

THE USE OF GC-MS AND FTIR SPECTROSCOPY COUPLED WITH MULTIVARIATE ANALYSIS FOR THE DETECTION OF RED GINGER OIL ADULTERATION

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ABSTRACT

The ginger oil traded worldwide could come from various sources. Standard quality is the most critical aspect of ensuring customer safety. This study aims to develop an analytical method for red ginger oil (RGO) authentication. Chemical compositions of red ginger oil were determined by Gas Chromatography-Mass Spectrometry (GC-MS). The Fourier Transform Infrared Spectroscopy (FTIR) coupled with multivariate analysis (discriminant analysis (DA), partial least square (PLS), and principal component regression (PCR) were used to identify and quantify the adulterant. The total terpenoid compounds were 55.72%, with the percentage of monoterpenes at 34.29% and sesquiterpenes at 21.43%. E-Citral (19.01%), Z-Citral (14.82%), Geranyl Acetate (11.90%), Geraniol (9.56%), 1,8-Cineole (5.84%), and camphene (4.92%) were identified as the main constituents. The best PLS model for quantifying the level of palm oil in RGO was at the wavenumber 3100–2700 cm⁻¹, while the region of 3100 – 2700 and 1850 – 650 cm⁻¹ was suitable for detection of soybean adulterants. FTIR spectroscopy coupled with chemometrics produced accurate and fast authentication of red ginger oil without the used solvent. Then, the GC-MS technique could identify the chemical constituents present in the red ginger oil.

Keywords: Red Ginger Oil, Authentication, Spectroscopy, Chemometrics, FTIR.

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INTRODUCTION

Ginger oil is among the most traded essential oils (EOs) worldwide and is one of the Indonesian export commodities.¹ It is used for various purposes in food products and packaging, pharmaceutical formulations, and aromatherapy since ginger oil has been awarded safe by Food and Drug Administration.²⁻⁷ These EOs was sold on many online shopping platforms without a proper label. Hence, the customers have easily obtained these EOs at lower prices. Nevertheless, only a few people know that their oils may be counterfeit.⁸ The supplier may adulterate the EOs by diluting them with plant-based oil such as palm oil, soybean oil, candlenut oil, etc., or organic solvent, such as triethyl citrate.^{9,10} The specific characteristics of ginger oil have been regulated in ISO 16928:2014. Still, it was specified for EOs of ginger cultivated in China, India, and West Africa. But, ginger oil's physicochemical properties and yield were affected by the cultivation location, variety, cultivation process, moisture levels during harvesting, extraction method, and plant age.¹¹ There is a need for the current analytical method for red ginger EOs (RGO) authentication. The fingerprinting approach using a combination of FTIR and chemometric tools offer various advantages, including rapid, low-cost, sensitive, and reliable.^{12,13} Meanwhile, the Gas Chromatography-Mass Spectrometry could identify and characterize the essential oil components.¹⁴ However, no study utilized these two approaches simultaneously to authenticate red ginger oil. Therefore, the current study aimed to use an analytical method including GC-MS and FTIR coupled with multivariate for authentication of red ginger oil.

EXPERIMENTAL

Material

The red ginger rhizomes were obtained from the local area (Lubuk Kailangan) in West Sumatera, Indonesia during June – September 2021. The rhizomes were cleaned and sliced using a cutter, then transferred into the distillation flask connected to the Clevenger apparatus. The hydrodistillation process was performed for 6 hours. The essential oils obtained were transferred to a dark bottle and placed at 4°C until further used for analysis. The adulterants were palm oil (PO) and soybean oil (SO) purchased from the local market.

Preparation of Binary Mixture Red Ginger Oil (RGO)/Adulterant

The binary mixtures were prepared by mixing RGO with palm oil (PO) and soybean oil (SO) at various concentrations from 0 percent to 100 percent v/v (Table-1). The authentic RGO and RGO diluted with SO and PO were labeled as "pure" or "adulterated."

