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Rapid Identification of Turmeric (*Curcuma longa*) Essential Oil Authenticity using FTIR Spectroscopy Coupled with Chemometrics

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Turmeric essential oil (TEO) exhibits diverse biological activities that include anti-inflammatory, cytotoxic, anti-arthritis, hepatoprotective, antifungal, antibacterial, and antioxidant properties. Problem statement: Many TEOs sold on online shopping platforms do not use proper labels. So, these TEOs could harm customers if they contain adulterated essential oils. Objective: This study aimed to authenticate TEO from vegetable oils, namely soybean oil (SO), palm oil (PO), and virgin coconut oil (VCO). Method: TEO was extracted using the hydrodistillation technique. Eleven binary mixtures of TEO with vegetable oils (0-100%) were prepared, and 13 commercial TEOs were obtained from online shopping platforms. All essential oils were then analyzed using FTIR spectroscopy coupled with chemometric analysis. Findings: The extracted TEO was pale yellow with a distinctive odor, yielding 0.21% (v/w). Discriminant analysis (DA) successfully distinguished pure TEO from adulterated TEO at specific wavenumbers (TEO-SO and TEO-PO models at 2000-650 cm^{-1} and TEO-VCO models at 4000-650 cm^{-1}). However, no commercial TEO was categorized as pure TEO using the developed DA models. For quantification purposes, the best PLS regression models for quantifying SO, PO, and VCO levels in TEO were in wavenumber ranges of 3500-2500 cm^{-1}, 2000-700 cm^{-1}, and 1500-700 cm^{-1}, respectively. Conclusion: FTIR spectroscopy combined with chemometrics is able to authenticate pure turmeric essential oil. The commercial turmeric essential oils were not detected as pure TEO.

Keywords: Turmeric; essential oil; FTIR Spectroscopy; Chemometrics; authentication

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The increasing demand and use of essential oils for various purposes encourage the practice of adulteration to increase profits and set competitive prices [1]. Essential oils are sold on various e-commerce platforms and sometimes unlabeled. So, consumers are unaware that the oil they buy may be counterfeit [2]. Essential oil quality and purity standards must be guided by governing institutions such as the International Standard for Standardization (ISO) or Indonesian National Standard (SNI) [3]. However, there is no standard to regulate the specific characteristics of turmeric essential oil.

Turmeric is a plant native to Southeast Asia and grows in tropical and subtropical regions [4], belonging to the family Zingiberaceae [5]. Turmeric is a perennial herb that can grow up to one meter tall. The rhizome is the main part of the turmeric plant, from which buds grow. The rhizome is branched, cylindrical, brightly colored yellow to orange, and fragrant. A visual representation of *Curcuma longa* is presented in Figure 1. Turmeric rhizome essential oil has several biological activities, such as anti-inflammatory, anticonvulsant, antidiabetic, antiplatelet, cytotoxic, anti-arthritis, hepatoprotective, sedative, anesthetic, antifungal, antibacterial, and antioxidant [6].

Commercial turmeric essential oils are sold on online shopping platforms without proper labels. Thus, the oil composition is unknown and this may harm customers. Therefore, it is crucial to authenticate essential oils. Additive substances may be added to the oil, including vegetable oils such as virgin coconut oil, soybean oil etc., or other solvents with similar physical characteristics. They may be less expensive than the essential oil. Authentication is aimed at ensuring quality and grade and avoiding the adulteration of traded essential oils. Several analytical techniques, such as chromatography (TLC, HPLC, and GC) and spectroscopy (UV-Vis, FTIR, NMR, and mass), have been used to authenticate essential oils. However, non-targeting analysis using fingerprinting with Fourier transform infrared (FTIR) spectroscopy has become popular as an alternative to classical GC-based methods, as it's fast, cost-effective, and environmentally friendly [7].

