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Halal authentication of sesame oil (*Sesamum indicum L.*) in market using GC-MS and FTIR spectrometry combination of chemometrics

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ABSTRACT: The benefits of sesame oils for therapeutic purposes lead to the adulteration of sesame oil samples with lard. This case was prohibited because it may harm consumers' health, and Muslim consumers don't consume haram products. Therefore, this research aims to determine the quality of isolated sesame oil sold in the Market using GC-MS and FTIR spectroscopy combined with chemometrics. Based on GC-MS, pure sesame oils and pork oil contain several similar dominant compounds, such as oleic acid, palmitic acid, and stearic acid; however, the fatty acid levels are different. Furthermore, this study reported that both sesame oils and lard have vibration fingerprinting at the wavelength of cm^{-1}. The partial least square produces a low error with a root means square error calibration (RMSEC) of 0.01 and a determination coefficient (R^2) 1. The validation method produces a low error with root means square error cross-validation (RMSECV) of 1.009 with R^2 of 0.9981 and root mean square error prediction (RMSEP) of 0.56 with R^2 of 0.9929. This study also reported that both oils can be separated; hence, FTIR spectroscopy combined with principal component analysis (PCA) successfully discriminates halal sesame oil. Furthermore, authentication of market samples shows that no market sesame oils detected contain pork oil adulterants.

KEYWORDS: Sesame; halal; GC-MS; FTIR; chemometrics; pork.

1. INTRODUCTION

Sesame oil from sesame seeds contains 42% to 60% (o/w) and is consumed as a flavoring oil and salad dressing in Asia and Europe. Consumers highly seek sesame-based samples due to their promising health benefits and bioactive compounds. Sesame seeds contain unsaturated fatty acids, vitamins, and antioxidants [1]. Therefore, sesame oil has many benefits in preventing oxidative processes; hence, it can be used as a food ingredient [2]. Another study by Hsu and Parthasarathy [3] reported that this oil can reduce low-density lipoprotein (LDL) levels and control high-density lipoprotein (HDL) levels. It has high nutritional value because it contains protein, lipids, and other active compounds responsible for treating several diseases. The fatty acids in sesame oil are oleic, linoleic, linolenic, palmitic, and stearic. Sesame oil also contains sesamin, sesamol, and sesamolin, which play an important role in oxidative stability and antioxidant activity [4]. Even though sesame oil is sold at a higher price than other vegetable oils, it could give rise to the potential for counterfeiting to reduce sampling costs. Therefore, these benefits might lead to the adulteration of sesame oil with other oils, including lard. However, fake sesame oil samples sold on the Market may harm consumers' health, and the Market's normal operations will be seriously damaged. As Muslims, we must keep our bodies to consume the haram material.

Many adulterants are added to sesame oil, such as rapeseed, soybean, cottonseed, and palm and corn oils [5-7]. Using lard as an adulterant of vegetable oils is a high candidate because it contains 46.2% saturated fatty acids, 45.2% monounsaturated fatty acids, and 11.0% polyunsaturated fatty acids. A previous study has reported that the adulteration of sesame oils using rapeseed oil, soybean oil, and cottonseed oil was detected in 4 samples, 1 sample, and two samples, respectively [7]. Besides, many cases have found pork fat mostly used as haram added to food products. Because of these problems, the development of an analytical method, which is a fast and effective method for detecting pork fat in food, was needed [7].

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Based on previous explanations, developing detection methods for vegetable oils is crucial to ensure whether these sesame oils are contaminated. However, efficiently detecting vegetable oils has become a research pillar in recent years. Many analytical methods have been developed to ensure the quality of sesame oils, including HPLC [8, 9], electronic nose [10], and GC-MS [6, 11, 12]. However, the previous methods only detect the main components or markers of the sesame oils or adulterants. Furthermore, Pan et al. [13] successfully analyzed the sesame oil's adulterants using 3D fluorescence spectra. Jiao et al. [12] successfully identified lignans in sesame oils using the near-infrared spectrometer. Therefore, this study analyses the adulteration of sesame oil with lard using FTIR spectroscopy combined with chemometrics. The principal component analysis (PCA) is one of the chemometric analyses used to discriminate between sesame oil and other vegetable oils. For quantitative analysis, partial least squares regression (PLSR) is the most appropriate method of chemometrics [14]. Furthermore, FTIR spectroscopy is used in this study due to its simpler procedure and fast, precise, and environmentally friendly analysis. It has great reports, including precision and accuracy for quickly detecting pork fat [15]. FTIR spectroscopy has also been proven to be an appropriate technique for determining various adulterants in food samples, such as lard in cakes and chocolate, animal fat mixtures, and other oil samples [16].

