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A review of the developed methods for the analysis of lard and pork in food and pharmaceutical products for halal authentication

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Abstract

The development of an analytical method for halal authentication analysis is urgent to assure the halalness of food and pharmaceutical products. Halal authentication analysis is widely applied for the analysis of non-halal components present in food and pharmaceutical products using some analytical methods. Non-halal components typically found in products are pig derivatives, any components derived from a pig (*Sus scrofa*), and alcohol. Pig derivatives such as lard and pork are widely applied in food and pharmaceutical products. Some methods based on chromatographic and molecular spectroscopy are widely applied for the analysis of pork and lard. The analytical responses obtained during the analysis of lard and pork are complex and involve a large dataset, therefore, the employment of multivariate data analysis is a must. This review described comprehensively the application of chemical analytical methods, mainly based on chromatographic and spectroscopic techniques, for the analysis of pork and pork fat (lard) in food and pharmaceutical products. The successful application of analytical methods for halal authentication can assist the regulatory bodies in confirming the absence of non-halal components in the products which support the implementation of halal certification.

1. Introduction

Today, the community of Muslim population is approximately 25% worldwide. It is estimated that the growth of the Muslim population is 35% over the next two decades, accounting for 1.6 billion to 2.2 billion by 2030 (Pew Research Center, 2011). In line with the Muslim population growth, the awareness of Muslims to use and consume only halal products (food, cosmetics and pharmaceuticals) has increased. The market size of halal products is increasing globally which challenges the industry to produce halal products complying with Syariah (Islamic law) requirements. The survey conducted by Yusuf and Yajid (2016) related the awareness of halal cosmetics in five Asian countries halal cosmetic products are high, with which 79% of respondents are aware of the term halal.

Not only Muslims but also non-Muslims are becoming more discerning and demanding the assurance that food and pharmaceutical products are of the highest quality. Thus, halal certification is mandatory to ensure the halalness and wholesomeness of products (Aziz *et al.,* 2014). According to Indonesian Act No. 33 regarding Halal certification, any products supposed to contain any non-halal components must be verified or confirmed using analytical methods in an accredited laboratory according to ISO/IEC 17025: 2017. Therefore, the availability of analytical methods for the detection and confirmation of non-halal components is a must.

The advancement in science and technology has led to the use of non-halal components in the products, and one of the most commonly non-halal components applied in food and pharmaceutical products is pig derivatives. The term pig derivatives (PDs) are any components derived from the pig (*Sus scrofa*) such as lard (pork fat), pork (pig meat), porcine placenta, porcine collagen and porcine gelatin (Wu *et al.*, 2003; Aida *et al.*, 2007; Erwanto *et al.*, 2018). Pork is typically found in meat-based foods such as meatballs, sausages and

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jerky, while lard is commonly found in lipid-based food and pharmaceutical products such as cream, lipstick and lotion (Alvarez and Rodríguez, 2000). The biomarkers in pork and lard such as protein and peptide compositions (for pork) as well as triacylglycerol and fatty acid (for lard) can be exploited as the basis for the development of analytical methods.

Several scientific publications have reported the application of physico-chemical and biological methods for the analysis of pork and lard including molecular spectroscopy (infrared spectroscopy, Raman, NMR), chromatographic-base techniques (liquid chromatography and gas chromatography hyphenated with mass spectrometric detector), differential scanning calorimetry, and electronic nose along with their analytical performances. The analytical responses from spectroscopic and chromatographic measurements involved a large data set, as a consequence, special tools called chemometrics were typically used. This review highlights the updates on analytical techniques in combination with multivariate data analysis for halal authentication analysis of food and pharmaceutical products containing pork or lard.

2. Multivariate data analysis (chemometrics)

Currently, the advances in science and technology have led to the development of powerful analytical instrumentation which offers the possibility to obtain high amounts of data (big data) on the objects or samples analyzed. Even, a single analysis such as gas chromatography for compositional analysis of fatty acids can obtain large data on fatty acids. Molecular spectroscopy also offers some chemical information contained in its peaks. Fortunately, a new discipline known as chemometrics has emerged as a powerful technique to treat big data. Chemometrics is also known as multivariate data analysis. In 1972, Svante Wold introduced chemometrics for extracting useful chemical information from complex experimental data (Oliveri and Forina, 2012).

Chemometrics refers to the employment of statistics and mathematics to treat chemical data. Here, chemical data can be spectra, chromatograms, or any measurement (particularly quantity subjected to measurement). The term chemometrics was also defined by the International Chemometrics Society (ICS) as “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 method” (Rohman and Windarsih, 2020). In relation to authentication analysis, the most used chemometrics techniques are exploratory data analysis, pattern recognition, and multivariate calibration (Moros *et al.*, 2010).

2.1 Exploratory data analysis

Exploratory data analysis (EDA) is widely applied to highlight the information contained in a data matrix to visualize the possible relationships between variables and samples. The objective of EDA is extracting the important variables from a large set of variables available in a data set, identifying the possible clusters of samples using certain variables (peak intensity, absorbances, concentration, fingerprinting), detecting the outliers and identifying the trends among samples and/or variables. Principal component analysis (PCA) and factor analysis are two methods commonly employed in EDA. EDA is typically the first step in the classification of evaluated samples, which is normally carried out by pattern recognition methods (Bosque-Sendra *et al.*, 2012).

