

Mini Review

The use of vibrational spectroscopy and chemometrics in the analysis of pig derivatives for halal authentication

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Abstract

Currently, issues related to halal products such as adulteration, trade, and preference are emerging issues among Muslim and non-muslim countries. Halal authentication of products is necessary in order to assure the halalness of products including food, pharmaceuticals and cosmetics, especially for Muslim communities. Due to the advancement of science and technology, some food may be adulterated or contaminated with non-halal components, therefore, the identification and, in some cases, quantification of non-halal components are obligatory for Muslim scholars. Several analytical methods have been developed, proposed, and standardized for analysis of non-halal components in some products such as chromatographic techniques, spectroscopic, and molecular biology techniques. Due to its simplicity and ease in operation, vibrational spectroscopies are the popular methods for analysis of non-halal components. In this review, we highlighted the use of vibrational spectroscopy (near infrared, mid infrared, and Raman) combined with chemometrics techniques for analysis of non-halal components in some products (food, cosmetics and pharmaceuticals).

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Introduction

Since the human civilization, food, cosmetics and pharmaceutical products are the essential human needs. It is an obligation for Muslim to not consume any consumer goods containing nonhalal components (Mursyidi, 2013). Non-halal products are absolutely forbidden for every Muslim except under extremely exceptional circumstances. Nonhalal components are grouped into the following nine categories: (i) carrion or dead animals, (ii) flowing or congealed blood, (iii) pig derivatives, including all its by-products such as lard, pork, and porcine gelatin (iv) animals slaughtered without pronouncing the name of God (Allah), (v) animals killed in a manner that prevents their blood from being fully drained from their bodies, (vi) all types of intoxicants, including alcohol and drugs, (vii) carnivorous animals with fangs such as lions, dogs, wolves, or tigers, (viii) birds with sharp claws (birds of prey) such as falcons, eagles, owls, or vultures, and (ix) land animals such as frogs or snakes (Riaz and Chaudry, 2003; Che Man and Shadzili, 2010). Among these nine categories, pig derivatives and intoxicants are frequently meet in food, cosmetics and pharmaceuticals products. In this review, we limited on pig derivatives because they are frequently met in consumer products.

Pig derivatives refer to any substances or

compounds resulting from or derived from a pig (*Sus scrofa*) such as lard obtained from pig fat, pork as a synonym for pig meat, and any gelatin produced from the bone and skin of pig. Derivatives of pig are usually cheaper than those of Sheep and cattle. For this reason, pig derivatives are used as a component in any consumer goods (Rohman and Che Man, 2008). Currently, there are numerous food, cosmetics and pharmaceutical products available in the market supplied to consumers in which the actual contents of these products may contain pig derivatives; consequently, the identification of pig derivatives in products is necessary for assuring the halalness of products (Mursyidi, 2013).

Vibrational spectroscopy

Vibrational spectroscopy (VS) deals with the interaction between electromagnetic radiation in infrared region with molecules. VS is the analysis of molecular properties based on vibrations at molecular level, associated with the chemical bonds in the sample, and it is highly selective (Diem, 1993; Lewis and Edwards, 2001). VS is used to describe the collective term of two powerful analytical techniques, namely: (1) infrared (IR) spectroscopy in near IR region (corresponding to wavenumbers of 14,000–4000 cm^{-1}), mid IR region (MIR, corresponding to wavenumbers of 4000–400 cm^{-1}), and far IR (in

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wavenumbers of 400–50 cm^{-1}), as well as (2) Raman spectroscopy (Moros *et al.*, 2010).

Infrared and Raman spectroscopy are rapid and sensitive enough, simple in operation, nondestructive and noninvasive methods that provide useful information about the molecular composition, structure, and interactions within a sample (Griffiths and Chalmers, 1999). One of the most important features of VS is the possibility to perform the simultaneous determination of different components in the same sample from a single instrumental measurement. Besides, VS can be used in the extended range of region covered and its excellent resolution together with the numerous bands associated with the structure of molecules that make up the samples (Moros *et al.*, 2010).

VS has been recognized as a fast analytical technique to detect and quantify pig derivatives in any samples such as food, cosmetics and pharmaceutical in the form of liquid, semisolid or solid (Harz *et al.*, 2009; Rohman and Che Man, 2012), because VS provides important information about the presence of certain functional groups responsible for peak and shoulder absorption (Che Man *et al.*, 2010). VS is also considered a “fingerprint technique,” meaning that there are no two oils with the same FTIR spectra either in the number of peaks, the maximum peak intensities and the exact frequencies of each bands and shoulders (Yap *et al.*, 2007). VS is also taken into account as green analytical chemistry because the use of toxic reagent and hazardous solvents is minimized or even eliminated (Moros *et al.*, 2010; Khanmohammadi and Garmarudi, 2011).