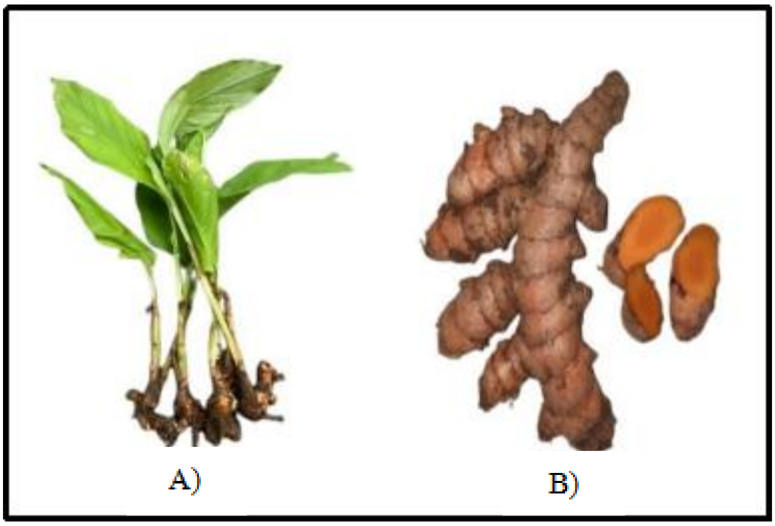

Figure 1. *Curcuma longa*. (A) Plant (B) Rhizome.

Fourier Transformation Infrared (FTIR) spectroscopy, when combined with chemometrics analysis, offers a powerful tool for authenticating essential oils. Chemometrics, in particular, provides significant advantages by enabling faster and more economical analyses [2]. It can be used for both qualitative and quantitative purposes, with pattern recognition methods like discriminant analysis for qualitative purposes and PLS (Partial Least Square) for quantitative analysis, which aim to establish a model for analyzing unknown samples [8].

The combination of FTIR spectroscopy with chemometrics such as discriminant analysis and partial least square (PLS) regression has been used for the authentication of four essential oils, namely wintergreen, rosemary, tea tree, and lemon eucalyptus oils, adulterated with either lemongrass essential oil or peppermint essential oil, and the authentication of oregano, thyme, chamomile, red ginger (2,9), and white turmeric oils adulterated with vegetable oils [10]. However, there are limited studies on utilizing FTIR spectroscopy coupled with chemometrics to identify turmeric as an essential oil. Therefore, this study aimed to authenticate TEO from vegetable oils, namely soybean oil (SO), palm oil (PO), and virgin coconut oil (VCO).

EXPERIMENTAL

Chemicals and Materials

The samples used were fresh turmeric (*Curcuma longa*) rhizomes, soybean oil (MamaSuka®), coconut oil (Barco®), and palm oil (Bimoli®), and commercial turmeric essential oils purchased through E-commerce platforms. An ATR-FTIR spectrometer (Thermo Scientific®) and the TQ Analyst™ software version 9 were employed in the analyses.

Characterization Methods

Sample Collection and Physical Characterization

Approximately 10 kg of turmeric (*Curcuma longa*) rhizomes was obtained from Kinali, Pasaman Barat, West Sumatra, Indonesia. A botanist at the ANDA herbarium, located in the Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, was consulted to identify the rhizome. The fresh rhizomes were washed thoroughly with water to remove any dirt. The clean rhizomes were then cut to the thickness of 2-3 mm and the essential oil was extracted by hydrodistillation method using a Clevenger-type apparatus for 6-8 hours. The essential oil was collected into an amber bottle. To remove any remaining water, anhydrous Na_2SO_4 was added. Then, the essential oil was stored at 4°C before further use. The physical properties of the turmeric essential oil (TEO) were assessed, which included its color, odor, specific gravity, and refractive index.

Preparation of Binary Mixtures of Turmeric Essential Oil and Vegetable Oil

Binary mixtures were prepared by mixing TEO with vegetable oils such as soybean oil (SO), palm oil (PO), and virgin coconut oil (VCO) at various concentrations (0%-100% (v/v)), as shown in Table 1. Pure TEO essential oil and the binary mixtures were labeled as "pure" and "mixed", respectively, to distinguish between TEO that is not diluted and TEO that is mixed with vegetable oils.

Table 1. Binary Mixtures of Turmeric Essential Oil with Vegetable Oils.