PCA is a data reduction technique in which a large data set can be reduced when there is a correlation among the studied variables. Therefore, PCA is not a useful technique if the original variables are uncorrelated (Ziegel, 2004). PCA can be employed as the basis for other more complex pattern recognition techniques. The assumption used in PCA is that a high variance value is synonymous with a high amount of information. Some parameters were typically reported during PCA including principle components (PCs) and loading plots. The first principle component (PC1) describes the most variations in the data set, the second principle component (PC2), accounts for the next largest variation and so on. Therefore, when there is a significant correlation in original variables, the new variables (represented by PCs) are much less than the number of original variables. PCs are also referred to as latent variables or hidden variables because the objects with similar PC1, PC2, PCn values have similar characteristics according to the original variables used (Miller and Miller, 2010). In addition, a loading plot indicated how strongly each characteristic variable influences PCs. If PCs are linear combinations of the original variables, the loading is the coefficients which multiply each variable and indicate the importance of loadings to given PCs. Weighted PCA or wPCA is a valuable alternative way to ordinary PCA as EDA, because wPCA offers some advantages including flexibility, the possibility for making differentiation between signal and baseline, and all results are displayed on the original scale of the data (Gad *et al.*, 2013).

2.2 Pattern recognition techniques

In pattern recognition techniques, two types were usually used (unsupervised pattern recognition and supervised pattern recognition). Pattern recognition is the methods which concentrate on the possibility of assigning the studied samples (objects) to a class based on the result of a set of measurements (Marini, 2009). Unsupervised pattern recognition (UPR) can be differentiated from EDA in that the purpose of UPR is to detect the similarities among objects, while with EDA there is no specific prejudice as to whether or how many classes will be found (Brereton *et al.*, 2017). One of the most commonly used in UPR is hierarchical cluster analysis (HCA). In HCA, a group of different objects (or samples) was divided into classes so that similar samples were in the same class. As in PCA, the groups are not known prior to the chemometrics analysis and there are no assumptions made about the variable distribution. HCA searches the objects which are close together in the variable hyperspace (Miller and Miller, 2010). The squared Euclidean distance and Ward's method are usually used to establish clusters which can be displayed as dendrograms (Sima *et al.,* 2018).

In supervised pattern recognition (SPR), the objects (samples) belonging to certain groups or classes are known. In SPR, a set of samples with known a priori information was used for making calibration or training sets to make the classification model. The calibration model was then used to predict the membership of sample sets which are independent of the training set, known as validation samples. The classification quality of validation samples can be used as a measure of models in terms of predictability, recognition capability, and robustness. If the number of samples was limited, a cross-validation using the leave one technique could be applied. Unfortunately, cross-validation may generate overestimated results (over-fitting) (Berrueta *et al.,* 2007). The most widely SPR techniques are linear discriminant analysis (LDA), partial least squares-discriminant analysis (PLS-DA), orthogonal projections to latent structures–discriminant analysis (OPLS-DA), artificial neural network (ANN), K-nearest neighbour classification (K-NN), and soft independent modelling class analogy (SIMCA) (Bosque-Sendra *et al.*, 2012).

Linear discriminant analysis (LDA) is typically employed for the reduction of data dimensionality and the classification of studied objects (samples). LDA is applied to predict class membership (for example halal food and non-halal food) of unknown samples based on the measurements of several variables (fatty acid compositions, FTIR spectra) (Messai *et al.*, 2016). The initial point of LDA is to find a linear discriminant function (LDF), *Y*, which is a linear combination of the original variables. In LDA, the original variables (*n variables*) for each sample are combined into a single value of *Y*, therefore, the original variables were reduced so the data have been reduced from n dimensions to another dimension. The coefficient values of the terms are selected in such a way that *Y* could reflect the maximum difference among groups. The samples in the same group would have similar values of *Y* and samples in different groups will have very different values of Y. Therefore, LDF or *Y* provides a means of discriminating between the two groups (Miller and Miller, 2010). PLS-DA could cover the correlated or multi-collinear variables in the data. In the initial step, the classified data is calibrated and the pre-defined classes are predicted with unknown samples. The algorithm of NIPALS (Non-Linear Iterative Partial Least Squares) is typically employed in PLS-DA (Gautam *et al.*, 2021). Furthermore, OPLS-DA was used as the improvement of PLS-DA intended to discriminate two or more classes of multivariate data. In OPLS-DA, a regression model is constructed between variables and the response which only contains class information. The evident advantage of OPLS-DA over PLS-DA is that a single component could serve as a predictor (Windarsih *et al.,* 2019).

Conceptually, k-NN method is a simple way for the classification of unknown objects based on the Euclidean metric when there are two or more groups of objects (samples) with a known class, typically called a training set. K-NN can be used when the classes cannot be separated by a plane. In k-NN, there is no assumption about the distributions in the classes and the samples were classified according to their k-nearest neighbors, in which k is an odd number. In its simplest form, k-NN method involves the assignment of the class members of the training set to the known classes. During performing k-NN, there are no outlier data or samples with unclear classification (De Carvalho *et al.,* 2020).

