

The application of FTIR spectra coupled with chemometrics for analysis of lard in food products for halal authentication: a mini review

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Article history:

Received: 29 September 2022

Received in revised form: 10 November 2022

Accepted: 8 December 2023

Available Online: 13 May 2024

Keywords:

Halal authentication,
Chemometrics,
Pork fat,
Food product,
Pharmaceutical product

DOI:

[https://doi.org/10.26656/fr.2017.8\(3\).489](https://doi.org/10.26656/fr.2017.8(3).489)

Abstract

Lard (LD) obtained from fat's extraction of adipose tissue of swine is a good component to be used in food and pharmaceutical products to provide specific functions. Lard, according to the Food and Drug Administration, is generally considered recognized as safe (GRAS) to be used in the products. However, LD is not allowed to be consumed or used in the products due to certain religious restrictions in which Muslims and Jews are not allowed to consume any products containing LD because LD is categorized as non-halal (Muslims) and non-kosher (Jews). Thus, the analysis of LD in food and pharmaceutical products is very urgent to ensure the product's halalness (and Kosherness) for Muslims and Jews. Some methods have been reported for the analysis of LD, including FTIR spectra, due to their property as fingerprinting analytical techniques capable of fast and reliable tools for screening LD. The responses obtained from the measurement of LD using FTIR spectroscopy are very large, involving big data analysis. Therefore, the chemometrics application to treat these chemical data is unavoidable. This present review highlighted the use of FTIR spectra and chromatographic-based techniques coupled with chemometrics for the analysis of lard in food and pharmaceutical products for halal authentication. The main findings of this review can assist regulators and analytical chemists in selecting the best method for analysis of LD in the products intended for Halal authentication analysis.

1. Introduction

From a religious point of view, Islam, Jews, Judaism, and Hinduism prohibit their followers from consuming products containing pig derivatives, including lard. From an Islamic perspective, even though the presence of the unlawful (haram) ingredients is less, it is still prohibited to be consumed (Harun, 2019), as clearly stated in the Holy Qur'an (9: 14-15) "*Then eat of what Allah has provided for you, which is lawful and good. And be grateful for the favor of Allah, if it is indeed Him that you worship. He has only forbidden to you dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah*".

Lard (LD), one type of animal fat obtained from the rendering of pig adipose tissue (*Sus scrofa*), is frequently

used with other plant oils such as olive and palm oils to produce shortenings, margarine, and other speciality food oils. LD can be viewed from 2 points of view, economics, and religion. From an economic perspective, in certain countries, the producers of food products prefer to blend vegetable oils with LD to reduce the production cost. From a religious perspectives, any food and pharmaceutical products containing LD are not allowed for religious reasons (Al-Kahtani *et al.*, 2014). Consequently, an analytical method for detecting LD is highly needed.

According to Codex Alimentarius (1999), lard is defined as "Pure rendered lard is the fat rendered from fresh, clean, sound fatty tissues from swine (*Sus scrofa*) in good health, at the time of slaughter, and fit for human

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consumption.” The tissues do not include bones, detached skin, head skin, ears, tails, organs, windpipes, large blood vessels, scrap fat, skimming, settlings, pressings, and the like, and are reasonably free from muscle tissues and blood. In addition, LD subjected to certain processing may contain refined lard, lard stearin, and hydrogenated lard (Codex Alimentari, 1999). Lard compositions and their physical characteristics vary according to diet, climate, and the overall physiological structure of the animals. Consequently, the amount and the composition of fatty acids in the feed affect the degree of unsaturation in lard (Heidari *et al.*, 2020).

Edible fats and oils can be characterized by some physico-chemical properties such as fatty acid (FA) composition and triacylglycerol profiles (TAG). The main FAs in lard are oleic, palmitic, and stearic acids. Besides, lard contains large quantities of linoleic acid. The structure of lard contains a high percentage of medium-melting triacylglycerols (TAGs) of disaturated-monounsaturated. These TAGs are largely in a symmetrical arrangement, which causes lard to crystallize in the β form. Generally, fats with uniform TG molecules have β crystals in a more stable state. This characteristic restricts the applications of lard requiring low structural properties but with high lubricity (Becker, 2017).