Mixture	**Percentage, % (v/v)**	
	Turmeric Essential Oil	**Vegetable Oil**
1	100	0
2	90	10
3	80	20
4	70	30
5	60	40
6	50	50
7	40	60
8	30	70
9	20	80
10	10	90
11	0	100

Analysis of Turmeric Essential Oil by ATR-FTIR (Attenuated Total Reflection Fourier Transform-Infra Red) Spectroscopy

A Thermo Scientific FTIR Nicolet iS10 spectrometer was used to acquire the FTIR spectra. The measurements were taken at the temperature of 25°C. Samples (TEO and commercial turmeric essential oils) were placed on the Smart iTR ATR surface and scanned in the MIR region of the 4000-650 cm^{-1} wavenumber range. Before measuring the TEO spectra, the background air was measured. The spectral measurements were repeated three times. The spectral analysis was performed using the OMNIC software version 9.

Chemometric Analysis

The TQ Analyst™ software version 9 was used for discriminant analysis and PLS (Partial Least Square) analysis. Discriminant analysis was used to classify turmeric essential oil with binary mixtures. While PLS was to create a quantitative prediction model to measure the purity of turmeric essential oil. The observed parameters include latent variables, correlation coefficient (R^2 value), standard error Root Mean Square Error Calibration (RMSEC) and Root Mean Square Error Prediction (RMSEP), and outlier diagnosis.

Table 2. Turmeric Essential Oil Characterization.

Parameters	**Current study**	**Indonesian Pharmacopeia, 2017** [14]	**Previous studies**
Color	Pale Yellow	-	-
Odor	Specific, pungent	-	Specific, pungent [12]
Yield, % (v/w)	0,21	1,85	0,36% [13]
Specific gravity (g/ml)	0,95	-	0,901-0,971 [19]
Refractive index	1,512	-	1,499-1,521 [19]

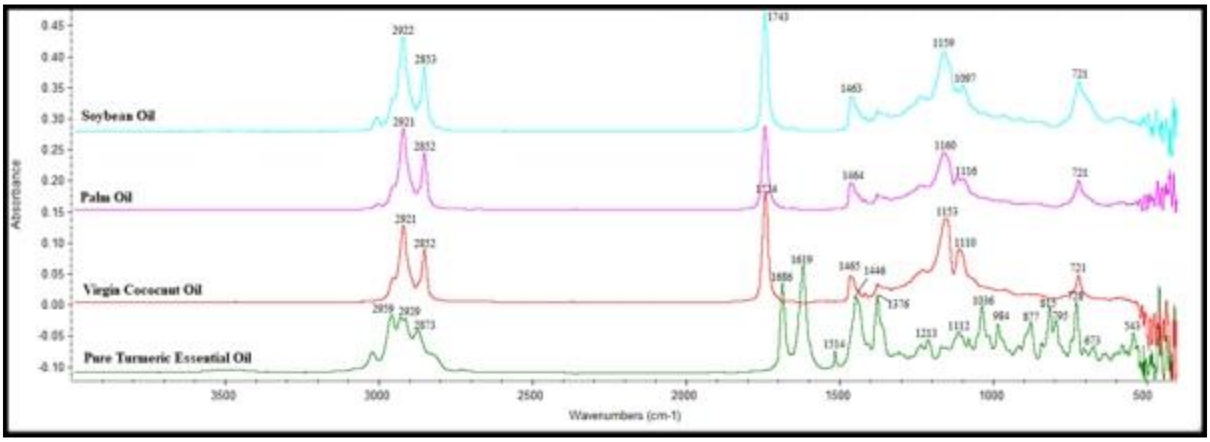

Figure 2. FTIR spectra of TEO, VCO, PO, and SO.