SIMCA is a very flexible technique for class modelling since this technique allows variation in a large number of original variable parameters including scaling or weighting, the components number, the range of scores, confidence level, and different distance weights from the model in the spaces (Forina *et al.*, 2008). The main essential SIMCA feature for class modelling is its ability to characterize the classification results of studied objects in a statistically sound way for estimating the errors of misclassification occurring theoretically (Pomerantsev and Rodionova, 2020). The SIMCA method relied on the application of PCA used for making the decision on the classification of extreme objects which can avoid over-and under-estimation. The models of SIMCA can be defined using the score ranges of objects in selected principle components.

Support Vector Machine (SVM), originally used for developing binary classification problems, is considered

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a supervised pattern recognition technique commonly used for the classification of objects in hyperplanes. Currently, SVM is applied to solve the problems of regression analysis. SVM was used for making the decision boundaries capable of separating data points of different classes. This chemometrics technique can able to handle simple, linear, and nonlinear classification problems (Xu *et al.,* 2006). The idea underlying SVMs is to map the original variables from the input space to a high dimensional space such that the classification problem becomes simpler in the feature space (Luts *et al.*, 2010).

Some performance characteristics were used for the evaluation of chemometrics of class modelling, such as sensitivity, specificity, and accuracy, as follows (Oliveri and Downey, 2012; Oliveri *et al.*, 2019):

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad (1)$$

$$\text{Specificity} = \frac{TN}{TN + FP} \qquad (2)$$

The parameter of model efficiency was also used, which is computed as the geometric mean of sensitivity and specificity;

$$\text{Efficiency} = \sqrt{\frac{TP \times TN}{(TP + FN) \times (TN + FP)}} \qquad (3)$$

The further parameters typically used for class modeling (chemometrics classification) are precision and accuracy.

$$\text{Precision} = \frac{TP}{TP + FP} \qquad (4)$$

$$\text{Accuracy} = \frac{TN + TP}{TN + TP + FN + FP} \qquad (5)$$

The parameter known as Matthews's correlation coefficient (r_M) was used as a comprehensive evaluation of model efficiency, considering all four possible outcomes (TP, TN, FP and FN).

$$\text{Matthews's correlation coefficient} = \frac{(TP \times TN) - (FP \times FN)}{\sqrt{(TP+FP) \times (TP+FN) \times (TN+FP) \times (TN+FN)}} \qquad (6)$$

2.3 Multivariate calibrations

Multivariate calibration is calibration which involves the use of multivariate data (the absorbance values at several wavelengths or wavenumbers or peak area in chromatogram) intended for the prediction of analyte(s) levels, pork and lard in this case. Multivariate calibration is usually used to develop calibration and validation models capable of correlating the actual values of lard or pork as determined with reference methods and predicted values using several variables assessed (Gao and Ren, 2010).

In quantitative analysis, various multivariate calibrations have been employed and optimized for the determination of pork and lard. The most commonly used are partial least square regression (PLSR), principle component analysis (PCR) and stepwise multiple linear regression (SMLR) (Bro, 2003). Some statistical parameters typically used for the evaluation of performance characteristics in multivariate calibrations are coefficient of determination (R^2) for the relationship between two variables, actual values in x-axis and predicted values using specific instruments, root mean square error of calibration (RMSEC) for evaluation error in calibration, root mean square error of prediction (RMSEP) for evaluation error in prediction or external validation. RMSEC and RMSEP were obtained using these equations:

$$\text{RMSEC} = \sqrt{\frac{\sum_{i=1}^{m}(\hat{Y}i - Yi)^2}{M-1}} \qquad (7)$$

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{Y}i - Yi)^2}{N}} \qquad (8)$$

Yi and $\hat{Y}i$ represent the actual and predicted value of analyte(s), while M and N are the number of data in the calibration and validation set (Pebriana *et al.*, 2017). The calibration models were also cross-validated using the leave-one-out technique (LOO) for detecting over-fitting. In the LOO technique, one of the calibration samples is left out from the calibration models developed, and the left calibration samples are crossed using a new calibration model. The left sample is computed using a newly developed calibration model. This procedure was repeated by leaving one by one of the calibration samples. The performance characteristics applied during the LOO technique are R^2, root mean square error of cross-validation (RMSECV), and Predicted Residual Error Sum of Squares or PRESS for evaluation of accuracy, precision and number of factors, respectively (Rohman and Anggita, 2019).

3. Application of molecular spectroscopy for analysis of pork and lard

Molecular spectroscopy is the study involving the interaction between electromagnetic radiation in certain frequencies or wavelengths with analyte(s) at molecular levels. Over the past few decades, molecular spectroscopy has been gaining interest to be applied in the quality control of food and pharmaceutical products. This method offers some main advantages including ease in sample preparation, low cost, less time analysis and require less chemical reagents and solvents which support the campaign to use green analytical chemistry (Cozzolino, 2014). The most commonly used molecular spectroscopic-based techniques used for the analysis of lard and lard extracted from pork in food and pharmaceutical products are vibrational spectroscopy

(Infrared and Raman) and nuclear magnetic resonance (NMR) spectroscopy.