Commercially, there are two instruments of vibrational spectrophotometers, namely dispersive and Fourier transform. Fourier transform infrared (FTIR) and FT-Raman spectrophotometer are based on interferometry. The Michelson interferometer used in the most FTIR and FT Raman spectrophotometers is composed of two perpendicular mirrors, namely a moving mirror that travels at a constant velocity and a stationary mirror. Between two mirrors, there is a beam splitter normally made up of KBr coated with germanium (for mid-IR) (Karoui, 2008). The interferogram contains all information required to produce an IR spectrum of the sample, but this information is in the time domain. In order to obtain a conventional IR spectrum, the interferogram must be converted to the frequency domain using Fourier transformation (Rohman *et al.*, 2014).

Chemometrics

The success of VS in the analytical purposes is the use of chemometrics. Chemometrics is branch

of sciences which use statistical and mathematical tools to extract the chemically relevant information from the chemical data which permit maximum collection and extraction of useful information (Bro *et al.*, 1997; Lavine, 1998; El-Gindy and Hadad, 2012). The International Chemometrics Society defines chemometrics as “the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods” (Gemperline, 2006).

The chemometrics procedure enables multidimensional calibration of selected spectroscopies techniques, mainly for interpretation of VS spectra, as well as for data obtained by other instrumental methods (El-Gindy and Hadad, 2012). Chemometrics is exploited for multivariate data collection and analysis protocols, calibration modelling, classification and cluster modelling, signal correction and compression, method optimization and statistical process control. Singh *et al.* (2013) stated that chemometrics is useful means for the real-time in-process testing and is a valuable process analytical tools. Generally, chemometrics are divided into two main categories, namely, for qualitative analysis (identification/classification) using unsupervised and supervised pattern recognition methods and multivariate calibration for quantitative purposes (Brereton, 2003; Miller and Miller, 2010).

According to Moros *et al.* (2010), in VS, there are three important chemometrics techniques commonly used, namely, (1) data processing such as derivatization, normalization, baseline corrections, standard normal variate, mean centering, derivatives, and multiplicative corrections; (2) classification techniques, which can be either supervised pattern recognition like discriminant analysis and partial least square-discriminant analysis (PLS-DA) or unsupervised pattern recognition such as principal component analysis (PCA) and cluster analysis; and (3) regression methods, in order to correlate between vibrational spectra to the quantifiable properties (concentration) of the analyte(s) of interest, mainly those based on multivariate regression through principal components (PC) known as principle component regression (PCR) and partial least squares (PLS) approaches. Some multivariate calibration techniques are also available, such as multivariate curve resolution algorithms, genetic multivariate calibration, or concentration residual augmented classical least squares (Miller and Miller, 2000).

Table 1. Some reported publications related to analysis of pig derivatives using FTIR spectroscopy along with chemometrics used