Some studies concerning the physico-chemical properties of LD have also been investigated using FTIR spectroscopy. Lard subjected to oxidative process reveals some changes in FTIR spectral peaks. A slight decrease in the peak intensity was observed for the band at 721 cm^{-1} , which implies the loss of *cis* double bonds during oxidation. This fact was supported by the decreased intensity at band 3006 cm^{-1} , corresponding to the same *cis*-double bonds in $\text{C}=\text{CH}$ (Bunaciu *et al.*, 2021). Due to the extensive use of LD in food products, the analytical methods capable of detecting lard have been continuously developed, including FTIR spectroscopy. Fourier transform infrared (FTIR) spectroscopy is a fingerprinting technique, and it's an ideal method for rapid authentication analysis, and combination with chemometrics of multivariate analysis, Fourier transform infrared (FTIR) offers rapid and reliable methods, fast analysis, simple in sample preparation, and require minimum solvent (green analytical chemistry) (Windarsih *et al.*, 2020; Lestari *et al.*, 2022). This review article highlights the application of FTIR spectroscopy in combination with chemometrics for analyzing lard in food products.

2. FTIR spectroscopy and chemometrics

Fourier transform Infrared (FTIR) spectroscopy is one of the vibrational spectroscopic methods widely

applied for authentication analysis of food and pharmaceutical products, including lard (LD) analysis. Apart from FTIR spectroscopy, vibrational spectroscopy includes near-infrared (NIR), Raman, and spectral imaging. Vibrational spectroscopy deals with the interaction of electromagnetic radiations in infrared regions with functional groups (chemical bonds) present in analytes or samples (objects) analysed, resulting in the vibrational transitions to get vibrational spectra. Therefore, not only functional groups present in analytes but also in samples can be detected, provided that functional groups are active in infrared radiations (Rohman, 2017). Table 1 provides the typical functional groups along with the mode of vibration and peak intensity of lard as recorded using FTIR spectroscopy in the mid-infrared region ($4000\text{--}650\text{ cm}^{-1}$).

The main principle of FTIR spectroscopy relies on the interactions between objects (samples) such as LD with electromagnetic radiation at the infrared region corresponding to the mid-infrared region ($4000\text{--}400\text{ cm}^{-1}$), resulting in vibrational transitions of molecules in the sample. FTIR spectroscopy using some sampling handling techniques has wide applications in the analysis of food and pharmaceutical products (Rohman, 2019). FTIR using the ATR (Attenuated Total Reflectance) spectroscopy technique for obtaining spectra of samples offers multiple advantages such as easy in-sample preparation; for instance, the samples can be directly placed on an ATR crystal for measurement, with less time analysis and requiring less solvent. Mid-infrared FTIR spectroscopy enables a wider range analysis of compounds; moreover, it showcases the fingerprint region in the wavenumber region of $1500\text{--}900\text{ cm}^{-1}$ that strongly supports the differentiation of halal and non-halal compounds. For this reason, FTIR spectra are widely applied for the analysis of LD in food products (Li *et al.*, 2019). However, the spectra generated from FTIR measurements are complex, which makes it difficult to be analysed, therefore, the use of powerful statistical analysis such as chemometrics of multivariate analysis is unavoidable to overcome this problem (Che Man *et al.*, 2011).

According to the definition set by the International Chemometrics Society (ICS), chemometrics is “the science of relating chemical measurements made on a chemical system to the property of interest (such as concentration) through the application of mathematical or statistical methods”. Chemometrics is typically employed to treat big data analysis obtained from the measurement of FTIR spectra (Shafii *et al.*, 2019). In line with the recent development of statistical software, computer technology, and analytical approaches, the chemometric technique has evolved as the leading tool

Table 1. Absorption peaks of common edible oils in the FTIR spectral region (4000-400 cm^{-1}) along with their assigned functional groups, mode of vibration, and intensities

Wavenumber (cm^{-1})	Functional groups	Mode of vibration	Peak and shoulder intensities
3468	-C=O (ester group)	Overtone	Weak
3025	=C-H (<i>trans</i> -)	Stretching	Very weak
3008	=C-H (<i>cis</i> -)	Stretching	Weak
2954	-C-H methyl (-CH ₃)	Asymmetrical Stretching	Medium
2924	-C-H methylene (CH ₂)	Asymmetrical Stretching	Very strong
2852	-C-H methylene (-CH ₂)	Symmetrical Stretching	Very strong
2730	-C=O (ester group)	Fermi resonance	Very weak
2678	-C=O (ester group)	Fermi resonance	Very weak
1743	-C=O (ester group)	Stretching	Very weak
1711	-C=O (acid)	Stretching	Very strong
1654	-C=C- (<i>cis</i> -)	Stretching	Very strong
1648	-C=C- (<i>cis</i> -)	Stretching	Very strong
1465	-C-H (-CH ₂ , CH ₃)	Bending (scissor)	Medium
1417	=C-H (<i>cis</i> -)	Bending (rocking)	Weak
1377	-C-H (-CH ₃)	Bending (symmetrical)	Medium
1228	-C-O	Stretching	Medium
1155	-C-O, -CH ₂ -	Stretching, Bending	Strong
1111	-C-O	Stretching	Medium
1098	-C-O	Stretching	Medium
1033	-C-O	Stretching	Very weak
962	-HC=CH- (<i>trans</i> -)	Out of plane bending	Weak
872	=CH ₂	Bending (wagging)	Weak
721	-(CH ₂) _n -	Bending (rocking)	Medium