RESULTS AND DISCUSSION

Physical Characterization of Turmeric Essential Oil

The quality of essential oil can be determined by its physicochemical characteristics. Table 2 provides the information on the physical qualities of turmeric essential oil, such as yield, color, refractive index, and specific gravity [11]. The parameters could refer to the international and national standards [3]. In general, the TEO obtained was a yellow to orange liquid with a fresh, spicy taste and an aromatic odor that resemble sweet citrus and ginger, as well as a sharp, burning bitterness [12]. In this study, the yield of the turmeric essential oil was 0.21% (v/w), while a previous study reported a yield of 0.36% [13]. In contrast, Indonesia Herbal Pharmacopedia (FHI) stated that the yield of turmeric essential oil is less than 1.85% (v/w) [14]. Several factors influence the yield of essential oils, including distillation method [15], time or drying process [16], distillation time [17], harvest time, geographic variation of plant origin, plant variety, raw material quality, and pre-use material management [18].

Turmeric Essential Oil and Vegetable Oil Analysis

Figure 2 shows that the FTIR spectrum of the turmeric essential oil exhibits a distinct pattern from that of the vegetable oils. However, the FTIR spectra of soybean oil (SO), palm oil (PO), and virgin coconut oil (VCO) show nearly identical patterns, with variations only in the intensity of each peak. This suggests that the chemical compounds present in these oils are virtually the same, differing only in concentration.

Table 3. Functional groups of turmeric essential oil and vegetable oils.

Wavenumbers (cm^{-1})	Functional Groups	Literature
2959, and 2873	Asymmetrical and symmetrical stretching vibration of methylene ($-CH_3$) group	[22]
2929, 2922, 2921, 2853, and 2852	Asymmetrical and symmetrical stretching vibration of methylene ($-CH_2$) group	[22]
1743, 1724, 1686, and 1619	Carbonyl C=O stretching vibration	[9]
1514	Aromatic ring bending vibration (C=C-C)	[22]
1465, 1464, 1463, and 1446	Methylene (CH2) bending vibration	[9]
1376	Methyl symmetric bending vibration (C-H3)	[9]
1213, 1160, 1159, 1153,	C-O carbonyl (ether) stretch vibration	[9]
1116, 1112, 1110, 1097, 1036	C-O ether bending vibration	[9]
987	-HC=CH-(Trans), out of plane vibration	[23]
877, 815, 795, 721, and 728	-HC=CH-(Cis), out of plane vibrations	[23]
673	-(CH2)n Stretch: -HC≡CH-	[23]

Most of the peaks and shoulders of the FTIR spectra of the turmeric essential oil and vegetable oils are caused by specific functional groups responsible for infrared absorption. The functional groups in the turmeric essential oil components will appear as peaks at specific wavenumbers, as listed in Table 3. The turmeric essential oil mainly consists of sesquiterpenes and monoterpenes. On the other hand, soybean oil (SO), palm oil (PO), and virgin coconut oil (VCO) typically consist of triglycerides, glycerol, and various fatty acids [20, 21].

Based on Table 3, the strongest peaks at 1743 and 1724 cm^{-1} are attributed to the result of carbonyl (C=O) stretching vibrations found in the vegetable oils and are identified as triglyceride groups [24]. However, in the pure turmeric essential oil, the peak is not as prominent.

Chemometrics Analysis

Discriminant analysis was used to differentiate between pure turmeric essential oil and turmeric essential oil adulterated with vegetable oils. The Mahalanobis distance was calculated using absorbance at 2000-650 cm^{-1} (DA TEO-SO and TEO-PO models) and 4000-650 cm^{-1} (DA TEO-VCO model). These absorbance values were used as variables for classifying pure and adulterated oils. Cooman plots were generated using this data.