Pig derivatives	Sample	Issue	Techniques	Chemometrics	References
Lard	Edible fats and oils	Lard characterization from other edible fats and oils	ATR-MIR at whole mid infrared region	-	Guillen dan Cabo (1997)
Lard	Animal fats	The presence of lard in other animal fats	ATR-MIR at 3009–3000 cm^{-1} (in lamb fat), 3006 cm^{-1} (in chicken fat), and 966.22 cm^{-1} (beef fat)	PLS	Che Man dan Mirghani (2001); Jaswir <i>et al.</i> (2003)
Lard	Animal fats	The presence of lard in other animal fats	ATR-MIR at 1,500–900 cm^{-1}	PLS, DA	Rohman and Che Man (2010)
Lard	Cake formulation	The use of lard in cake formulation	ATR-MIR at 1117–1097 cm^{-1}	PLS	Syahaniza <i>et al.</i> (2005)
Lard	Chocolate and chocolate products	The adulteration of lard in chocolate and chocolate products	ATR-MIR at 4000–650 cm^{-1}	PLS	Che Man <i>et al.</i> (2005)
Lard	Biscuit	The sue of lard as one of component in biscuit	ATR-MIR at 3500–2900 cm^{-1}	PLS	Syahaniza (2006)
Lard	Cod liver oil	Authentication of cod liver oil from lard	ATR-MIR at 1035–1030 cm^{-1}	PLS, DA	Rohman dan Che Man (2009)
Lard	Virgin coconut oil	Authentication of cod liver oil from lard	ATR-MIR at 1,120–950 cm^{-1} ,	PLS, DA	Mansor <i>et al.</i> (2011)
Lard	Vegetable oils	Adulteration of high price vegetable oils with lard	ATR-MIR at 1,500–1,000 cm^{-1}	PLS, PCR, PCA	Rohman <i>et al.</i> (2011) ^b
Lard	Cream cosmetics	The use of lard mixed with olive oil as base in cream cosmetics	ATR-MIR at 1200–100 cm^{-1}	PLS, PCR	Rohman <i>et al.</i> (2014)
Lard	French fries	The presence of lard mixed with palm oil as frying oil in French fries	MIR-ATR at 3100–1050 cm^{-1}	PLS and DA	Che Man <i>et al.</i> (2014)
Lard	Meatball broth	The use of pork as beef substitute in meatball	MIR-ATR 1018–1284 cm^{-1} .	PLS and PCA	Kurniawati <i>et al.</i> (2014)
Pork	Liver and Chicken	Authentication of pork from liver and chicken	NIR	PLS	Fan <i>et al.</i> (2010)
Pork	Beef meatball	Authentication of beef meatball from pork	MIR-ATR at 1200–1000 cm^{-1}	PLS and DA	Rohman <i>et al.</i> (2011) ^a
Pork	Chinese ham sausages	Discrimination of halal and nonhalal Chinese ham sausages	KB disk-MIR at whole mid infrared region (4000–400 cm^{-1})	PLSDA and LS-SVM	Xu <i>et al.</i> (2012)
Pork	Beef jerky (dendeng)	Authentication of beef jerky from pork	ATR at 4000–700 cm^{-1}	LDA, SIMCA, SVM	Kuswandi <i>et al.</i> (2015)
Pork	Veal sausage	Adulteration veal sausage with pork and pork fat,	NIR at 6037–5576 cm^{-1}	PCA, SVM	Schmutzler <i>et al.</i> (2015)
Gelatin	Porcine and bovine Gelatin	Differentiation of porcine and bovine gelatin for halal authentication studies	MIR-ATR at combined wavenumbers of 3290–3280 cm^{-1} and 1660–1200 cm^{-1}	PCA	Hashim <i>et al.</i> (2010)

PLSDA = Partial least squares discriminant analysis; LS-SVM = least squares support vector machine; DA = Discriminant analysis; LDA = linear discriminant analysis; SIMCA = soft independent modelling class analogy; SVM = support vector machines;

Analysis of pig derivatives using vibrational spectroscopy and chemometrics

VS can produce a spectral fingerprint of samples, therefore, this technique is suitable for analysis of any materials for identification, characterization, structure elucidation, reaction monitoring, quality control, quality assurance, and halal authentication studies. Table 1 listed the use of vibrational spectroscopy along with chemometrics techniques for the characterization of lard as well quantification of lard and other pig derivatives in some products (food, cosmetics, pharmaceuticals).

Characterization of pig derivatives using vibrational spectroscopy

Guillen and Cabo (1997) have identified functional groups responsible for IR absorption, represented by

peaks and shoulders which appear in FTIR spectra. Figure 1 is typical FTIR spectra of lard scanned at mid infrared region of 4000–400 cm^{-1} with resolution of 4 cm^{-1} . Table 2 compiled wavenumbers of peaks and shoulders absorption along with its functional groups. The similar wavenumbers basically appear in all edible fats and oils, because fats and oils consisted of triacylglycerols with different fatty acid composition (Bosque-Sendra *et al.*, 2012). As a consequence, fats and oils have similar wavenumbers but with different peak intensities at certain peaks and shoulders. This is not surprising due to the property of FTIR spectra as finger print tools for differentiation of samples (Rohman *et al.*, 2014a). For this reason, Che Man *et al.* (2011) have characterized FTIR spectra of lard and other animal fats and vegetable oils in terms of peak intensities at 16 wavenumbers. For classification and similarity identification of lard