Table-1: Preparation of Binary Mixture RGO with PO/SO

Mixture	Percentage (%v/v)	
	RGO	PO/SO
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

Compositional Analysis of Red Ginger Oil by Gas Chromatography-Mass Spectroscopy (GC-MS)

The red ginger oil (RGO) compositions were analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS) (Shimadzu GCMS-QP 2010 SE). The experimental condition was similar as previously reported.¹⁵ The compound was identified using the "WILEY library" available in the GC-MS software.

ATR-FTIR Spectra Measurement

The FTIR spectra were obtained from (Nicolet iS10 FTIR spectrometer, Thermo Nicolet Corp, Madison, WI). The sample was put on the ATR (Smart iTR) surface and scanned in the MIR region with wavenumbers of 4000–650 cm⁻¹ at a controlled room temperature of 25°C. It was run with scanning for 32 scanners and a resolution of 8 cm⁻¹. The resulting spectra were automatically adjusted or corrected by previously measured air as background. The spectra measurement was performed in triplicate. All spectra obtained were saved and interpreted by OMNIC software (Version 8. 0, Thermo Nicolet, Madison, WI).

Chemometric Analysis

TQ analystTM version 9 was used for discriminant analysis and PLS and PCR analysis for quantitative prediction model for the authenticity of EOs. The parameters observed were included latent variables, correlation coefficient (R² value), standard error (RMSEC and RMSEP), and outlier diagnostics.

RESULTS AND DISCUSSION

Chemical Composition Red Ginger Oil

The chemical constituents of red ginger oil were characterized by GC-MS. Fig.-1 revealed the RGO chromatogram, which showed about 70 compounds. They consisted of sesquiterpenes, monoterpenes, and other aliphatic compounds. In this study, oxygenated monoterpene E-Citral (19.01%), Z-Citral (14.82%), Geranyl Acetate (11.90%), Geraniol (9.56%), 1,8-Cineole (5.84%), and hydrocarbon monoterpenes Camphene (4.92%) were the main constituent of the RGO. The total terpenoid compounds were 55.72%, with the percentage of monoterpenes at 34.29% and sesquiterpenes at 21.43%. Nevertheless, a previous study in

Malaysia found the RGO had 81.9% monoterpene consisting of camphene (14.5%) as the principal constituent, followed by citral (14.3%) and geranyl acetate (13.7%), as seen in Table-2.¹⁶ The constituent of red ginger oil extracted from *Zingiber officinale var rubrum* cultivated in West Sumatera, Indonesia, contained higher monoterpene compounds than sesquiterpenes. This finding was similar to those reported by other studies conducted in Aceh, which found 60.55% of monoterpene dominated by 1,8-Cineole (15,1%), E-Citral/Geraniol (12%), Z-Citral/Neral (7,1%), Borneol (6,8%), and sesquiterpenes were ar-curcumene (16,9%), β -sesquiphellandrene (6,8%), β -bisabolene (6.4%).¹⁷ In contrast, another study in a different area of Indonesian regions showed ar-curcumene, zingiberene, cedrelanol, geraniol, selina-6-en-4o, geranyl acetate, noniphenol, trans-sesquisabinene hydrate, and citral as the main component.¹⁸ The ISO standard 16928:2014 also revealed the different constituents for Ginger oil originated from the *Zingiber officinale* Roscoe (White ginger) from three different countries (India, China, and West Africa). For India origin, five major compounds namely Zingiberene (35-40%), β -sesquiphellandrene (11.5-13.5%), ar-curcumene (6.5-9%), camphene (5-8%) and β -bisabolene (2.5 – 5.5%) should be in the acceptable percentage. For the neral compound, chinese origin Ginger oil contained 0-0.5%, while the Indian and West African origin contained neral in the percentage of 0.1-0 and 0.2-2%, respectively. In addition, Chinese ginger oil should contain geraniol 0 – 0.6%, while Indian and West African in 0.5 – 3.5%.¹⁹