3.1 Analysis of lard and pork using FTIR spectroscopy

FTIR spectroscopy is considered a fingerprinting analytical technique and is the ideal technique for the analysis of analyte(s). In the analysis of edible fats and oils including lard, each peak and shoulders in FTIR spectra correspond to the functional groups contained in the analyzed samples. Indeed, the functional groups in triacylglycerols are dominant because the main components composed of edible fats and oils are approximately 98% TAG with 2% minor components including sterols, soluble vitamins and phenolics. The representative FTIR spectra of lard and other animal fats are shown in Figure 1.

Analysis of lard and pork using FTIR spectroscopy was typically performed by (1) direct analysis of lard without any extraction steps, especially in the mixture with other edible fats and oils for authentication analysis and (2) analysis of extracted fats (lipid fraction) in meat-based food and pharmaceutical products. Two chemometrics techniques (pattern recognition and multivariate calibration) were normally used for the identification and quantification of lard and pork in this model. The analytical steps involving the use of multivariate calibration for the analysis of lard and pork in food and pharmaceutical products are schematically depicted in Figure 2.

Table 1 compiles the application of FTIR spectroscopy for direct analysis of lard in the mixtures with other fats and oils along with chemometrics techniques used for modelling and data analysis. Lard (non-halal fats) has been differentiated from other animal fats and edible oils (chicken fat, mutton fat, tallow fat and palm oil) using FTIR spectra and classification chemometrics (PCA, k-means CA and LDA). The evaluated samples were subjected to temperatures of 120, 180 and 240°C at different times (30, 60, 120 and 180 min). FTIR spectra at wavenumbers of 4000-650 cm $^{-1}$ were used as variables for chemometrics of PCA, k-means CA and LDA). The combination of PCA with k-mean CA was capable of differentiating the heated fats and oils according to their origin. In addition, LDA could classify animal fats and edible oils with an accuracy rate of 80.5% in its group (Salleh *et al.*, 2018).

Lard can be found in food products such as cakes and chocolates and pharmaceutical products such as cream, lotion and lipstick. Lard may be obtained from extracting lipid components in pork-based food such as meatballs and sausages. Lard in food and pharmaceutical products can be extracted using some extracting techniques including liquid-liquid extraction, Soxhlet, Bligh and Dryer. FTIR spectroscopy in combination with PCA has been used for the identification of pork in meat-based foods labelled with Halal and Non-halal. The absorbance values at wavenumbers of 3007-1236 cm^{-1} were used as variables during PCA modelling. Pork fat (lard) could be clearly separated from other animal fats. PCA has also been used for the identification of meat-based foods labelled as Halal and non-halal and score plots of non-halal food are closer to those of pork fat, while score plots of halal meat-based food located near chicken fat (Saputra *et al.,* 2018).

The presence of lard in sausages was fruitfully analyzed using ATR-FTIR spectra and chemometrics. Fats (lipid fractions) in sausages were extracted using Soxhlet with n-hexane as extracting solvents at a temperature of 70°C. ATR-FTIR spectra of evaluated

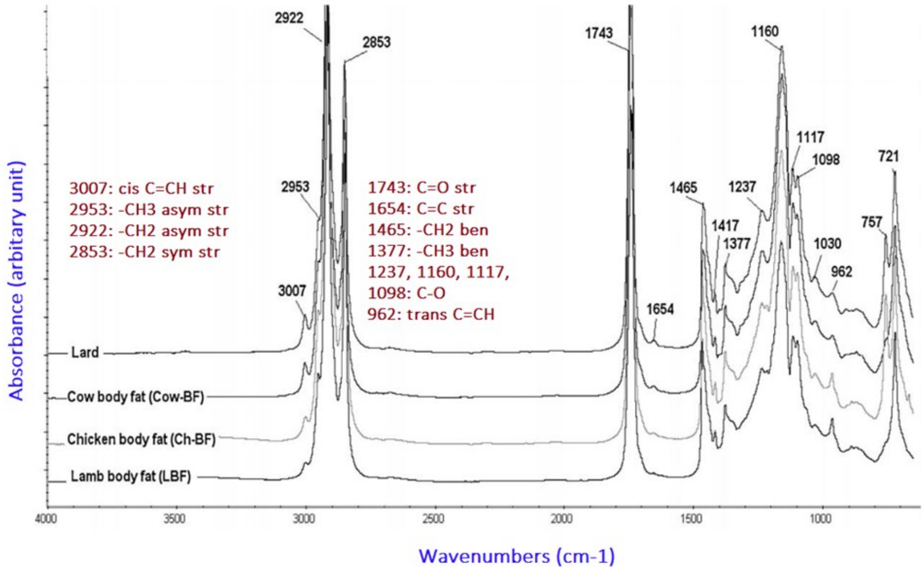

Figure 1. FTIR spectra of lard and other animal fats scanned using attenuated total reflectance at mid-infrared regions (4000-650 cm^{-1}) (Che Man *et al.,* 2011; Rohman and Che Man, 2011).

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Table 1. Analysis of lard in the mixtures with other animal fats and edible oils using FTIR spectroscopy and chemometrics during the last ten years.