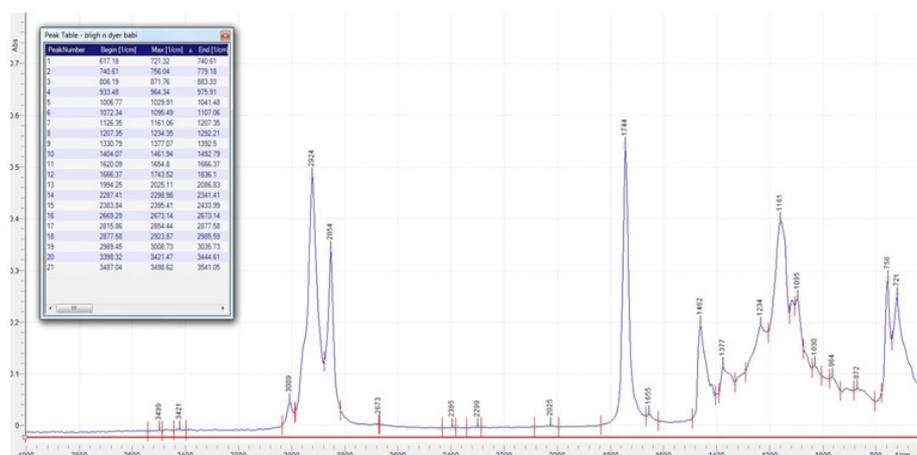


Figure 1. FTIR spectra of lard along with wavenumbers of peak and shoulders at 4000 – 650 cm^{-1} . The identification of functional groups for each peaks and shoulders is compiled in Table 2

and others, the chemometrics techniques of principle component analysis (PCA) and cluster analysis (CA) are used. Based on PCA score plot profile using absorbancies at 16 wavenumbers as variables, lard has the close similarity to extra virgin olive oil and pumpkin seed oil. Using CA with same variables used in PCA, lard, cod liver oil, corn, soybean, rice bran, sesame, sunflower, extra virgin olive, pumpkin seed, walnut, and canola oils can be joined in one cluster.

Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) for the characterization of lard has been demonstrated by Yang and Irudayaraj (2000). The optimum measurement of FTIR-PAS is as follows: a scanning speed of 5 kHz, number of scans of 256 scans/sample, and a resolution of 4 cm^{-1} . The FTIR spectra obtained for lard is compared with soybean oil. The result showed that FTIR spectra of lard and soybean oil are similar because they have similar functional groups as described by Guillen and Cabo (1997), but with different intensities of peaks and shoulders.

FTIR spectroscopy (FTIR) in whole mid infrared region, namely 4000-400 cm^{-1} has been used for the structural characterization of pork in the level of 10%, 30%, 50% and 70% (W/W) pork-in-camel and pork-in buffalo mixtures. All the samples were homogenized and dried using phosphorous pentoxide. The relative content of protein to lipid in the samples was determined using the absorbance ratios of carbonyl (C=O) group (amide I) at 1654 cm^{-1} and N-H bending band at 1540 cm^{-1} (amide II) to C-H stretching at 2924 cm^{-1} . A comparison between these ratios for any given samples was carried out. The deconvolution of the FTIR spectra in the region 2000 cm^{-1} -1000 cm^{-1} was used for more characterization of the samples using the area under peak. The data showed that the relative protein to lipid content

of pork-in-camel and pork-in-buffalo mixtures decreased with increasing pork content in these mixtures (Lamya, 2013).

Analysis of specific matters in pig derivatives

There are some reports regarding the analysis of certain compounds in non-halal matters using VS, indeed, these specific compounds can be explored as a tools for identification of non-halal matters. Infrared spectroscopy in near infrared (NIR) region and mid infrared (MIR) region has been used to analyse saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), C16:0, C18:0, C18:1 and C18:2 contents in fat extracts on pork back and breast fat. The results obtained with 80 back and 80 breast samples showed the validity of MIR and NIR spectroscopies to estimate these fatty acids. The coefficients of determination (R^2) for such correlation were around 0.91 and 0.98 for MIR and around 0.85- 0.96 for NIR (Ripoche and Guillard, 2001).

Specific fatty acids known as marine fatty acids namely C22:5n3 + C22:6n3, in pork have been analysed using FTIR spectroscopy combined with PLS regression. The result obtained was compared with official method of gas chromatography. The pre-processing spectra using first derivative and normalized spectra at wavenumbers region of 3080–3000 cm^{-1} offer the best predictive capability. The R^2 and root mean square error of cross validation obtained are 0.982 and 0.062%, respectively (Flatten *et al.*, 2005).