2. RESULTS

2.1. The Identification of the dominant component of sesame oils and pork oil

The analysis of sesame seed oil components using the GC-MS method will show the results of the oil components and the percentage of sesame seed oil compound content. The AUC (Area Under the Curve) shown in the chromatogram is directly proportional to the concentration of each component contained in the sample. Gas chromatography analysis will obtain the possible number of oil components and their levels while determining the type of oil component. This is carried out using mass spectrometry, which is then identified using spectra originating from NIST and WILEY. Each peak was further identified by comparing the mass spectrum with reference compounds in the GC-MS database. Sesame oil components are determined based on the S.I. (Similarity Index) value, which refers to the suitability of the fragmentation pattern of the compound [17]. The results of the GC-MS analysis are shown in Table I, which shows the presence of 4 fatty acids in sesame oil. The identified fatty acids were classified into saturated and monounsaturated fatty acids. Oleic acid (78.27%) was the main fatty acid identified, followed by palmitic acid (7.23%), stearic acid (11.50%), and lauric acid (3.04%).

Table 1. The dominant fatty acid components were analyzed using GC-MS

No	tR (minute)	Fatty acid types	Formula (n)	%Area					SI
				Sesame oil	Pork oil	Sample 1	Sample 2	Sample 3	
1	13.347	*Hexadecanoic acid*	C16:0	7.23	23.73	6.58	1.85	3.61	95
2	15.428	*9-Octadecenoic acid*	C18:1	78.27	59.36	78.21	23.18	49.62	96
3	15.682	*Octadecanoic acid*	C18:0	6.33	15.35	6.33	2.02	3.53	96

2.2. The identification of the functional groups of sesame oils and pork oil

Based on the type of dominant fatty acid in sesame oil and pork oil, there is no difference, but the fatty acid content of each type is different. Therefore, this research developed a halal analysis method that is simple, easy to use, and non-destructive. Based on Figure 1, the FTIR spectrum obtained is in the wave number range of 4000-600 cm^{-1}. There is no significant difference between the fat spectrum of sesame oil and pork oil because the dominant components of both fats are the same, as given in Table 1.

The results of ATR-FTIR spectra analysis (Figure 1) show that functional groups were detected including (A) O.H. stretching, (B) > aliphatic CH_2, (C) > aliphatic CH_2, (D) O=C=O stretching (carbon dioxide), (E) C=O stretching (carbonyl ester), (F) > CH_2->CH_3 bending, (G) C-O, (H) -CH_2.

2.3. Optimization and Quantification analysis using Partial least square

The optimization process of the quantification analysis was performed to obtain the best model to identify pork oil in sesame oil products. Based on this study, the chosen wave number is 3000-2850 cm^{-1} of the FTIR spectra, producing a coefficient of determination (R^2) of 1 and an RMSEC value of 0.01% with linear regression y = 1x + 0.0003. The optimization results show that the accuracy between the predicted and actual

values is 100%. Furthermore, this model contains the cross-validation parameter (RMSECV) of 1.009% with an R^2 of 0.9981 and a linear regression value of y = 1x + 0.0003. Besides, this model has an RMSEP value of 0.56% with a linear regression equation y = 0.9929x + 0.5661 with a coefficient of determination (R^2) of 0.9929.