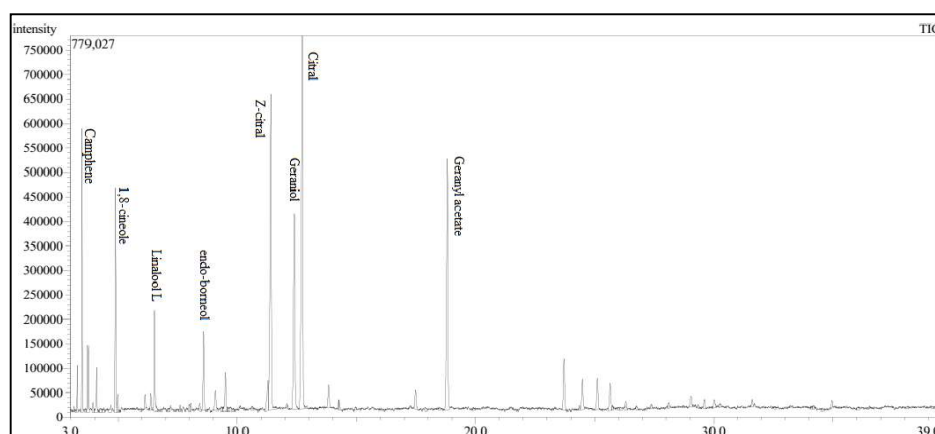


Fig.-1: Total Ion Chromatogram of Red Ginger Oil

Table-2: Chemical Composition of RGO (current study and previously reported)

Compound	Percentage of Relative Area (%)	
	Current study	Previous research ¹⁶
Citral	19.01	14.3
Z-citral	14.82	7.7
Geranyl acetate	11.90	13.7
Geraniol	9.56	7.3
1,8-cineole	5.84	5
Camphene	4.92	14.5
Linalool L	3.24	2.3
Endo-Borneol	2.73	2.9
Ar-Curcumene	2.43	1
β -Citronellol	1.69	0.4
α -Terpineol acetate	1.63	0
Zingiberene	1.50	3.2
β -Bisabolene	1.46	0
β -Sesquiphellandrene	1.39	1.6
6-Methyl-5-hepten-2-one	1.31	0.2
β -Myrcene	1.10	0
Endobornyl acetate	1.04	1.4

FTIR Analysis

Figure-2 shows FTIR spectra of essential oils of pure red ginger oil and adulterant (PO and SO) scanned at wavenumbers 4000-650 cm^{-1} . Since the main components in both oils differed, the FTIR spectra revealed some differences. RGO was mostly made up of monoterpenes¹⁶, whereas palm and soybean oils were mostly made up of triglycerides, glycerol esters, and various fatty acids.^{20,21} The spectral bands were designated in accordance with previous research.

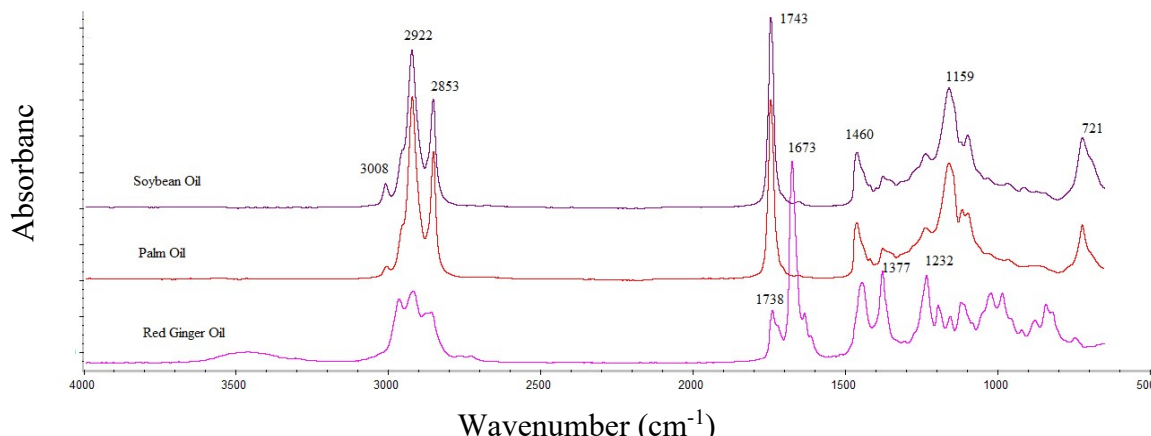


Fig.-2: FTIR spectra of red ginger oil (RGO), palm oil (PO) and soybean oil (SO)