Olsen *et al.* (2007) have used Raman spectroscopy coupled with multivariate calibration of partial least square (PLS) for analysis of fatty acid composition in 77 samples from pork adipose tissue and melted fat from the same tissue. As a reference method, they

Table 2. Some functional groups responsible for IR absorption of each band shoulders of lard (Guillen and Cabo, 1997)

Wavenumbers	Functional group
3468 cm ⁻¹	ester carbonyl, overtone of the glyceride ester carbonyl
3025 cm ⁻¹	stretching vibration of <i>trans</i> olefinic double bonds
3006 cm ⁻¹	stretching vibration of <i>cis</i> olefinic double bonds
2953 cm ⁻¹	methyl asymmetrical stretching vibration
2924 cm ⁻¹	methylene (-CH ₂) asymmetrical stretching vibration
2853 cm ⁻¹	methylene (-CH ₂) symmetrical stretching vibration
2730 and 2677 cm ⁻¹	Fermi resonance of the carbonyl group
1746 cm ⁻¹	Carbonyl group (C=O) of triglycerides
1711 cm ⁻¹	Free fatty acids
1654 and 1648 cm ⁻¹	C=C stretching vibration of disubstituted <i>cis</i> C=C of acyl groups of oleic and linoleic acids
1465 cm ⁻¹	Bending vibration of methylene group (scissoring band)
1418 cm ⁻¹	rocking vibrations of CH bonds of <i>cis</i> -disubstituted olefins
1377 cm ⁻¹	symmetrical bending vibration of methyl groups
1350 and 1150 cm ⁻¹	twisting and wagging vibrations of the CH ₂ groups
1238, 1163, 1118, and 1097 cm ⁻¹	stretching vibrations of the C-O group in esters
1033 cm ⁻¹	stretching vibrations of the C-O group in esters
968 cm ⁻¹	bending vibration out-of-plane of <i>trans</i> disubstituted olefinic groups
914 cm ⁻¹	ending vibration of <i>cis</i> disubstituted olefinic groups
723 cm ⁻¹	overlapping of the methylene rocking vibration and the out-of-plane bending vibration of <i>cis</i> -disubstituted olefins

used gas chromatography (GC). The polyunsaturated fatty acids (PUFA) ranged from 7.8% to 31.7% and monounsaturated fatty acids (MUFA) ranged from 35.2% to 51.5% of total fatty acids were obtained. Using pre-processed spectra of melted fat combined with PLS regression with cross-validation, the correlation coefficient (R) for the relationship between actual value of PUFA as determined by GC and FTIR predicted value is 0.98, while root mean square error of cross-validation (RMSECV) of 1.0% of total fatty acids, using 5 PLS factors. MUFAs were predicted with R of 0.96 and RMSECV of 1.0% of total fatty acids, using 9 PLS factors.

Analysis of pig derivatives for halal authentication

Pig derivatives are any components derived from pig such as pork, lard and porcine gelatin. Due to its properties as a good component to be used in certain products, pig derivatives may be met in food, cosmetics and pharmaceuticals. Besides, pig derivatives generally have low price, therefore pig derivatives can be potential adulterants in products.

Analysis of lard in food and cosmetics products

FTIR spectroscopy combined with multivariate

calibration of partial least square (PLS) has been used for quantification of lard in the mixture with lamb, chicken and beef fats (Che Man and Mirghani, 2001). The percentage of lard in the mixture with lamb fat was performed at wavenumbers of 3009–3000 cm⁻¹, using the equation for the relationship between actual value of lard (x-axis) and FTIR predicted value (y-axis) of $y = 0.1616x + 3002$. The R² and standard error of prediction (SEP) values obtained were 0.9457 and 1.23%, respectively. Lard in chicken fat was analyzed using band absorbance at 3006 cm⁻¹ using the equation of $y = 0.0071x + 0.1301$ (R² = 0.983, SEP = 0.012 %). Meanwhile, lard mixed with beef fat was determined using absorbance band at 966.22 cm⁻¹ with the equation, R², and SEP obtained were $y = -0.0050x + 0.3188$; 0.9831, and 0.01%, respectively. The similar study was also conducted by Jaswir *et al.* (2003).