Source: Li *et al.* (2019), Jamwal *et al.* (2020), Amit *et al.* (2020).

among analytical chemists in order to obtain faster analysis results and shorter product development times (Salleh *et al.*, 2018a). In halal authentication, various chemometric techniques are commonly applied and provide an alternative way to analyse complex chemical data, namely, chemometric classification analysis and multivariate calibration (Bunaciu *et al.*, 2021). Some data pre-processing, such as Savitzky-Golay-based derivatization, standard normal variate, baseline corrections, mean centering, signal correction and compression, spectra normalizations, and multiplicative correction, are also used to treat molecular spectra before being subject to chemometrics analysis. Besides pre-processing data, two other chemometrics techniques applied to FTIR spectroscopy are chemometrics of classification and multivariate calibrations.

Chemometrics of classification (pattern recognition) is the most common chemometric technique applied in halal authentication, especially to classify products with and without non-halal components. It is typically performed using three approaches, namely, exploratory data analysis, unsupervised pattern recognition, and supervised pattern recognition techniques, as revealed in Figure 1. Exploratory data analysis and unsupervised pattern recognition are commonly used to simplify groups of samples by reducing the amount of original

data and gaining better knowledge of chemical data sets (Rohman and Windarsih, 2020).

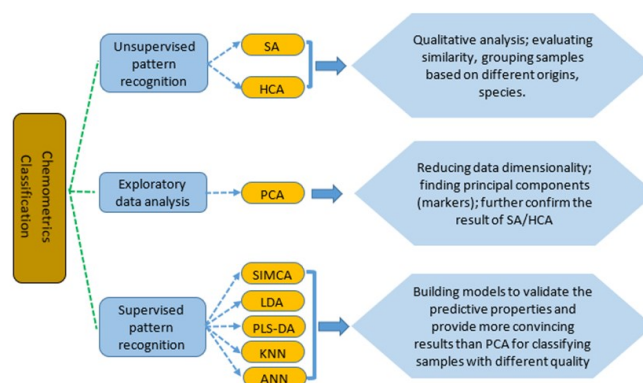


Figure 1. Some chemometrics techniques are typically applied for the classification of objects such as the classification of food products with and without lard in its formulation. SA: similarity analysis, HCA: hierarchical clustering analysis, PCA: principal component analysis, SIMCA: soft independent modeling of class analogy, LDA: linear discriminant analysis, PLS-DA: partial least squares discriminant analysis, KNN: k-nearest neighbors, and ANN: artificial neural networks. Source: Rohman and Windarsih (2020).

The chemometrics of supervised pattern recognition (SPR) tries to make the class membership of the samples to a certain group known as training sets. It enables classifying new unknown samples (test samples) in one of the known classes on the basis of the variables used.

The most-used class modeling method is known as SIMCA or soft independent modeling of class analogy. SIMCA considers the objects that fit the class model for a category as part of the class model and classifies as non-members those that do not. Discrimination models include linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA), artificial neural networks (ANNs), and k-nearest neighbors (KNNs). All these discrimination models are used to build models using certain variables (for example, FTIR spectra) based on all the categories concerned in the discrimination, whereas disjoint class-modeling methods create a separate model for each category. The most reported discrimination methods are discriminant analysis, either using linear or partial least square algorithms. LDA is based on linear discriminant functions (LDFs) in which the variance ratio of between-class membership of objects is minimized, while the variance ratio of within-class objects is maximized. PLS-DA is intended to find the variables and directions in the multivariate space capable of discriminating the established classes in the calibration set (Gad *et al.*, 2013).