Issues	FTIR spectra condition	Chemometrics	Results	References
Identification of lard from 16 edible fats and oils	Normal ATR-FTIR spectra at mid-infrared region (4000-650 cm^{-1})	PCA and HCA	Using absorbance values at whole IR regions, PCA and HCA could identify lard from others. Using PCA, a 74.3% variance could be described using PC1 and PC2.	Che Man *et al.* (2011)
Lard adulteration in Cod Liver Oil (CLO)	FTIR spectra using absorbance values of 1035–1030 cm^{-1} (quantification) and 1500–1030 cm^{-1} (for discrimination)	PLSR for quantification and LDA for discrimination	PLSR was successful in facilitating the prediction of lard in CLO providing a good relationship between the actual and predicted value of lard with R^2 of 0.996 (calibration), 0.987 (validation), RMSEC of 1.04% and RMSECV of 1.605%	Rohman and Che Man (2009)
Lard in ternary mixtures with animal fats of lamb, cow and chicken	FTIR spectra were scanned at mid-infrared region (4000–650 cm^{-1}) with resolution of 4 cm^{-1} and 32 scanning	PLSR and PCR. The composition of animal fats was selected randomly using Excel.	PLSR using the first derivative FTIR spectra at 1500–1000 cm^{-1} provides the best prediction with R^2 of 0.997 and SEC of 0.773%	Rohman and Che Man, 2011)
Lard adulteration in palm oil	ATR-FTIR spectra at wavenumbers of 1480-1085 cm^{-1}	PLSR	The relationship between the actual value of lard and FTIR value resulted linear relationship with R^2 of 0.998, RMSEC value of 1.69 %, and RMSECV of 2.87 % (v/v).	Rohman *et al.* (2012)
Analysis of lard as an adulterant in palm oils and animal fats	ATR-FTIR spectra at wavenumbers of 3007-1236 cm^{-1}	PLSR for quantification and PCA for classification	FTIR spectra using PCA have been shown to be a powerful tool to detect lard adulteration. PLSR was successful in the prediction of lard contents	Saputra *et al.* (2018)
Analysis of lard as adulterant in Virgin Coconut Oil (VCO)	ATR-FTIR spectra at wavenumbers of 3030-2992 cm-1	PLSR	A good square correlation coefficient of 0.997 was obtained in the prediction of lard	Shimadzu (2017)
Analysis of lard in binary mixture with vegetable oils of canola oil, corn, sunflower, and soybean oils	FTIR spectra at wavenumbers of 1500-1000 cm^{-1}	PLSR and LDA	Using PLSR, the lard as low as 1% could be detected. LDA based on Coomans plot is successful for the classification of vegetable oils and those adulterated with 1–50% of lard.	Rohman, Yaakob, Hashim *et al.* (2011)
Analysis of lard as an adulterant in VCO	The combined frequency regions of 3020–3000 and 1120–1000 cm^{-1}.	PLSR and LDA	PLSR could predict the levels of lard as an adulterant in VCO with an R2 of 0.9990. LDA can classify VCO and adulterated with lard with an accuracy rate of 100%.	Mansor *et al.* (2011)
Analysis of lard as an adulterant in Bovine milk fat (BMF) is	Normal FTIR spectra at 3098-669 cm^{-1} (LDA); The first derivative spectra at the combined wavenumber regions of 3033-2770 and 1510-692 cm^{-1}	PLSR and LDA	LDA could perfectly classify authentic BMF and adulterated BMF with lard. PLSR allowed the quantification of lard in BMF with R^2 (>0.99) in calibration and validation models with RMSEC of 0.631% and RMSEP of 1.94%.	Windarsih *et al.* (2020)
Detection of lard in pure ghee (heat-clarified milk fat)	The combined wavenumbers of 3030-2785, 1786-1680, and 1490-919 cm^{-1}	PCA, SIMCA and PLSR	PCA and SIMCA using variables of absorbance at selected wavenumbers could classify ghee and ghee adulterated with lard with a classification efficiency of 100%. PLSR statistically prove that the said region is good for detecting the presence of lard in ghee.	Upadhyay *et al.* (2018)