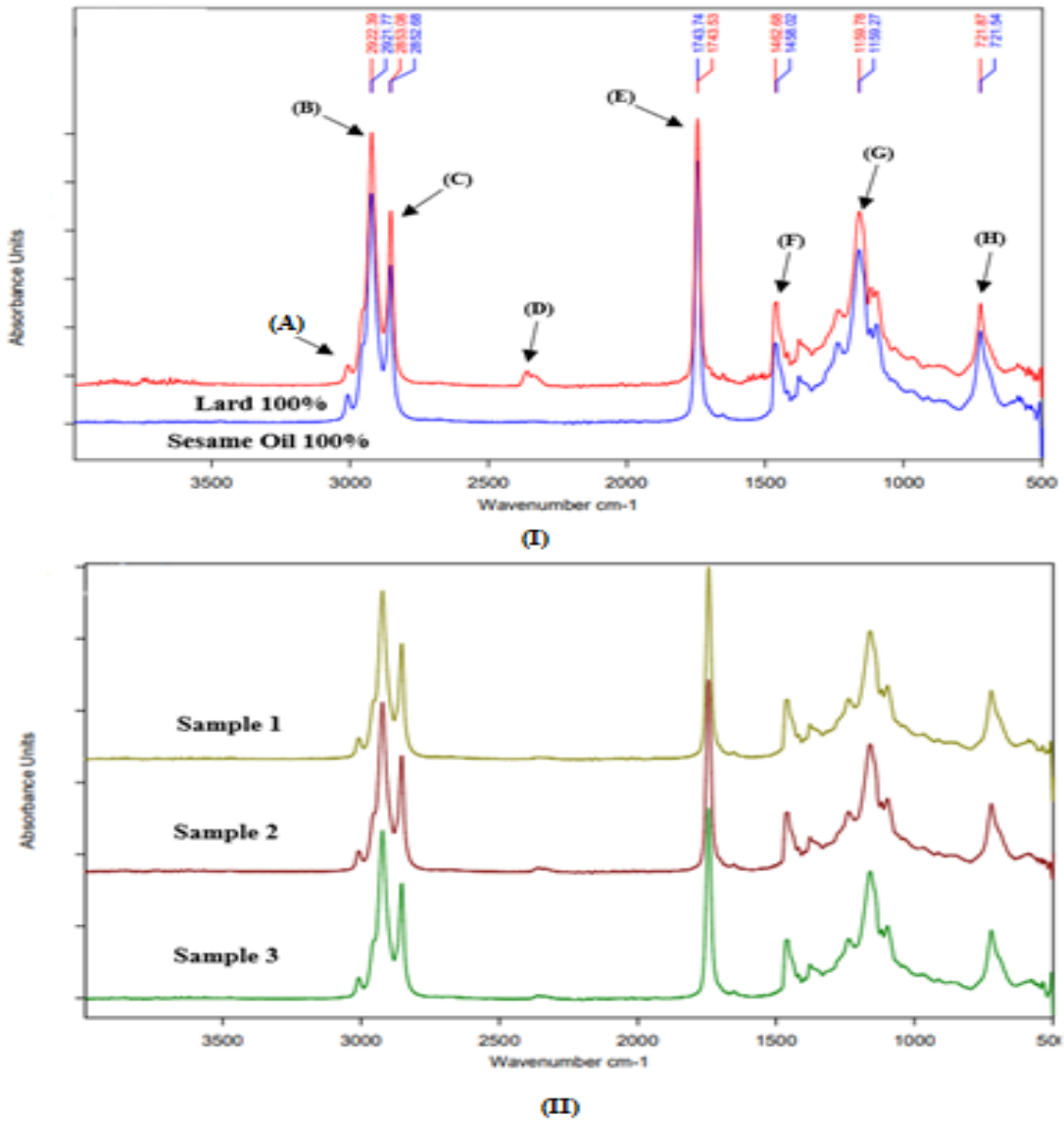

Figure 1. ATR-FTIR Spectra: (I) oils standards; (II) Commercial samples

2.4. Discriminant analysis of the commercial oil samples

The discriminant analysis used PCA to project the separation between sesame and pure pork oil. This study successfully separated sesame oil and pork oil. the PCA results show significant differences between sesame oil (A) and lard oil (B). This gives a good picture of the results to see the differences between the samples and whether any samples contain pork oil adulterants.