For quantitative analysis of LD in food products, the multivariate calibrations are typically applied. The chemometrics of multivariate calibration uses several variables, such as employing the absorbance values at several wavelengths or wavenumbers regions. Multivariate calibration is commonly used to develop calibration and validation models capable of correlating the actual values of analytes as determined by the reference method and predicted values using several variables assessed (Riyanta *et al.*, 2020). Various multivariate calibrations have been used for quantitative analysis of non-halal components, including stepwise multiple linear regression (SMLR), principal component regression (PCR), and partial least square regression (PLSR). The accuracy of calibration and validation models using multivariate calibration was evaluated by the coefficient of determination (R^2) for the relationship between two variables, while the precision of models was assessed by the root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) (Rohman *et al.*, 2021). RMSEC and RMSEP were obtained using these equations:

$$RMSEC = \sqrt{\frac{\sum_{i=1}^m (\hat{Y}_i - Y_i)^2}{M - 1}} \quad (1)$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (\hat{Y}_i - Y_i)^2}{N}} \quad (2)$$

Where Y_i represents the actual value of analytes, \hat{Y}_i represents the predicted value of analytes, M is the

number of data in the calibration set and N is the number of data in the validation sets.

The main advantage of multivariate calibration is the reliability of prediction results for unknown samples obtained. However, multivariate calibrations have the main disadvantage of over-fitting of the model. Over-fitting is the over-optimistic performance of multivariate calibrations in calibration datasets, but the performance in validation datasets is not acceptable. Consequently, cross-validation of the leave-one-out technique can be used to assess this problem. In cross-validation, one of the calibration samples is left out from the multivariate calibration models used, and the remaining calibration samples are exploited to develop a new calibration model. The removed sample is then calculated using the newly developed PLS model. This procedure was repeated by leaving one by one of the calibration samples. The statistical parameters used to evaluate the performance of cross-validation is R^2 (for accuracy of the model), as well as the root mean square error of cross-validation (RMSECV) and the predicted residual error sum of squares or PRESS (for the precision of the model) (Miller *et al.*, 2018).

3. Application of FTIR spectroscopy and chemometrics for analysis of lard

Analysis of lard in food and pharmaceutical products using FTIR spectroscopy and chemometrics intended for confirmation analysis of lard, qualitative analysis of lard, and quantitative analysis of lard. The analysis can be met using two approaches, direct method and indirect method by extracting lard using some lipid extracting techniques such as Soxhlet, Bligh and Dyer methods. FTIR spectroscopy in combination with two unsupervised pattern recognition techniques of principal component analysis (PCA) and hierarchical cluster analysis has been successfully applied for identification of lard from other edible fats and oils. FTIR spectra of lard and others were subjected to ATR-FTIR spectral measurement at wavenumbers ($1/\lambda$) 6000-650 cm^{-1} . Using PCA, lard could be clearly separated from other fats and oils using the first principal component (PC1) and second principal component (PC2), which are linear combinations from original variables (absorbance values at 16 wavenumbers). PC1 and PC2 accounted for 44.1% and 30.2%, respectively, therefore 74% data variance can be described using PC1 and PC2. The other output of PCA is a loading plot used to identify the most variable contributing to lard separation on PC1 and PC2, and the results showed that variables of absorbance values at wavenumbers 2853, 2922, and 1465 cm^{-1} contributed significantly to such separation. On the other hand, HCA based on Euclidean distance is successfully applied for

making the clustering lard and others using the same variables used in PCA (Che Man *et al.*, 2011). Based on the results of PCA and HCA, the combination of FTIR spectra and chemometrics provides a rapid analytical method for screening the presence of lard in the analysed samples. The most popular application of FTIR spectroscopy in lard analysis is quantitative analysis, especially in combination with chemometrics of multivariate calibrations. Table 2 lists the application of FTIR spectroscopy and chemometrics for analysis of lard in food and pharmaceutical products along with sample preparation used to extract lard in the products.

Fourier transform infrared (FTIR) spectroscopy, in combination with Attenuated Total Reflectance (ATR) and Partial Least Square (PLS) regression, was used to detect the presence of lard in the chocolate formulation. Based on spectral data at the frequency region 4000–650 cm^{-1} , using the equation $y = 0.9225x + 0.5539$. The coefficient of determination (R^2) was 0.9872 with a standard error (SE) of 1.305. FTIR spectroscopy is a fingerprinting technique and a rapid analytical tool for the quantitative determination of adulterants, especially lard in chocolate (Che Man *et al.* 2005).