Che Man and Mirghani (2001) as well as Jaswir *et al.* (2003) have used different wavenumbers rather than using one wavenumbers region for analysis of lard mixed with other animal fats. Besides, both authors did not perform discriminant analysis (DA) for the classification of lard and lard in the mixture with animal fats. For this reason, Rohman and Che Man (2010) have developed FTIR spectroscopy combined with PLS and DA for the quantification and classification of lard in the mixture with lamb fat, chicken fat and beef fat. Two FTIR spectral regions, namely using whole mid infrared regions (3,300–700 cm⁻¹) and at selected fingerprint regions (1,500 – 900 cm⁻¹) were optimized for the developing of PLS calibration model. Finally, the frequency region of 1,500- 900 cm⁻¹ was chosen for the quantification of lard in the mixtures with other animal fats with R² > 0.99 and root mean square error of calibration (RMSEC) value of 0.98% (lamb fat), 0.61% (chicken fat), and 0.73% (beef fat). This study also demonstrated that DA can classify lard and lard in the mixture with other animal fats in the region of 3,300–800 cm⁻¹ according to their fat classes.

FTIR spectroscopy combined with multivariate calibration of PLS regression has been used to quantify the presence of lard in selected food products, namely cake (Syahariza *et al.*, 2005), chocolate (Che Man *et al.*, 2005), and biscuits (Shahariza, 2006). The spectral bands associated with lard when used to prepare cake, cocoa butter, and biscuits, and their blends with other oils were scanned at mid infrared region of 4000 – 400 cm⁻¹ and identified. A semi-quantitative approach is proposed to measure the percentage of lard in the blends with food systems on the basis of spectral data at frequency regions of 4000–650 cm⁻¹ (chocolate), 1117 – 1097 cm⁻¹

(cake), and at frequency regions of 3500 – 2900 cm^{-1} (biscuits). The equations obtained for the relationship between actual values of lard (x-axis) and FTIR predicted values (y-axis) are: $y = 0.9225x + 0.5539$; with coefficient of determination (R^2) of 0.9872 and standard error of measurement (SEM) of 1.31% (chocolate); $y = 0.9937x + 0.1980$ with R^2 of 0.9937 and SEM of 2.26% (cake); and $y = 0.9962x + 0.1396$, with R^2 and SEM of 0.9974 and 2.82%, respectively for biscuits.

Lard can be a potential adulterant in the high priced oils such as cod liver oil (CLO) and virgin coconut oil (VCO). FTIR spectroscopy combined with PLS and discriminant analysis (DA) has been developed for quantification of lard in CLO (Rohman and Che Man, 2009) and in VCO (Mansor *et al.*, 2011). The wavenumbers of 1,035 – 1030 cm^{-1} were used for quantification of lard in CLO. The relationship between actual value of lard (x) and FTIR predicted value of lard (y) can be expressed with an equation of $y = 0.872x - 0.392$ with R^2 and RMSEC values of 0.967 and 1.61 % (v/v), respectively. Furthermore using 7 factors, DA in the frequency region of 1,500 – 1,030 cm^{-1} able to classify lard and lard in the mixture with CLO with no misclassification reported. Lard and VCO share some similarities such as having a transparent to yellowish color and are solid at room temperature (20°C); consequently, lard can be a potential adulterant in VCO. The presence of lard in VCO can be easily analyzed using FTIR spectroscopy with the aid of PLS calibration and DA. Using the frequency region of 1,120 – 950 cm^{-1} , PLS was capable of predicting the contents of lard in VCO with the equation of $y = 0.999x + 0.006$; for the relationship between the actual value of lard (x) and FTIR predicted value (y) with R^2 of 0.999. DA was successfully exploited for the classification of VCO and VCO adulterated with lard. The accuracy of such classification is 100% meaning that there is no misclassification reported between lard and lard in VCO, containing at least 1% of lard.

Rohman *et al.* (2011b) used FTIR spectroscopy for quantification and classification of lard in selected vegetable oils, namely canola oil (Ca-O), corn oil (CO), extra virgin olive oil (EVOO), soybean oil (SO), and sunflower oil (SFO). Due to its ability to give the highest values of R^2 and the lowest values of RMSEC compared with other spectral regions or their combinations, FTIR spectra at selected fingerprint regions (1,500 – 1,000 cm^{-1}) were used for PLS calibration models of lard in these vegetable oils. The normal and first derivative spectra gave better results in terms of high R^2 (> 0.99) and low values of RMSEC and root mean square error of prediction

(RMSEP) than the derivative spectra. Meanwhile, the presence of lard in EVOO is best quantified using the first derivative spectra.

