry with Principal Component Analysis (PCA) effectively distinguishes black cumin oil from corn and soybean oil. This is evidenced by the results of chemometric analysis, which demonstrate distinct groupings of oil samples. These groupings are based on the similarities in their physical and chemical properties. Additionally, the three software programs used for the chemometric analysis did not reveal significant differences in the

PCA results, as illustrated in the scree plot and score plot curves. The only variation observed was in the presentation of the score plot data.

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AUTHOR CONTRIBUTION

THW: Concepts or ideas; design; definition of intellectual content; literature search; experimental studies; data analysis; manuscript preparation.
YNA: literature search; experimental studies; data analysis; manuscript preparation.
MSF: Concepts or ideas; design; definition of intellectual content; literature search; experimental studies; data analysis; manuscript preparation.
NEE : Concepts or ideas; design; definition of intellectual content; literature search.
MWSP : Concepts or ideas; design; definition of intellectual content; literature search.

ETHICS APPROVAL

None

CONFLICT OF INTEREST (If any)

None to declare

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