Lipid fractions (mainly lard) obtained from the corned pork-beef samples with various concentrations of pork were analysed using FTIR spectroscopy and chemometrics (PCA and PLSR). The Extraction was carried out using Soxhlet applying *n*-hexane as an extracting technique for 4 - 5 hrs at 69 - 70°C. PLSR using absorbance values at wavenumbers region of 1180 - 730 cm^{-1} was selected to analyse lard obtained from the lipid extraction of corned pork-beef samples with R^2 value of 0.9833, RMSEC of 2.06%, RMSEP of 1.65% and RMSECV of 2.22%. Furthermore, PCA could classify samples in different quadrants according to their group (corned pork 100%, corned beef 100% and the mixture of corned pork-beef. This indicated that FTIR spectroscopy in combination with chemometrics is effective tools for analysis of lard extracted from pork corned and beef corned samples (Guntarti *et al.*, 2020).

4. Conclusion

The authentication analysis of halal food products is a must to ensure that those food products consumed by the Muslim community are free from non-halal components. Lard, one of the non-halal components, is typically found in food products as a substitute for halal components. Therefore, some analytical methods capable of detecting lard in food products are continuously developed, validated, and applied to commercial samples that contain lard. FTIR spectroscopy is a fingerprinting analytical technique combined with chemometrics of pattern recognition and multivariate calibration it is

proven to be an effective tool for detecting and quantifying lard in food products.

Conflict of interest

No potential conflict of interest was reported by the authors.

Acknowledgements

The authors thank the students and researchers from Centre of Excellence of Halal Institute and Systems for their continuous efforts to develop analytical methods intended for Halal authentication.

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Table 2. The use of FTIR spectroscopy in combination with chemometrics for analysis of lard in food products.

Issue	Sample preparations and FTIR condition	Chemometrics	Results	References
Differentiation of LD from fats extracted from chicken (CF), mutton (MF), tallow (TF) and palm oil.	Fats and oils were heated at different temperatures and at different times. ATR-FTIR at 4000-650 cm ⁻¹ , with a resolution of 4 cm ⁻¹ and 32 scans.	PCA, k-mean CA and LDA for classification and differentiation.	The combination of PCA with k-mean CA using absorbance values at the entire mid IR was able to differentiate the heated fats (120, 180 and 240°C) and different times (30, 60, 120 and 180 min) according to its types. LDA method classify successfully fats and palm-based shortening with an accuracy rate of 80.5% of samples in its group.	Salleh <i>et al.</i> (2018)
Analysis of lard extracted from pork in sausages.	Lard in sausages was extracted using Soxhlet at ±70°C for 6 h using n-hexane. ATR-FTIR spectra at 4000-650 cm ⁻¹ .	PCA is for the classification of beef sausages and pork sausages, while PLS-R for the prediction of lard contents in sausages.	PLSR and PCA analysis was performed using variable of absorbance values at 1200-1000 cm ⁻¹ . PLSR provided the linear regression for the relationship between actual (x-axis) and FTIR predicted value (y-axis) with the equation of $y = 0.921x + 4.623$ to obtain R ² of 0.985 and RMSEC of 2.094% and RMSEP of 4.77% with internal validation RMSECV of 5.12%. PCA is able to classify beef sausages and pork sausages according to their classes.	Guntarti and Zelinda (2019)
Analysis of LD in ternary mixture with chicken fat (CF) and beef fat (BF).	These fats were extracted using rendering from the fresh adipose tissue of corresponding animals. ATR-FTIR spectra were scanned at 4000-650 cm ⁻¹ .	PLSR was used to assist the prediction model of lard. The pre-processing FTIR spectra using Savitzky-Golay derivatives were applied.	Normal FTIR spectral mode using absorbance values at combined wavenumbers of 3100-2750 and 1800-660 cm ⁻¹ is successfully applied for analysis of LD in ternary mixtures with CF and BF with R ² values of 0.9971 (calibration) and 0.9874 (validation) with RMSEC of 0.0156 and RMSEP of 0.0328. This indicated that the combination of FTIR spectra and PLSR provide accurate and precise results for the prediction of LD.	Lestari <i>et al.</i> (2022)
Analysis of LD in the mixture with chicken. LD has similar characteristics to CF in terms of FA composition and FTIR spectra.	LD and CF were obtained from the rendering of adipose tissues of pork and chicken. ATR-FTIR spectra were scanned at 4000-650 cm ⁻¹ .	PLSR was used for the quantification of LD; LDA was used for the discrimination of CF and CF added with LD.	Normal FTIR spectra using variables of absorbance values at combined wavenumbers of 3100-2750 and 1500-660 cm ⁻¹ provided R ² for the relationship between actual and FTIR predicted values of 0.9994 (calibration) and 0.9918 (validation) with an error in calibration of 0.008% (RMSEC) and error in validation of 0.032% (RMSEP). LDA is capable of discriminating LD and LD in the mixture with CF with an accuracy level of 100%.	Irnowati <i>et al.</i> (2022)
Analysis of LD as an adulterant in bovine milk fat (BMF)	Samples were directly prepared by mixing BMF with LD in the range of 0-100% of LD. ATR-FTIR at 4000-650 cm ⁻¹ .	Multivariate calibrations of PLSR and PCR for quantitative analysis of LD; LDA for classification of BMF and BMF adulterated with LD.	LDA using normal FTIR spectra at 3098-669 cm ⁻¹ perfectly classified the authentic BMF and BMF adulterated with LD. PLSR was preferable to PCR for the prediction of LD using absorbance values of 1 st derivative FTIR spectra at a combined region of 3033-2770 and 1510-692 cm ⁻¹ with R ² of >0.99 either in calibration or validation models with low RMSEC of 0.631 and RMSEP of 1.94. FTIR spectroscopy coupled with LDA and PLSR could be effective tools for the detection and quantification of LD in BMF.	Windarsih <i>et al.</i> (2020)