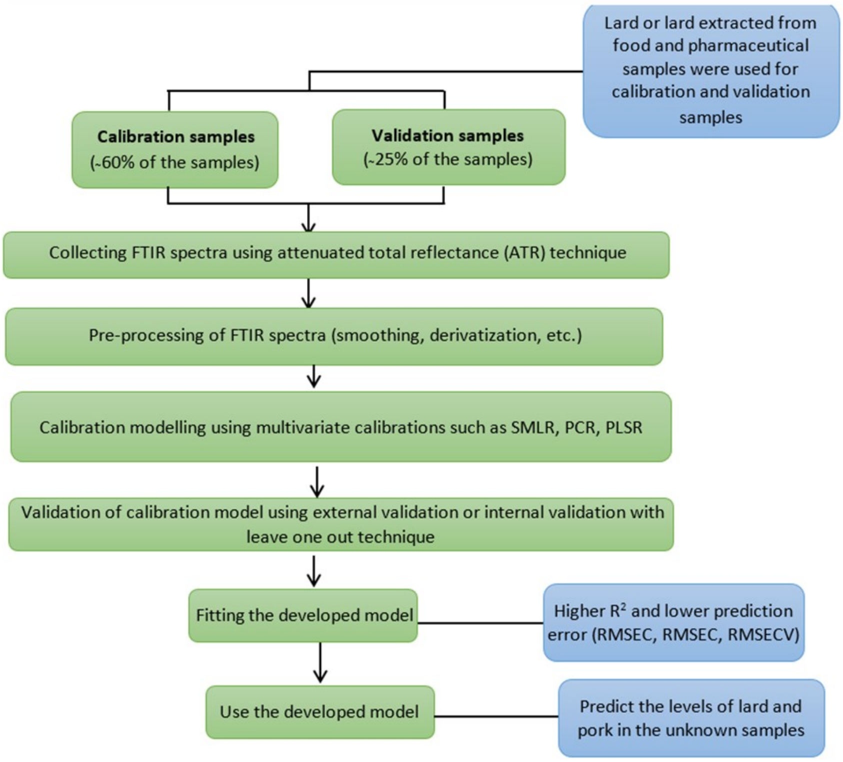

Figure 2. The schematic diagram related to the application of FTIR spectroscopy and multivariate calibration for analysis of lard and pork in food and pharmaceutical products (Rohman *et al.*, 2020).

samples were subjected to PLSR for quantification of lard in sausages and PCA was used for classification between sausages with lard and without lard. The absorbance values at optimized wavenumber regions of 1200–1000 cm^{-1} were employed for PLSR which resulted in the equation for the relationship between actual (x-axis) and FTIR predicted value (y-axis) of $y = 0.921x + 4.623$ with R^2 of 0.985 and RMSECV of 5.12%. PCA using the same variables used in PLSR could differentiate 100% pork sausage and 100% beef sausage. This result supports the applicability of FTIR spectra for the analysis of pork intended for halal authentication analysis (Guntarti *et al.*, 2019).

The combination of FTIR spectra and chemometrics is also used for the analysis of lard in printing materials typically used in food and pharmaceutical products for labelling. Two wavenumber regions include the full mid-IR region (4000-650 cm^{-1}) and the combined regions of 3110–2630, 1940–649 cm^{-1} were optimized for discrimination and classification of lard, commercial gravure ink, and the mixture of both using supervised pattern recognition of SIMCA and unsupervised pattern recognition of PCA. PCA could explain 87% variance using 7 PCs. SIMCA could classify lard and others according to classes. Some commercial printing samples were evaluated using the developed method, and one of the samples (sample 2) was deduced to have the highest possibility of lard, while others may be not-lard containing samples. This finding informed that the combination of FTIR spectra and chemometrics could provide a rapid method for detecting lard in the ink of foodstuff packaging without excessive sample preparation (Ramli *et al.*, 2015).

In cosmetic products, lard may be found in some preparations. The safety assessment revealed that lard is safe to be used in cosmetic products (Becker, 2017). Waskhito *et al.* (2016) analyzed lard in lipstick formulation containing castor oil. Three different extraction methods include (1) saponification method followed by liquid-liquid extraction (LLE) with extracting solvent of hexane-dichloromethane-ethanol-water, (2) saponification method followed by LLE with dichloromethane-ethanol-water, and (3) Bligh and Dyer extraction method using chloroform-methanol-water. The chemometrics of PCA and PLSR were used for the classification and quantification of lard respectively. Using three extraction methods, PCA was capable of identifying lard using wavenumbers of 1200-800 cm^{-1} in which the method (3) provides the best results as indicated the most distinct separation cluster between lipstick containing lard and castor oil. The extraction method (3) also gave the best prediction models of lard quantification as analyzed using PLSR employing absorbance values at 1200-800 cm^{-1} with the statistical parameters of R^2-values of 0.9956 and 0.9970 (in calibration and validation models) and low values of RMSEC and RMSEP. This indicated that the combination of PLSR and FTIR spectra provides an accurate and precise method for the prediction of lard. The employment of FTIR spectroscopy and chemometrics for the analysis of pork and its lipid fraction in food and pharmaceutical products are compiled in Table 2.

3.2 Raman spectroscopy

As the complementary technique to IR spectroscopy, Raman spectroscopy (RS) uses the same wavenumbers used in the IR region. RS is one of the vibrational spectroscopies widely applied for quality controls of food and pharmaceutical products. RS is relied on inelastic scattering which results in frequency (wavenumbers) shifting. In RS, the samples are subjected to photons, resulting in excitation from a ground state to the excited state, then undergoing a wavelength shift (Craig *et al.,* 2013). Some Raman spectroscopy techniques existed including FT-Raman spectroscopy, dispersive RS, spatially offset RS, and surface-enhanced RS. In addition, RS provides an imaging technique for fingerprinting and profiling of compounds in samples, which is very useful for sample differentiation. Therefore, the application of RS in combination with chemometrics is useful for halal authentication analysis and is noticeably promising. The combination of RS with chemometrics was applied for analysis of lard in mixtures with other oils and fats including beef tallow, chicken fat, and duck oil. The energy source used was laser diode 785 nm and Raman spectra were scanned at the wavenumber region of 1800–700 cm^{-1} applying a resolution of 1.25 cm^{-1}. PCA was successful in being applied to the classification of lard with animal fats (Lee *et al.*, 2018).