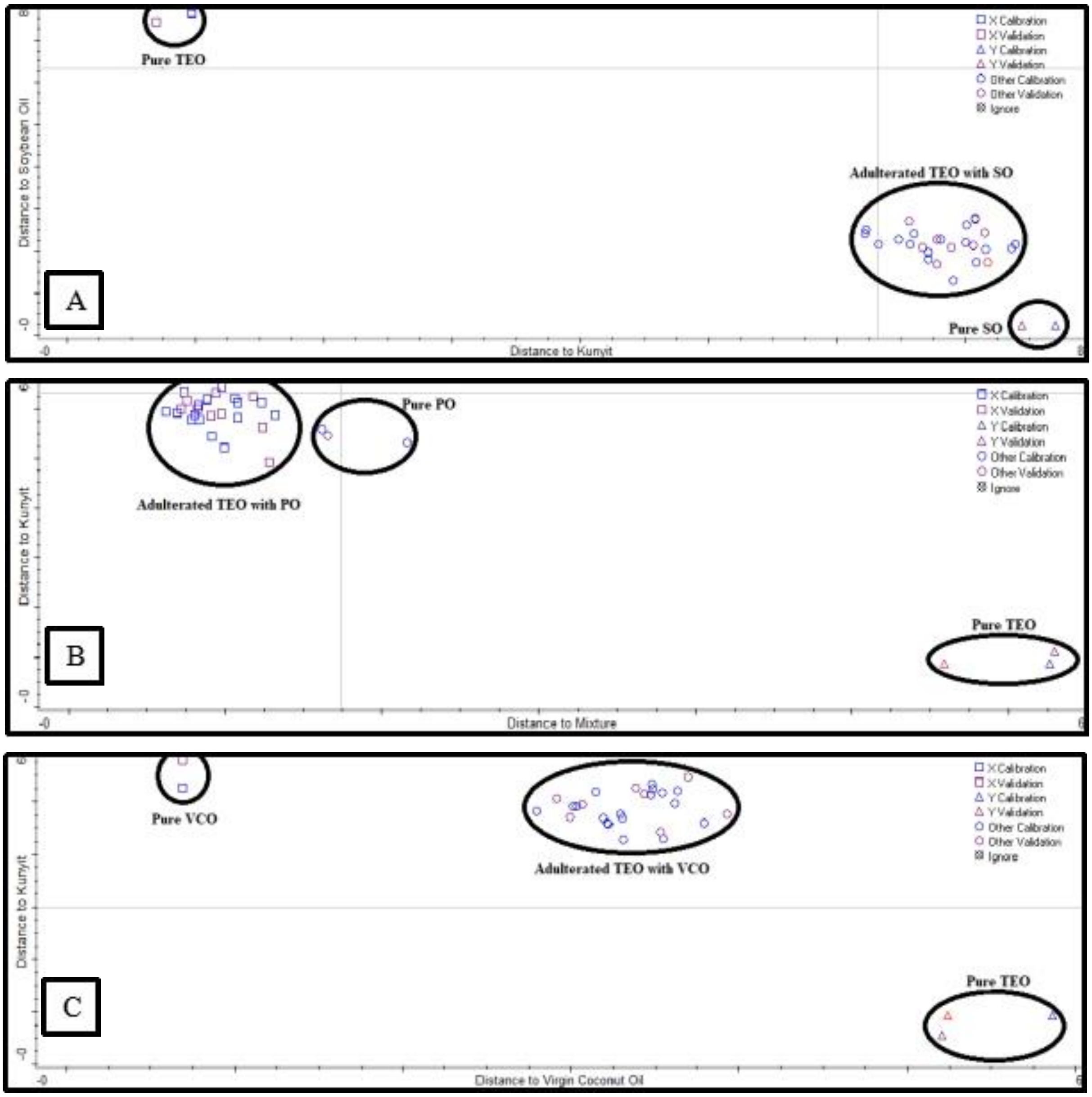

Figure 3. Cooman Plots for Pure TEO and Binary Mixtures: (A) soybean oil (SO), (B) palm oil (PO), and (C) virgin coconut oil (VCO).

Table 4. The best PLS Model from Multivariate Calibration of Each Binary Mixture.