LD: lard, SIMCA: Soft Independent Modelling of Class Analogy, M-SVM: Multiclass Support Vector Machine, PLSR: Partial Least Square regression, PCR: Principle Component Regression, RMSEC: Root Mean Square Error of Calibration, RMSEP: Root Mean Square Error of Prediction, RMSECV: Root Mean Square Error Cross-Validation, PCA: Principal Component Analysis, DA: Discriminant Analysis, LDA: Linear Discriminant Analysis.

Table 2 (Cont.). The use of FTIR spectroscopy in combination with chemometrics for analysis of lard in food products.

Issue	Sample preparations and FTIR condition	Chemometrics	Results	References
Analysis of LD in vegetable oils (sunflower, canola, coconut, olive, and mustard oils).	LD was mixed with these vegetable oils to provide calibration and validation datasets.	PLSR for quantification of lard in vegetable oils.	The absorbance values at wavenumbers of 1078-1247 cm^{-1} were successfully applied to make PLS correlation between actual and predicted values with an R^2 value of 0.9577 and RMSEC of 0.0488.	Munir et al. (2019)
Analysis of LD as an adulterant in lard in palm olein oil (POO)	The binary mixtures of LD and POO (10 training sets); ATR-FTIR spectra at f 4000-525 cm^{-1} .	Simple linear regression (SLR), MLR and PLSR for prediction of LD in POO	The marker bands at 3006, 2852, 1117, 1236, and 1159 cm^{-1} were identified as the marker bands as determined using Fisher's weights. The absorbance values at wavenumbers 3020-2990 cm^{-1} (I) and 1130-1100 cm^{-1} (II) were used for LD prediction and resulted the satisfactory predictive ability using PLSR providing %RMSE of 16.03 and 13.26%, respectively.	Sim et al. (2018)
Analysis of LD in frozen French fries and vegetable oils (corn, sunflower, palm and olive oils).	LD in binary mixtures with vegetable oils ranging from 1 to 20% LD were prepared; ATR-FTIR at 4000-650 cm^{-1} .	PLSR for quantification of LD in vegetable oils and in products (frozen French fries).	The FTIR spectral regions at 1405-1365, 1260-1198, 935-910, 877-857 and 857-833 cm^{-1} provided the basis for the identification of LD as well as for the prediction of LD in binary mixtures with vegetable oils or LD extracted from frozen French fries. FTIR was able to detect levels of LD as low as 1% in all mixtures.	Al-Kahtani et al. (2017)
Analysis of LD as an adulterant in palm oil.	LD was mixed with PO in the range of 1-60% (v/v) of LD in PO. All samples were scanned at 4000-400 cm^{-1} .	PLSR for quantification of LD in PO.	The absorbance values at regions of 1480-1085 cm^{-1} with the aid of PLSR provide a good relationship between the actual value of LD and FTIR predicted value with R^2 of 0.998, RMSEC of 1.69%, and RMSECV of 2.87% (v/v).	Rohman et al. (2012)
Analysis of LD in the extracted ink of printed food packaging.	The sample mixtures were subjected to an ATR-FTIR spectrometer and scanned at 4000-650 cm^{-1} .	PCA for choosing the numbers of PCs; SIMCA for the classification of LD, commercial gravure ink, and the mixture of LD-ink.	SIMCA using absorbance values of full FTIR spectra, could classify LD, commercial gravure ink, and the blends of both using 7 PCs obtained from optimization result of PCA. Using SIMCA, Si versus Hi plots showed that the calibration standards can be classified as lard-containing standards. One sample was deduced to have the highest possibility to contain LD, two samples cannot be confirmed, and the other samples did not contain LD. It cannot be classified as lard-containing samples.	Ramli et al. (2015)
Analysis of LD as an adulterant in BF, MF, and CF with the ratio of 10-50% v/v of LD in the mixtures.	The fats were obtained by the rendering process. Analysis for all samples was carried out using the combined wavenumbers of 1700-1800 and 2800-3000 cm^{-1} .	PCA was used for the determination of the number of PCs; M-SVM was used for the classification of pure lard and lard added to other fats.	M-SVM using 3 PCs as a result of PCA capable of explaining 97.31% variance could identify the samples according to their classes (pure and in the mixtures). The overall accuracy for the classification of pure samples was 81.25% and 71.21% for the mixtures with the adulteration ratio of LD above 10%. BF and MF provide the highest classification accuracy (85%) either in pure or adulterated samples, while CF had the lowest accuracy value of 78% for each category. M-SVM model provides the accurate classification of LD adulteration in different animal fat samples.	Siddiqui et al. (2021)