3. DISCUSSION

3.1. The Identification of the dominant component of sesame oils and pork oil

In the Indonesian National Standard regarding sesame oil as edible oil, it is stated that the fatty acid composition in sesame oil is oleic acid (35-50%), palmitic acid (7.0-12%), stearic acid (3.5-6.0%), lauric acid (<0.4%), and myristic acid (<0.5%) [17]. The data obtained by researchers on pure sesame oil show a quite significant difference in oleic acid, namely 78.27%. This can occur due to many factors that influence the intensity of the compound content in sesame oil, including environmental factors, the region of origin of

sesame, and differences in method. Harvesting, post-harvest treatment, extraction process, and storage of sesame oil [18]. In Table I, it is shown that all detected components have S.I. values of> 90%. This shows that the sample's oil type matches the comparative spectrum. The base peak is the peak with the greatest abundance in the spectrum, with a value of 100%. If the S.I. value is close to 100% or more than 90%, the detected compound is similar to the comparative data.

In detail, sesame oil mainly consists of palm acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, and behenic acid. Among them, oleic acid has the highest content, which aligns with previous research findings [7]. The proportion of unsaturated fatty acids in the samples was 78.21%-78.27%, corresponding to the range reported in previous studies [19]. In product C, a peak of tetradecanoic acid (myristic acid) was detected at 3.7%. In previous research, the myristic acid content was 0.5-2.0% [7], 1.1-2, 5% [19], and 1.6% [20], which is the concentration of constituent components in palm oil, so it is suspected that Product C contains adulterants in the form of palm oil.

3.2. The Identification of the Functional groups of sesame oils and pork oil

The FTIR spectra produced from sesame oil and pork oil were then interpreted, and a band with a strong intensity of 2922.39 was produced: 2921.77 cm^{-1} and 2853.08; 2852.67 cm^{-1} caused by vibrations >CH2, namely asymmetric (aliphatic) stretching. -CH(CH3), asymmetric strain -CH(CH2)-, and symmetric strain -CH(CH3). The strong band at wave number 1743.74; 1743.53 cm^{-1} comes from C=O (carbonyl ester) stretching triglyceride vibrations. Strong and moderate intensity bands are located at 1462.67 and 1458.02 cm^{-1} caused by cis-vibrations (bending) of -CH2- and -CH3, respectively. Bands at wave numbers between 1159.78 and 1159.27 cm^{-1} are related to C-O stretching vibrations. The last band located at 721.87; 721.54 cm^{-1} represents bending vibrations (rocking mode) of methylene (-CH2) and bending vibrations (out of plane) of cis-disubstituted olefins [21]. From the functional groups that appear, it can be concluded that in pressed sesame oil, methyl ester group compounds provide a general description of the content of sesame oil and lard, which consist of fatty acids.

Sesame oil and pork oil have similarities in functional fat groups. Carbonyl groups, C-H and C-O appear in the FTIR spectrogram [22]. These results are also in accordance with the research of Suparman et al. [23], which stated that the FTIR spectra of pork fat and chocolate when observed visually, will look similar, but there are still differences in the intensity of the resulting bands and maximum absorption frequencies that are different from each other. Differences in the fatty acid composition of lard and chocolate fat cause this. The difference in peak intensity of pork fat from FTIR analysis shows a type of vibration of pork fat molecules that is not visible in brown fat. The detailed functional groups' vibration from sesame oil, pork oil, and commercial oil is illustrated in Table 2.

Table 2. The functional groups vibration from sesame oil, pork oil, and commercial oil

No	Wavenumbers	Functional groups	Intensity				
			Sesame oil	Pork oil	Sample 1	Sample 2	Sample 3
A	3110.50	*Alkenes*	Low	Low	Low	Low	Low
B	2922.39	*Alkanes*	High	High	High	High	High
C	2853.08	*Alkanes*	High	High	High	High	High
D	2350.40	*Amines*		High	High	High	High
E	1743.74	*carbonyl (-C=O)*	High	High	High	High	High
F	1159.78	*ester (-C-O)*	High	High	High	High	High
G	721.87	*Alkyl (CH_2)*	High	High	High	High	High

3.3. Optimization and Quantification analysis using Partial least square

Based on the functional groups detected in Table 2, no visible difference exists. Therefore, it is necessary to conduct an analysis using FTIR spectroscopy combined with chemometrics. One of the chemometric analyses is quantitative determination using PLS. To produce more accurate quantification, selecting the FTIR spectroscopy wave number that most specifically shows the difference between sesame and pork oil is necessary. Therefore, it is necessary to optimize the quantification process. Number optimization in PLS is needed so that model performance can reach optimum even though this method can calculate the entire computerized spectrum [24]. The choice of wavelength and type of spectrum is based on its ability to produce the highest R^2 value and the lowest RMSEC value [25]. Selecting the fingerprint region can also be

used as an initial target for selecting the wave number as a unique absorption band for each molecule [26]. The detailed optimization processes are illustrated in Table 3.