Multivariate Calibration	Wavenumbers (cm^{-1})	Spectra	Calibration		Validation	
			R^2	RMSEC	R^2	RMSEP
PLS (TEO-SO)	3500-2500	**Normal**	**0,9949**	**0,0319**	**0,9981**	**0,0282**
		1st derivate	0,9997	0,00816	0,9958	0,0315
		2nd derivate	0,9979	0,0205	0,9959	0,0369
PLS (TEO-PO)	2000-700	**Normal**	**0,9970**	**0,0244**	**0,9943**	**0,0358**
		1st derivate	0,9983	0,0182	0,9889	0,0492
		2nd derivate	0,9993	0,0117	0,9827	0,0611
PLS (TEO-VCO)	1500-700	Normal	0,9908	0,0428	0,9795	0,0647
		1st derivate	**0,9986**	**0,0167**	**0,9921**	**0,0399**
		2nd derivate	0,9974	0,0227	0,9898	0,0497

Figure 3 reveals the Cooman plots for the classification results between pure turmeric essential oil and binary mixtures. The discriminant analysis achieved 100% accuracy with no misclassifications. A similar study conducted by Syafri et al. used the essential oil from white turmeric, a plant also belonging to the Zingiberaceae family. In their research, discriminant analysis distinguished between pure white turmeric essential oil, mixtures, and vegetable oils. The spectral range for the discriminant analysis of white turmeric essential oil and its adulteration with vegetable oils (soybean oil, virgin coconut oil, and palm oil) was observed at wavenumbers in the range of 4000-650 cm^{-1} [10].

Additionally, determining the level of impurities in mixtures of turmeric essential oil involves using PLS multivariate regression analysis. The acceptability of the FTIR spectral region and multivariate calibration is evaluated based on achieving a high coefficient of determination (R2) close to 1 and minimizing the Root Mean Square Standard Error of Calibration (RMSEC) and Root Mean Square Error of Prediction (RMSEP) close to 0 (22). The best PLS model for each binary mixture is shown in Table 4.

The research findings indicated that the best calibration and prediction model of the binary mixture of turmeric essential oil with soybean oil were by using the spectral information in the 3500-2500 cm^{-1} region. On the other hand, the binary mixture of turmeric essential oil with palm oil showed the best calibration and prediction using the spectral information in the 2150-650 cm^{-1} range. In the case of the binary mixture of turmeric essential oil with virgin coconut oil, the best calibration and prediction of the PLS model utilized the spectral information in the range of 1500-700 cm^{-1}. A previous study on TEO, soybean oil, and sunflower oil found that spectral range analysis of the 3200 - 500 cm^{-1} region results in a better performance in the predictive ability of the PLS model (25). Additionally, another study on white turmeric oil (WTO) and adulterants (vegetable oils) found that the best PLS Regression occurred at wavenumbers in the ranges of 4000-1100 cm^{-1} (WTO-SO models), 1400-1050 cm^{-1} (WTO-VCO models), and 2100-650 cm^{-1} (WTO-PO models) [10]. In addition, the study on red ginger oil (RGO) revealed the best PLS Regression model at wavenumbers in the range of 3100-2700 cm^{-1} for palm oil, while wavenumbers in the 3100-2700 cm^{-1} and 1850-650 cm^{-1} regions were the best PLS model for quantifying soybean oil in RGO [2].

The study used discriminant analysis (DA) and PLS models to forecast the purity of 13 commercial turmeric essential oil samples (Brands A-M). The DA model was employed to classify these samples, with the Mahalanobis distance calculated for each commercial turmeric essential oil and are presented in Table 5. Based on the distance, the software determines whether a commercial turmeric essential oil belongs to the pure turmeric essential oil group. If it falls within the same group as pure turmeric essential oil, it is classified as a pass, indicating that the commercial turmeric essential oil is pure. Conversely, if it fails, it suggests that the commercial turmeric essential oil is not pure and may be adulterated. An external validation test using pure turmeric essential oil samples not used in building the model was conducted to validate the DA model for classification.

Table 5. Summary of Discriminant Analysis (DA) Parameters.