The presence of lard mixed with palm oil as frying oil in French fries pre-fried was analyzed with FTIR spectroscopy at wavenumbers of 3100–1050 cm^{-1} with the aid of PLS. The coefficient of determination (R^2) obtained for the relationship between actual value of lard (x-axis) and FTIR predicted value (y-axis) is 0.9791 with 0.5% of detection limit. The equation obtained is $y = 0.9351x + 0.1843$. The error in calibration expressed with RMSEC was 0.979%. In addition, the error obtained during cross validation was 2.45%. A discriminant analysis test was success for differentiation between fries samples adulterated with lard and samples pre-fried with palm oils (Che Man *et al.*, 2014).

The meat used in meatball can be analyzed via corresponding fat. Kurniawati *et al.* (2014) used FTIR spectroscopy and chemometrics of PLS and principal component analysis (PCA) for quantitative analysis and classification of lard extracted from pork meatball broth. Lard in meatball broth was quantitatively analyzed at wavenumber region of 1018–1284 cm^{-1} . The values of R^2 and RMSEC obtained were 0.9975 and 1.34% (v/v), respectively. Furthermore, the classification of lard and beef fat in meatball broth at wavenumber region of 1200–1000 cm^{-1} , and meat beall with lard and beef could be classified correctly. Previously, Rohman *et al.* (2011) have analyzed pork in beef meatball. The selected fingerprint regions of 1200 - 1000 cm^{-1} was used for pork analysis. The equation obtained for the relationship between actual value (x-axis) and FTIR predicted values (y-axis) of pork was $y = 0.999x + 0.004$, with R^2 and RMSEC of 0.999 and 0.442%, respectively. The RMSEP value obtained was 0.742%. The work showed that FTIR spectroscopy can be used for the detection and quantification of pork in beef meatball formulation for halal verification purposes.

In pharmaceutical products and cosmetics formulation, some investigators also used FTIR spectroscopy for analysis of pig derivatives. Lard is a good component to be used in cream cosmetics as oily bases, either alone or along with other oils. Food and drug administration (FDA, 2006) has compiled lard as one of the generally recognized as safe substances (GRAS) used in cosmetics products. Rohman and Che Man (2011) have analyzed lard along with virgin coconut oil (VCO) in cream cosmetics using FTIR spectroscopy combined with PLS and discriminant analysis (DA). Analysis was done at the combined wavenumbers region of 3,020 – 2,995 cm^{-1} and 1,200 – 1,000 cm^{-1} . The PLS calibration model obtained

for the relationship between actual value of lard (x-axis) and FTIR predicted (y-axis) values of lard in cream cosmetics was $y = 0.997x + 0.065$, with R^2 and RMSEC of 0.997 and 0.81% (v/v), respectively. In addition, DA can successfully classify creams containing lard in its formulation using 9 principal components.

FTIR spectroscopy coupled with PLS has been optimized for rapid determination of lard mixed with palm oil in a cosmetic lotion formulation. Lard, palm oil, and its binary mixture were extracted from matrix samples using liquid-liquid extraction using hexane as extracting solvent. The concentration of lard in lotion formulation was quantified at wavenumbers of 1,200–1,000 cm^{-1} . PLS calibration model reveals good correlation between the actual value of lard (x-axis) and the FTIR predicted value (y-axis) with a R^2 of > 0.99 . Furthermore, the classification between lotions with and without lard in their formulation was performed using PCA using the same frequency region used for quantification (Lukitaningsih *et al.*, 2012).

FTIR spectroscopy coupled with PLS and PCA has been used for quantitative analysis and classification of lard mixed with extra virgin olive oil as oil base in cream formulations. The extraction of lipid component in cream was performed using liquid-liquid extraction using hexane as extracting solvent, and the lipid obtained was subjected to FTIR spectra measurement, using horizontal attenuated total reflectance as sampling technique. The result showed that FTIR spectroscopy in combination with PLS can be used to quantify the levels of lard in creams using the combined wavenumbers region of 1785–702 cm^{-1} and 3020–2808 cm^{-1} . Furthermore, PCA using absorbance intensities at 1200–1000 cm^{-1} as variables has been successfully used for the classification of cream with and without lard in the formulation.