Functional group of each compound absorbs the IR radiation and would appear as peak at specific wavenumber. The peak at 3464 cm^{-1} is as a result of the stretching vibration of O-H, while the peaks at 3008 cm^{-1} is regarded as cis C=CH stretching. The asymmetric stretching vibrations of methyl (-CH₃) and methylene (-CH₂) groups are appeared at 2953 cm^{-1} and 2920 cm^{-1} respectively. Peaks at 2873 cm^{-1} correspond to the symmetric stretching vibration of the methyl (-CH₃) group. The peak at 1673 cm^{-1} and 1232 cm^{-1} are only observed in RGO which correspond to C = C, stretching. However, the broad peak at 1159 cm^{-1} was observed in PO and SO attributed to C-O ether, bending.²²⁻²⁴

Chemometric Analysis

Comparing IR spectra allows adulterants in red ginger EOs to be easily detected. On the other hand, the prediction of adulterant content in binary EO mixtures necessitates the use of multivariate regression analysis of PLS and PCR. The parameters used to determine whether to use FTIR spectral regions and multivariate calibration is based on a high R_2 value and the lowest standard error for calibration (RMSEC) and prediction (RMSEP).²⁴ The PLS and PCR model of RGO in PO/SO adulterant were developed in different regions in FTIR spectral data. The best calibration and prediction of PLS model were acquired using spectral information from the 3100-2700 cm^{-1} range. For the calibration and validation model, the R_2 values were 0.9973 and 0.9969, respectively. In addition, the lowest RMSEC (0.0242) and RMSEP (0.0261) values were also observed at this wavenumber region (Fig.-3A). On the other hand, the best calibration and prediction of the PCR model in the 1850-650 cm^{-1} region, the coefficient determination R_2 was 0.9944, RMSEC and RMSEP 0.0347 and 0.0351 for calibration and validation, respectively. The SO adulterant prediction model showed that the 3100 – 2700 and 1850 – 650 cm^{-1} region was the best for the PLS prediction model with the R_2 was 0.9999, RMSEC 0.00571 for the calibration, and the R_2 was 0.9991.

Discriminant analysis (DA) is used to distinguish between pure and adulterated RGO.²⁵ The Mahalanobis distances were then calculated using these absorbances to generate Cooman's plot. Cooman's plot illustrates the classification of RGO and RGO adulterated with PO and SO (Fig.-4A dan 4B). It shows 100% accuracy and without misclassification. The implementation of the FTIR technique combined with chemometrics has successfully separated pure and adulterated RGO as applied to patchouli oil in the other study.²⁶ Moreover, other studies on authentication of *Curcuma mangga* EOs using PLSR model revealed that 1614-1068 cm^{-1} was the best wavenumber for quantifying the adulterant. The discriminant analysis also differentiated the categorization of pure *C. mangga* EOs and adulterated *C. mangga* EOs with 100% accuracy.