3.3 Nuclear magnetic resonance spectroscopy

NMR spectroscopy relies on the interaction of molecules with certain radio waves, resulting in changes in spin direction. NMR spectra provide fingerprint spectra, which makes it useful for the differentiation of lard and pork with other edible fats and oils. The main advantage of NMR spectroscopy is the fact that this technique does not involve extensive reagents and solvents which support the application of green analytical technique. In addition, NMR spectroscopy is easy in-sample preparation, robust and reproducible and can be used for the simultaneous analysis of heterogeneous samples. Proton-NMR (1H-NMR) and Carbon-NMR (13C-NMR) in combination with chemometrics are the most common spectroscopic techniques applied for halal authentication analysis (Rolin *et al.*, 2013). NMR spectra can generate large data sets and are widely applied for fingerprinting profiling and metabolomics studies in the field of food and pharmaceutical products (Rocchetti and O'Callaghan, 2021).

1H-NMR and 13C-NMR spectra combined with chemometrics were successful for the authentication of butter from lard. Certain Peaks in the region of 2.60–2.84 ppm revealed the special characteristics which only observed in lard. The peak at δ 2.63 corresponding to the chemical shift of double-allylic methylene protons is a unique characteristic in lard. Some signals 1H-NMR were correlated with 13C-NMR spectra for the confirmation of signals (Fadzillah *et al.*, 2017).

4. Gas Chromatography-Mass Spectrometry

Recently, GC-MS in combination with chemometrics of PCA, HCA, and PLS-DA has been used for the detection of lard adulteration in wheat biscuits. Lard was extracted from biscuit samples using Soxhlet according to the AOAC method (2007) using petroleum ether as extracting solvent. GC-MS was used for analysis of fatty acids derived as methyl esters. The profiles (types and levels) of fatty acids were used as variables during chemometrics analysis (PCA, HCA, PLS-DA). PCA and HCA could classify lard, wheat biscuits without lard and lard-adulterated biscuit samples using those variables. Random forest and PLS-DA also could discriminate lard, wheat biscuits lard and biscuit samples adulterated with lard in which random forest outperformed PLS-DA by giving prediction accuracy of 100% and 92% for random forest and PLS-DA, respectively. Furthermore, linoleic acid (C18:3n6) is proposed as the potential biomarker in discriminating pure wheat biscuits and biscuits adulterated with lard because of its dose-dependent composition with lard addition (Azizan *et al.*, 2021).

GC-MS is a suitable approach for the analysis of lard and lipid components extracted from pork in food and pharmaceutical products (Chin *et al.*, 2009). The combination of two-dimensional gas chromatography applying two different columns with different polarity (GC×GC) combined with time-of-flight mass spectrometry (TOF-MS) has been successfully applied for the identification of lard by finding some fatty acid markers which are only present in lard. The specific fatty acid markers in lard as discriminant from other animal fats are methyl-*trans*-9,12,15-octadecatrienoate (C18:3 n3t), methyl-11,14,17-eicosatrienoate (C20:3 n3t), and methyl-11,14-eicosadienoate (C20:2n6) (Indrasti *et al.*, 2010). This technique is suitable for the analysis of lard in any product (food, cosmetics and pharmaceuticals).

5. Conclusion

Halal authentication analysis is a must to be developed and standardized to assure that food and pharmaceutical products consumed or used by Muslims are free from non-halal components. Some analytical methods based on spectroscopic and chromatographic techniques in combination with multivariate data

Table 2. Analysis of pork and its lipid fraction (lard) in food and pharmaceutical products using FTIR spectroscopy and chemometrics.

Issues	FTIR spectra condition	Chemometrics	Fat extractions	Results	References
Analysis of lard in broth meatballs	ATR-FTIR spectra at wavenumbers of 1018–1284 cm^{-1} (PLS) 1200-100 cm^{-1} (PCA)	PCA for classification and PLS for quantification	Meatball broth was extracted using liquid-liquid extraction using n-hexane	FTIR spectra could be used for the prediction of lard in meatball broth with R^2 of 0.9975 and RMSEC of 1.34% (v/v). PCA could classify meatball broth containing lard and beef fat.	Kurniawati *et al.* (2014)
Analysis of pork in Halal and non-Halal Chinese ham Sausages	Transmittance FTIR spectra at wavenumbers of 4000-400 cm^{-1}	FTIR spectra were subjected to derivatives and SNV. PLS-DA and LS-SVM were used for the classification	The samples of ham sausage were finely ground and added with KBr to form pellets to be subjected to FTIR spectral measurement.	FTIR spectra treated with second derivative-SNV offered good spectral treatment to be used as variables during PLSDA and LS-SVM. The sensitivity and specificity for discrimination between halal and non-halal sausages were 0.913 and 0.929 (PLSDA) as well as 0.957 and 0.929 (LS -SVM).	Xu *et al.* (2012)
Analysis of pork in beef meatballs	ATR-FTIR spectra at wavenumbers of 1200-1000 cm^{-1}	PCA for exploratory data analysis and multivariate calibrations (PLSR and PCR)	Meatballs were ground, hydrolyzed with concentrated HCl and subjected to liquid-liquid extraction using a mixture of petroleum ether and petroleum benzene (1:2 v/v).	PLSR offered the best correlation between the actual value and FTIR predicted value with an R^2-value of 0.997 and SEC of 0.04%. PCA could classify pork meatballs and beef meatballs.	Rohman *et al.* (2017)
Analysis of pork in meatballs formulation	ATR-FTIR spectra at wavenumbers of 1200-1000 cm^{-1}	PLSR	Meatball was cut into small pieces, ground and subjected to extraction using the Soxhlet method	The method provided a good relationship between the actual value and FTIR predicted values with the equation of predicted value = 0.999 x (actual value) + 0.004; R^2, RMSEC and RMSEP values of 0.999, 0.442 and 0.742, respectively with 4 principal components.	Rohman, Sismindari, Erwanto *et al.* (2011)
Analysis of pork adulteration in *dendeng* (beef jerky)	Normal ATR-FTIR spectra (4000–700 cm^{-1})	Class modelling using LDA, SIMCA, and SVM	Dendeng was cut into small pieces, blended to form powder, and subjected to FTIR analysis	Among class modelling techniques used, LDA offered the best classification between pork and beef dendeng with an accuracy level of 100%. The results obtained using FTIR spectra showed good agreement with those using the ELISA method.	Kuswandi *et al.* (2015)
Analysis of pork in the mixtures with raw minced camel and buffalo meat	FTIR spectra in wavenumbers of 2000 -1000 cm^{-1}	Simple linear regression using absorbance ratios at1654/2924 cm^{-1} (pork in camel) and 1540/2924 cm^{-1} (pork in buffalo meats)	The meats were minced, homogenized, dried, and then subjected to FTIR spectral measurement in the form of KBr pellet	Pork could be predicted using FTIR spectroscopy with values of R^2 0.9174 and 0.942 for pork in camel and buffalo, respectively.	Lamyaa (2013)