Table 3. Optimization of Wave Numbers for PLS Multivariate Calibration Showing the Relationship Between Actual Values (X-Axis) and Predicted Values (Y-Axis).

Bilangan gelombang (cm^{-1})	R^2	Persamaan regresi	RMSEC
750-650	0.9999	y = 0.9999x + 0.0005	0.10225
800-700	0.9996	y = 0.9996x + 0.0248	0.80630
1300-1100	0.9995	y = 0.9995x + 0.0296	0.88697
1350-1050	0.9976	y = 0.9976x + 0.1551	2.02974
1500-1400	0.9996	y = 0.9996x + 0.0275	0.85440
1800-1650	0.9692	y = 0.9692x + 1.9783	7.24770
3000-2850	**1.0000**	**y = 1.0000x + 0.0003**	**0.01203**
3000-2800	0.9982	y = 0.9982x + 0.1128	1.73106
3100-2850	0.9980	y = 0.9980x + 0.1288	1.84927

The obtained PLS model shows a coefficient of determination (R^2) of 1 and an RMSEC value of 0.01% with linear regression y = 1x + 0.0003 at 3000-2850 cm^{-1} (Figure 2a). Based on this study, the accuracy between the predicted and actual values is 100%. The value of R^2=1 means the correlation between the two variables will be better, meaning that there is a very strong correlation between concentration and absorbance [27], while the smaller the RMSEC value (closer to 0) indicates that the smaller the error that occurs [28]. The RMSECV cross-validation parameter (Figure 2b) obtained an RMSECV of 1.009% with an R^2 of 0.9981 and a linear regression value of y = 1x + 0.0003 (Figure 2 a). A smaller RMSECV value indicates a smaller error, so the model built has better capabilities [29]. Based on the previous study, the adulteration of sesame oils with soybean oil and sunflower seed oil can be detected and classified clearly in wavenumbers ranging from 1,800-650 cm^{-1} [30]. Another study also showed that a classification technique to identify the adulterant of sesame oils with some vegetable oils such as hazelnut, canola, and sunflower oils was analyzed in wavenumbers range of 1267-1209 cm^{-1}, 1121-1045 cm^{-1}, 876-814 cm-1 [31]. Therefore, the use of FTIR wavenumbers for classifying and authenticating sesame oils depends on adulterant types added.

Based on Figure 2c, a linear regression equation is obtained with y = 0.9929x + 0.5661 with a coefficient of determination (R^2) of 0.9929 and an RMSEP value of 0.56%. A low RMSEP value indicates success in creating a regression model. This validation is often referred to as external validation. The results of this validation model show that the validation model carried out can be applied to new samples.

3.4. Discriminant analysis of the commercial oil samples

Based on the PLS results, the quantification process can be carried out in the 3000-2850 cm-1 range. Therefore, the next analysis process was carried out using PCA. In the PCA analysis, the wave number chosen is the wave number that has been previously optimized, namely the wave number 3000-2850 cm^{-1}. The selection of this wave number is based on its ability to produce a clear grouping between pure sesame oil, pork oil as an adulterant, and samples on the Market seen from the results of R^2, linear regression, RMSEC, RMSECV, and RMSEP values. PCA results showed a perfect separation between sesame oil and pure pork oil. Figure 3 (a) shows that PCA can classify pure sesame and pork oil. Figure 3 (a) shows the PCA results where significant differences were found between sesame oil (A) and lard oil (B). This gives a good picture of the results to see the differences between the samples and whether any samples contain pork oil adulterants.