LD: lard, SIMCA: Soft Independent Modelling of Class Analogy, M-SVM: Multiclass Support Vector Machine, PLSR: Partial Least Square regression, PCR: Principle Component Regression, RMSEC: Root Mean Square Error of Calibration, RMSEP: Root Mean Square Error of Prediction, RMSECV: Root Mean Square Error Cross-Validation, PCA: Principal Component Analysis, DA: Discriminant Analysis, LDA: Linear Discriminant Analysis.

Table 2 (Cont.). The use of FTIR spectroscopy in combination with chemometrics for analysis of lard in food products.

Issue	Sample preparations and FTIR condition	Chemometrics	Results	References
Differentiation of bread with LD at 1% wt/wt and without LD in their composition.	Lipids containing LD were extracted from bread using Bligh and Dyer method employing CHCl_3 - CH_3OH (1:2, v/v) as extracting solvent.	NR	ATR-FTIR spectra at combined wavenumbers of 1120-1090 and 990-950 cm^{-1} could differentiate LD, bread without LD, and LD in bread at levels as low as of 1% wt/wt.	De Cicco et al. (2019)
Analysis of LD in French fries pre-fried in palm oil (PO).	Lipids in commercial and prepared French fries were extracted using the Soxhlet method using Petroleum ether.	All spectra were scanned at 4000 to 650 cm^{-1} with 64 scans at a resolution of 4 cm^{-1} . LD was predicted using PLSR. LDA was used for the discrimination of French fries.	PLSR was successfully applied for the prediction of LD lard in French fries pre-fried in palm oil, providing R^2 of 0.9791 with a 0.5% of the detection limit. RMSEC and RMSECV values were 0.979% and 2.45%, respectively. LDA test was able to distinguish between French fries samples adulterated with LD and French Fries pre-fried with palm oils.	Che Man et al. (2014)
Analysis of LD in grilled and steamed sausages.	Lipid was extracted using Soxhlet employing n-hexane as an extracting solvent. ATR-FTIR at 4000-400 cm^{-1} .	PLSR for the prediction of the levels of LD in sausages; PCA was used for the classification of sausages with and without LD.	The optimization results showed that LD in steamed sausages was analysed in the range of 1000-791 cm^{-1} and for grilled sausages at 1070-796 cm^{-1} . For steamed sausage, the levels of LD were predicted using PLSR to obtain the equation of predicted value (y) = 0.9977 x actual value + 0.1166 (R^2 of 0.9977; RMSEC of 1.22%; RMSEP of 0.22% and RMSECV of 1.26%). For grilled sausage, LD was predicted using the equation of predicted value (y) = 0.9972 x actual value + 0.1379 (R^2 of 0.9972; RMSEC of 1.27%; RMSEP of 0.42%, and RMSECV of 0.18%). PCA could classify sausages with and without LD in the formulation.	Guntarti et al. (2019)
Analysis of LD in crackers prepared by buffalo skins and pig skins	Lipid was extracted using Soxhlet employing n-hexane as an extracting solvent. ATR-FTIR at 4000-650 cm^{-1} .	PCA was used for the classification of crackers containing LD from pig skin and crackers prepared from buffalo skins. PLSR was used for the prediction of LD.	FTIR spectra using regions at 1200-1000 cm^{-1} were selected for PCA and PLSR analysis. The results showed that the relationship between the actual value and FTIR predicted value provides R^2 of 0.96, RMSEC of 2.56, and RMSEP of 1.10. PCA models successfully classify types of buffalo skin, pig skin, and commercial crackers. The PLS calibration model and PCA can be used to classify and quantification of the various types of used skin lipid.	Muttaqien et al. (2016)