SNV: standard normal variate, PLSDA: partial least squares discriminant analysis, LS-SVM: least squares support vector machine, SIMCA: soft independent modelling of class analogy, SVM: Support Vector Machines.

Table 2 (Cont.). Analysis of pork and its lipid fraction (lard) in food and pharmaceutical products using FTIR spectroscopy and chemometrics.

Issues	FTIR spectra condition	Chemometrics	Fat extractions	Results	References
Detection of pork adulteration in beef-based formulation	FTIR spectra at 1480-1360 cm^{-1} (PCA) and the combined wavenumbers region of 1760-1710 cm^{-1} and 1210-1190 cm^{-1} (HCA)	PCA and HCA	The meats were minced, homogenized, dried, and then subjected to FTIR spectral measurement in the form of KBr pellet	PCA using absorbance values of 1480-1360 cm^{-1} separate beef-based food and food adulterated with pork. HCA could make clustering between beef and beef adulterated with pork with sensitivity and specificity of 100%.	Candogan *et al.* (2020)
Analysis of lard in Rambak crackers	FTIR spectra at 1200–1000 cm^{-1}	PCA and PLS	The lard in crackers was extracted using Soxhlet extraction with hexane as an extracting solvent	PLS relationship between the actual value of lard and FTIR predicted provides an R^2 value of 0.946 with low errors in calibration and validation. PCA can be successfully used for the classification of lard in "Rambak" crackers.	Erwanto *et al.* (2016)
Discrimination of lard from other animal fats and edible oils in biscuit products	The combined frequency regions of 3050–2800, 1800–1600, and 1500–650 cm^{-1}	LDA	The lipid fractions containing fats/oils from biscuits samples were extracted using the Soxhlet method	The Cooman plot revealed that all edible oils and animal fats, including lard, are clustered in a distinct group. LDA was also used for discriminating biscuits with lard and without lard.	Man *et al.* (2011)
Analysis of lard in binary mixture with EVOO in cosmetics products (cream)	FTIR spectra at a combined frequency of 1785-702 and 3020-2808 cm^{-1}. PCA was analyzed using a frequency of 1200 – 1000 cm^{-1}	PCA and PLS	10 g cream was added with HCl, separated, and extracted using 2 × 15 mL of hexane.	The levels of lard in the mixture with EVOO in cosmetics creams have been successfully predicted with the aid of PLS. PCA could classify creams with and without lard.	Rohman *et al.* (2014)
Analysis of lard in binary mixture with palm oil in cosmetics products (cream)	FTIR spectra at wavenumbers of 1200 – 1000 cm^{-1}	PLSR and PCA	Samples of lotion (10 g) were added with HCl and extracted using chloroform.	PLSR could predict lard in the mixture with palm oil in lotion cosmetics with R^2 of 0.99059, and RMSECV of 1.72% v/v. PCA could classify lotions with lard and palm oil, respectively with an accuracy level of 100%.	Lukitaningsih *et al.* (2012)

SNV: standard normal variate, PLSDA: partial least squares discriminant analysis, LS-SVM: least squares support vector machine, SIMCA: soft independent modelling of class analogy, SVM: Support Vector Machines.

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analysis (chemometrics) are successfully validated and applied for analysis of pork in food products such as meatballs and sausages. The identification of specific markers in lard is the best approach for authentication of pharmaceutical products from lard. Some laboratories and government agencies should collaborate to make the proficiency testing for comparing the analytical results obtained using the developed methods.

Conflict of interest

The authors declare no conflict of interest.

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