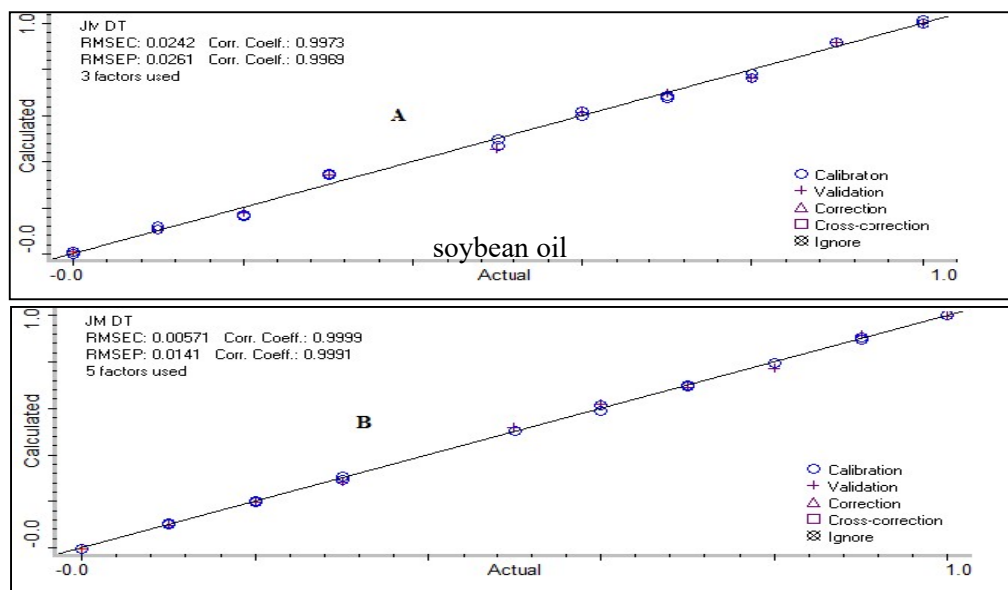


Fig.-3: Partial Least Square Regression (PLS) Prediction Model of Red Ginger Oil in (A) Palm Oil (B) Soybean Oil

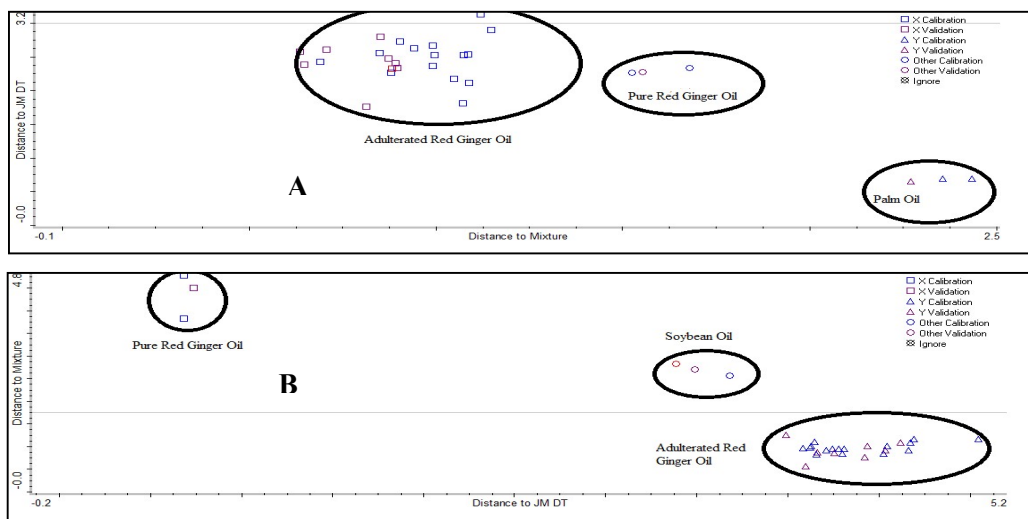


Fig.-4: The Cooman Plots for Pure RGO and Adulterated RGO with (A) Palm Oil and (B) Soybean Oil

CONCLUSION

The characterization of RGO by GC-MS technique identified E-Citral (19.01%), Z-Citral (14.82%), Geranyl Acetate (11.90%) as the main component. To authenticate red ginger essential oil, a combination of FTIR spectroscopy and chemometrics could be used. It promised fast, robust, and more economically than other analytical techniques. Besides, it was environmentally friendly due to no solvent used and produced less waste. The FTIR spectra were used as a fingerprint to discriminate the authentic and adulterated red ginger oil by evaluating the Cooman plots. For quantification purposes, PLS and PCR model was developed. The best model for quantifying palm oil in RGO at the region of 3100–2700 cm^{-1} , while the region of 3100 – 2700 and 1850–650 cm^{-1} for soybean as an adulterant.

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