No	Samples	Model	PI	No of Components	Variability (%)	Class Name	Distance	Pass/Fail
1.	Validation Test	TEO-SO	91,7	9	99,99	TEO	2,98	Pass
		TEO-PO	91,6	10	99,99	TEO	2,03	Pass
		TEO-VCO	93,8	10	99,99	TEO	2,09	Pass
2.	Brand A	TEO-SO	91,7	9	99,99	TEO	22,01	Fail
		TEO-PO	91,6	10	99,99	PO	10,35	Fail
		TEO-VCO	93,8	10	99,99	Mixture	5,19	Fail
3.	Brand B	TEO-SO	91,7	9	99,99	TEO	18,61	Fail
		TEO-PO	91,6	10	99,99	Mixture	15,97	Fail
		TEO-VCO	93,8	10	99,99	TEO	8,45	Fail
4.	Brand C	TEO-SO	91,7	9	99,99	Mixture	9,81	Fail
		TEO-PO	91,6	10	99,99	PO	16,67	Fail
		TEO-VCO	93,8	10	99,99	Mixture	8,18	Fail
5.	Brand D	TEO-SO	91,7	9	99,99	TEO	11,48	Fail
		TEO-PO	91,6	10	99,99	TEO	18,13	Fail
		TEO-VCO	93,8	10	99,99	TEO	7,24	Fail
6.	Brand E	TEO-SO	91,7	9	99,99	TEO	24,51	Fail
		TEO-PO	91,6	10	99,99	Mixture	18,71	Fail
		TEO-VCO	93,8	10	99,99	TEO	20,08	Fail
7.	Brand F	TEO-SO	91,7	9	99,99	Mixture	13,26	Fail
		TEO-PO	91,6	10	99,99	PO	8,33	Fail
		TEO-VCO	93,8	10	99,99	Mixture	4,91	Fail
8.	Brand G	TEO-SO	91,7	9	99,99	SO	25,71	Fail
		TEO-PO	91,6	10	99,99	Mixture	26,10	Fail
		TEO-VCO	93,8	10	99,99	VCO	9,98	Fail
9.	Brand H	TEO-SO	91,7	9	99,99	TEO	9,50	Fail
		TEO-PO	91,6	10	99,99	PO	15,73	Fail
		TEO-VCO	93,8	10	99,99	TEO	6,08	Fail
10.	Brand I	TEO-SO	91,7	9	99,99	TEO	8,25	Fail
		TEO-PO	91,6	10	99,99	PO	14,92	Fail
		TEO-VCO	93,8	10	99,99	TEO	6,34	Fail
11.	Brand J	TEO-SO	91,7	9	99,99	Mixture	13,22	Fail
		TEO-PO	91,6	10	99,99	PO	8,40	Fail
		TEO-VCO	93,8	10	99,99	Mixture	5,34	Fail
12.	Brand K	TEO-SO	91,7	9	99,99	Mixture	23,56	Fail
		TEO-PO	91,6	10	99,99	Mixture	23,03	Fail
		TEO-VCO	93,8	10	99,99	VCO	16,86	Fail
13.	Brand L	TEO-SO	91,7	9	99,99	Mixture	9,65	Fail
		TEO-PO	91,6	10	99,99	PO	16,03	Fail
		TEO-VCO	93,8	10	99,99	Mixture	7,04	Fail
14.	Brand M	TEO-SO	91,7	9	99,99	Mixture	5,26	Fail
		TEO-PO	91,6	10	99,99	PO	12,02	Fail
		TEO-VCO	93,8	10	99,99	Mixture	5,09	Fail