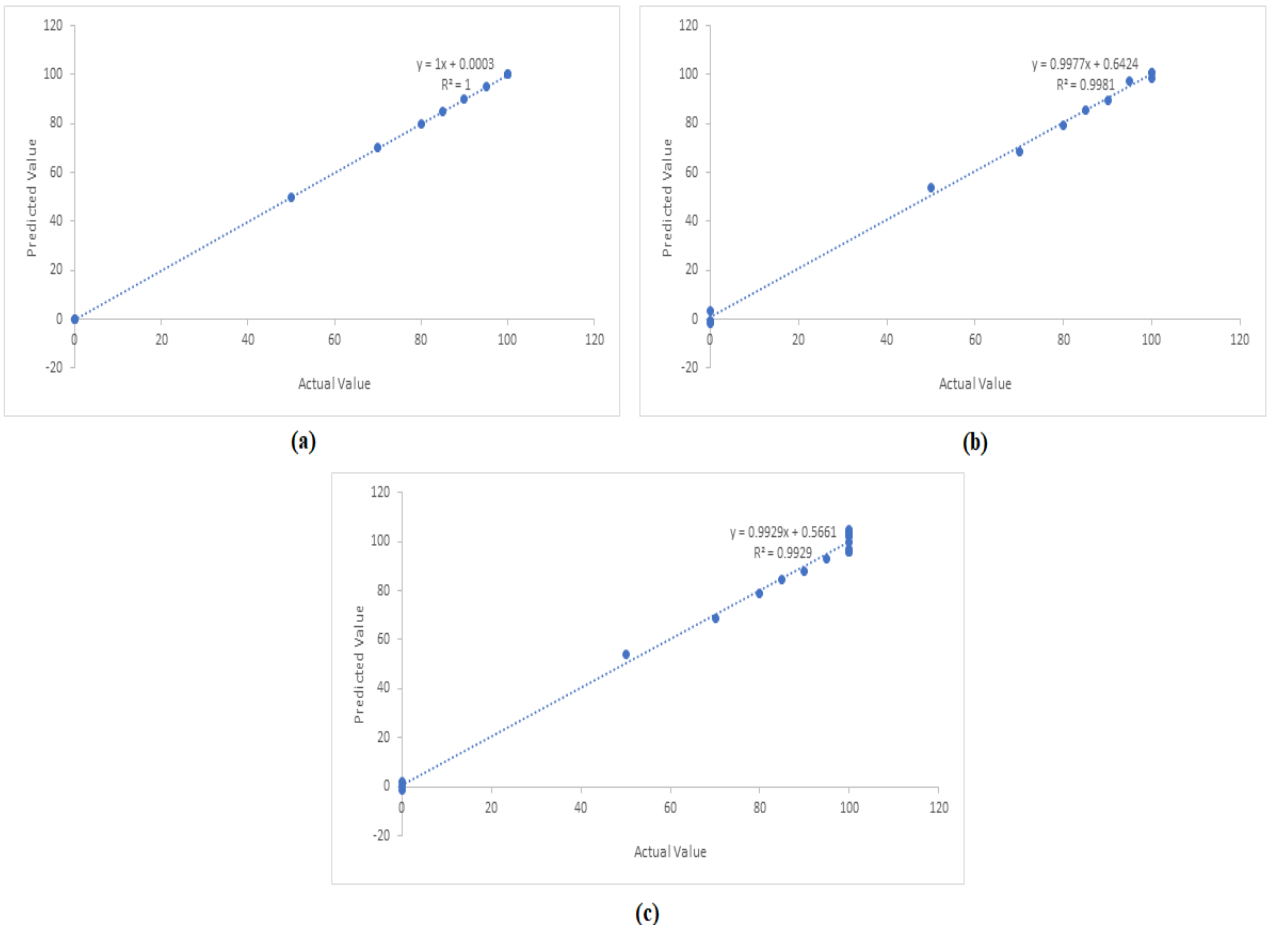

Figure 2. (a) Optimization curve, (b) internal validation curve with calculated values from cross-validation, and (c) External Validation calculated at waves 3000-2850 cm^{-1}

Furthermore, Figure 3 (b) shows the grouping of pure sesame oil, pork oil, and samples A, B, and C on the Market. The scanning results (Figure 3b) show that four quadrants can differentiate between pure sesame oil, pork oil, and samples on the Market. In Figure 3 (b), pure sesame oil and pork are in different quadrants, where the further the distance between the oils, the greater the difference in sample characteristics [32]. Sample A is in the same quadrant as sample B and sample C; this shows that sample A likely has the same physical and chemical characteristics as samples B and C because it is processed by roasting sesame oil and then extracting the oil. Meanwhile, the pure sesame oil resulting from pressing is in a different quadrant to samples A, B, and C, which can, of course, be influenced by the treatment without roasting the sesame seeds in the pure sesame oil so that the compounds contained in the sesame seeds are not extracted optimally, This is following previous research which stated that pyrazine compounds mainly exist in the form of alkylated pyrazines, and generally have a strong aroma when roasted and nutty [33].