LD: lard, SIMCA: Soft Independent Modelling of Class Analogy, M-SVM: Multiclass Support Vector Machine, PLSR: Partial Least Square regression, PCR: Principle Component Regression, RMSEC: Root Mean Square Error of Calibration, RMSEP: Root Mean Square Error of Prediction, RMSECV: Root Mean Square Error Cross-Validation, PCA: Principal Component Analysis, DA: Discriminant Analysis, LDA: Linear Discriminant Analysis.

Table 2 (Cont.). The use of FTIR spectroscopy in combination with chemometrics for analysis of lard in food products.

Issue	Sample preparations and FTIR condition	Chemometrics	Results	References
Analysis of LD in “Rambak” crackers	Cracker containing cow skin (beef fat) and pig skin (lard) with different concentrations were prepared. ATR-FTIR at 4000-650 cm^{-1} .	PLSR for quantitative analysis of LD in crackers; PCA was used to classify crackers with and without LD.	FTIR using absorbance values at regions of 1200-1000 cm^{-1} was successfully employed for the quantitative analysis of LD in “Rambak” crackers. The relationship between the actual value and FTIR predicted values of LD with an R2 value of 0.946 with low errors in calibration and validation models. PCA could classify LD and cow fat in commercial “Rambak” crackers.	Erwanto et al. (2016)
Detection of pig body fat (LD) in pure ghee (heat clarified milk fat).	Pure mixed ghee was spiked with LD at levels of 3, 4, 5, 10, and 15% level. All ATR-FTIR spectra were scanned at 4000 - 500 cm^{-1} .	SIMCA was used for the probable class membership of pure ghee, and that was added with LD. PLSR was used for the prediction of LD.	Using variable absorbance values at combined regions of 3030-2785, 1786-1680, 1490-919 cm^{-1} . SIMCA could classify 90% of samples according to their respective class (pure ghee, LD, pure ghee added with LD). PLSR using the same regions could predict LD in pure ghee with R2 values of >0.99 either in both calibration or validation sets with a detection level of 3%.	Upadhyay et al. (2018)
Analysis of lard extracted from pork in beef meatballs.	The lipid extraction of pork in meatballs was carried out using solvent extraction using petroleum benzene, previously hydrolysed using concentrated HCl.	PLSR and PCA were used for quantification and classification of pork and beef meatballs using absorbance values at 1200-1000 cm^{-1} .	PCA was successfully used for the classification of pork in beef meatballs. PLSR is reliable for the prediction of pork levels through LD analysis. The relationship between the actual value and predicted value of lard (lipid fraction obtained from meatballs containing pork) exhibited a good correlation, with an R ² value of 0.997 and SEC of 0.04%. The results of FTIR spectroscopy were also confirmed using real-time PCR.	Rohman et al. (2017)
Analysis of LD in meatballs broth	Lipids in meatballs were extracted using liquid-liquid extraction using n-hexane. ATR-FTIR at 4000-650 cm^{-1}	PLSR and PCA were used for the quantification and classification of LD and BF extracted from meatball broths.	FTIR spectra using absorbance values at 1200-1000 cm^{-1} combined with PCA are able to classify LD and BF in meatball broth. LD in Lard in meatball broth was predicted using absorbance values at 1018-1284 cm^{-1} , providing R2 of 0.9975 and RMSEC of 1.34% (v/v). The results indicated that the combination of FTIR spectra and chemometrics are effective tools for the analysis of LD intended for Halal verification studies.	Kurniawati et al. (2014)

LD: lard, SIMCA: Soft Independent Modelling of Class Analogy, M-SVM: Multiclass Support Vector Machine, PLSR: Partial Least Square regression, PCR: Principle Component Regression, RMSEC: Root Mean Square Error of Calibration, RMSEP: Root Mean Square Error of Prediction, RMSECV: Root Mean Square Error Cross-Validation, PCA: Principal Component Analysis, DA: Discriminant Analysis, LDA: Linear Discriminant Analysis.

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