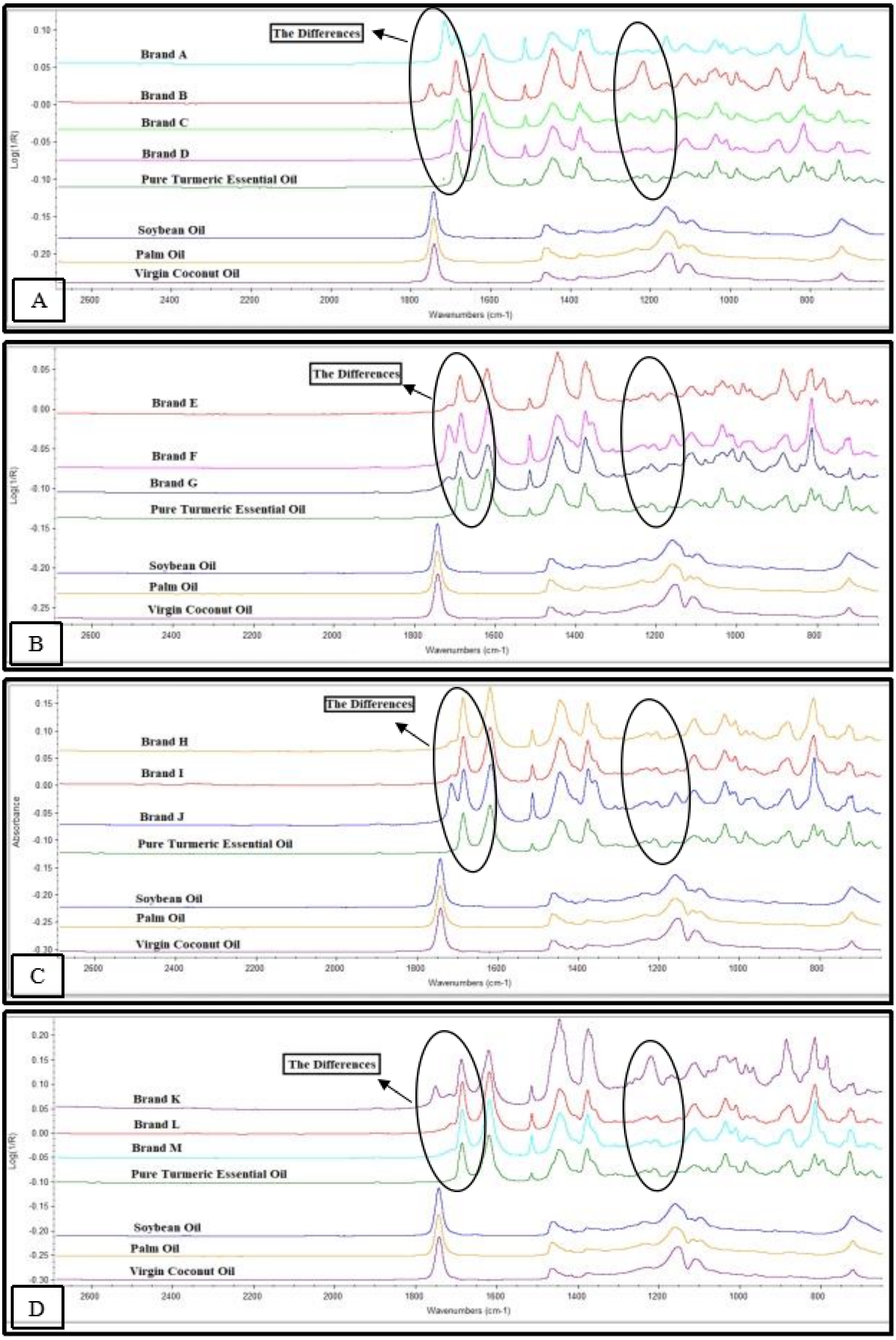

Figure 4. Differences in Spectral Shape of Commercial Turmeric Essential Oils (Brands A-M) with Pure Turmeric Essential Oil and Vegetable Oils.

The findings showed that the commercial oils have Mahalanobis distances far from pure turmeric essential oil, so they are stated as fails, which means that the oil group does not belong to pure or mixed turmeric essential oil. The length of Mahalanobis distances may be due to adulterated commercial products or the TEO originating from different locations and extraction methods. Some commercial TEOs originating from Indonesia and India are extracted using the steam distillation method. Also, it can be seen in Figure 4 that the spectral patterns of Brands A-M turmeric essential oils show some differences from pure turmeric essential oil.

CONCLUSION

The combination of FTIR spectroscopy with chemometrics can be used to authenticate turmeric rhizome essential oil in soybean oil, palm oil, and coconut oil. From the analysis, it was found that some commercial turmeric essential oils are not grouped as pure turmeric essential oil. Hence, this method can be used as an initial screening for turmeric essential oil authentication, demonstrating its practical application. Further evaluation is necessary to ensure the purity of turmeric essential oil, and this can be achieved using GC-MS spectroscopy.

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