In addition, pyrazines mostly originate from the Strecker degradation pathway in seeds during high-temperature roasting [34, 35]. No pyrazine compounds were found in sesame oil samples extracted without roasting, possibly because they were sampled at a lower temperature [36]. Pork oil is in a different quadrant to pure sesame oil, samples A, B, and C, which gives the possibility that samples A, B, and C do not contain a mixture of pork oil adulterants. Therefore, to find out the specific content of pure sesame oil, samples A, B, and C, and pork oil, further analysis is needed, such as using PCR and E-Nose.

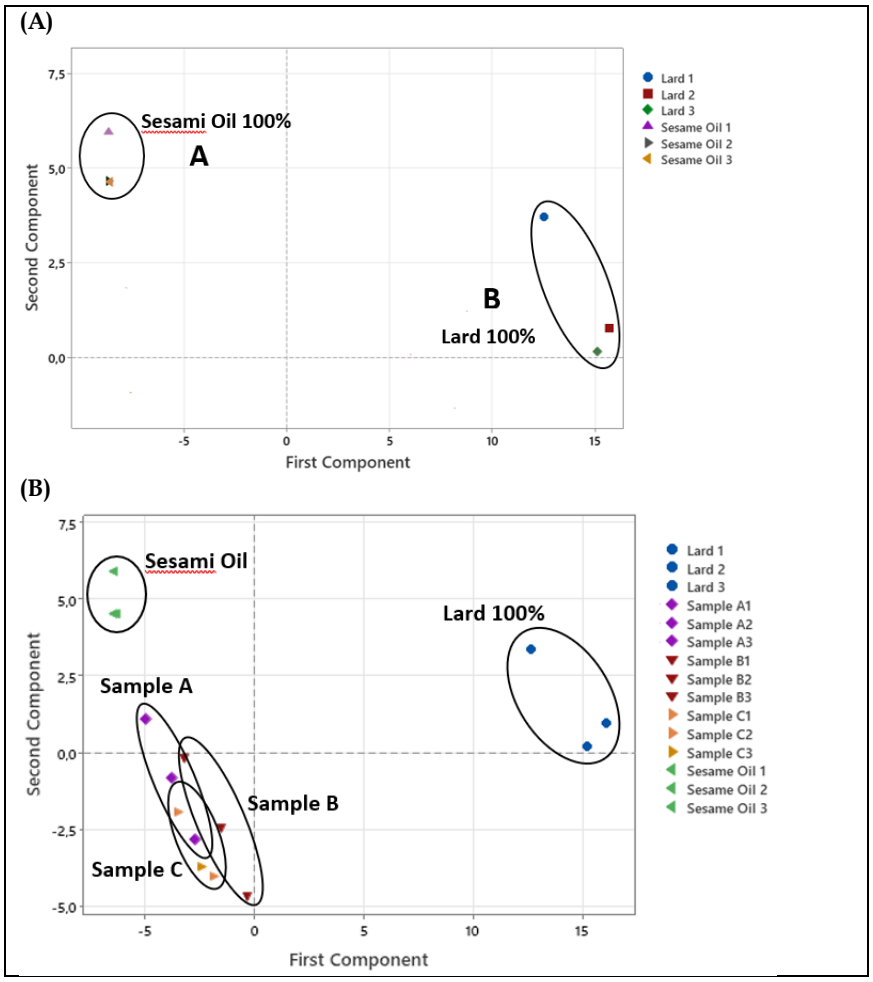

Figure 3. (a) PCA score plot of 100% pure sesame oil (A) and 100% pure pork oil (B), and (b) PCA score plot of 100% pure sesame oil, 100% pure pork oil, and market samples (A B C)

4. CONCLUSION

This study concludes that the contamination of sesame oils with pork fat can be determined using FTIR spectroscopy combined chemometrics. The quantitative analysis was performed using PLS at wave numbers 3000-2850 cm^{-1}. The quantitative parameters have a coefficient determination (R^2) of 1 with an RMSEC of 0.01%, an RMSECV value of 1.009%, and an RMSEP value of 0.56%. Furthermore, commercial sesame oils are in a different quadrant from pure sesame oil and pork fat. Based on this study, sesame oil products did not contain pork fat; all products were not pure sesame oils. FTIR spectroscopy combined with chemometrics has successfully classified and identified sesame products as not pure oils.

5. MATERIALS AND METHODS

5.1. Tools

The tools used in this research were a press, oven, beaker, measuring pipette, micropipette, basin, filter cloth, filter paper, flannel cloth, centrifuge, Whatman paper, pycnometer, Abbe refractometer (*Atago DR-A1-Plus*), 2 ml vial, refrigerator, Eppendorf tube, and FTIR-ATR (*Bruker type alpha II*).

5.2. Ingredients

The ingredients used in this research were dried sesame seeds obtained from the Intisari Yogyakarta shop and lard obtained from the Pathuk Yogyakarta market. Sesame oil brands A, B, and C, sampled from 3 countries (China, Korea, and Singapore), were obtained from Yogyakarta City minimarkets. Anhydrous Na_2SO_4 (*sigma*), ethanol P.A. (*merck*), chloroform (*Merck*), acetic acid (*Merck*), potassium iodide (K.I.), 0.5% starch solution, indicator, 0.1 N sodium thiosulphate (*merck*), iodine reagent (iodine 1% chloride in glacial

acetic acid) (*Merck*), K.I. 15%, distilled water, 95% ethanol (*Merck*), P.P. indicator, 0.1 N ethanolic KOH, n-hexane (*bratachem*), NaOH (*Merck*), methanol (*Merck*).

5.3. Process of the research

5.3.1. Sesame seed extraction

Sesame extraction is performed mechanically using the cold press method [37]. The stages in sesame extraction are preparing 1 kg of seeds and sorting them. The sesame seeds are pressed with a pressure of 300 kg/cm^2. The pressing is repeated two times. Sesame oil is filtered and left for 24 hours, then filtered again. Sesame oil is stored in a dark-colored bottle that is tightly closed.

5.3.2. Pork oil extraction

Lard extraction is carried out by dry rendering [38]. Prepare 1 kg of pork fat tissue, cut it into small pieces, and put it into a beaker glass. Heat in the oven at a medium temperature of 90-100°C for 1-1.5 hours. Filtered through flannel cloth, anhydrous Na_2SO_4 was added and centrifuged at 3000 rpm for 20 minutes. Filtered using Whatman paper and placed anhydrous Na_2SO_4.

5.3.3. The vibrational analysis using Fourier Transform-Infra Red (ATR FT-IR)

The sesame oils and pork oil were analyzed using ATR-FTIR spectroscopy (*Bruker type alpha II*) that was carried out at a frequency of 4000–650 cm^{-1}. The small oil samples (~5 µL) were determined on the surface and in contact with the ATR sample base plate at controlled room temperature (25˚C). Measurements were performed on 32 scans at a resolution of 4 cm^{-1}. The plate was carefully cleaned twice with hexane, followed by acetone, and dried with soft tissue before loading with the next sample [39], [40].

5.4. Statistical analysis

5.4.1. Partial least square analysis

The FTIR data was collected and analyzed with the Minitab 21 multivariate calibration program using the PLS technique for quantification. The data were processed based on a linear regression between the sample formula concentration series, market samples (A, B, and C), and the FTIR spectral profiles of pure lard and pure sesame oil [15]. The partial least squares (PLS) was used to determine linearity. Microsoft Excel 2022 software spreadsheet was also used to compare actual samples with estimated sample concentrations. The accuracy of the PLS model is evaluated by the coefficient of determination (R^2), and the data analysis method is evaluated using the Root Mean Standard Error of Calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP) [41].

5.4.2. Principal component analysis

The data from the PLS results were then analyzed by grouping the components of sesame oil and lard, as well as samples of sesame oil on the Market (A, B, and C) using Principal Component Analysis (PCA) followed by biplot using Minitab 21 software [42].

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