Analysis of pork and other nonallowed meat

The presence of pork and pork fat in veal sausage has been analyzed using Fourier transform near infrared (FT-NIR) spectroscopy with chemometrics of PCA and support vector machine (SVM) for laboratory, industrial and on-site analysis (Schmutzler *et al.*, 2015). The level of pork and pork fat used is in the range of 0–50% of pork in sausage. During the preprocessing of NIR spectra, it is found that Savitzkyev Golay second derivative offers the best data pre-treatments for the laboratory setup, industrial, and on site analysis. During the adulteration analysis, three different wavenumber regions for the three different setups were used, namely 6028–480

cm^{-1} for the laboratory analysis, 5784–5736 cm^{-1} during industrial analysis (using quartz cuvettes) or 6028 - 5480 cm^{-1} (for measurements through the polymer packaging) and the wavenumbers of 6037–5576 cm^{-1} for all measurements using the handheld NIR spectrophotometer. The result showed that adulteration practice to the down level of 10% pork could be detected.

FT-NIR spectroscopy has been developed for analysis of pork adulteration with liver and chicken in 10% increments. Quantitative analysis was done with the aid of partial least squares (PLS) regression with standard normal variate (SNV) pretreatment. For analysis of chicken as adulterant, the values of correlation coefficient (r) for the correlation between actual value of liver in pork and FTIR predicted value, RMSEC, and RMSEP obtained were 0.97706, 0.067% and 0.073%, respectively. PLS with the raw spectra was used for analysis of pork meat adulterated with chicken with r, RMSEP and RMSEC values of 0.98614, 0.053%, and 0.122%, respectively (Fan *et al.*, 2010).

FTIR spectroscopy in mid infrared region combined with chemometrics of partial least squares discriminant analysis (PLS-DA) and least squares support vector machine (LS-SVM) has been exploited for fast discrimination of pork in halal and nonhalal Chinese ham sausages (Xu *et al.*, 2012). FTIR spectra in transmittance mode was read at 4000 - 400 cm^{-1} . The analyzed samples are 73 Halal and 78 nonhalal Chinese ham sausages. The samples were mixed with KBr IR grade to make KBr disk. Some preprocessing spectra are taken, including smoothing, derivatives and standard normal variate (SNV). The results indicate that removal of spectral background and baseline plays an important role in discrimination. The pretreatment of SNV can improve classification accuracy and reduce the complexity of PLS-DA.

Kuswandi *et al.* (2015) developed FTIR spectroscopy coupled with chemometrics of linear discriminant analysis (LDA), soft independent modelling class analogy (SIMCA), and support vector machines (SVM) for prediction of pork adulteration in the beef jerky (dendeng in Indonesia). Some optimization was conducted including the selection of chemometrics type and wavenumbers region. Finally, spectra in the whole region (4000–700 cm^{-1}) and LDA model offer the best prediction model, since it could classify and predict the sample tested with accuracy of 100%. The LDA model was applied toward the real samples of the beef jerky marketed in Jember, East Java, Indonesia. The results showed that FTIR spectroscopy and chemometrics were in good agreement with those obtained using ELISA method.

Currently, due to the expensive price of beef in Indonesian market, some unethical seller may added rat's meat into beef meatballs in order to gain economical profit. FTIR spectroscopy in combination with chemometrics of PLS and PCA is used for quantitative analysis and classification of rat's meat (Rahmania *et al.*, 2015). Some frequency in mid infrared region were optimized, and finally, the frequency region of 750–1000 cm^{-1} was selected during PLS and PCA modelling. For quantitative analysis, the relationship between actual values (x-axis) and FTIR predicted values (y-axis) of rat meat is described by the equation of $y = 0.9417x + 2.8410$ with coefficient of determination (R^2) of 0.993, and root mean square error of calibration (RMSEC) of 1.79%. Furthermore, PCA was successfully used for the classification of rat meat meatball and beef meatball.

Another meat used as substitutes in meatball is wild bear meat (WBM). Guntarti *et al.* (2015) used FTIR spectroscopy combined with PLS and PCA for analysis of WBM in beef meatball. The wavenumbers region used for quantitative analysis with PLS and classification with PCA is 1250-1000 cm^{-1} . The relationship between actual value of wild boar (x-axis) and FTIR predicted value (y-axis) at optimized wavenumber region is $y = 0.9749 x + 1.4658$, with coefficient of determination (R^2) of 0.988 and root mean square error of calibration (RMSEC) of 2.0%. Furthermore, PCA was successfully used for the classification of WBM and beef meatball.

Analysis porcine gelatin

FTIR spectroscopy is an ideal technique for classification of gelatin sources. Hashim *et al.* (2010) have developed FTIR spectroscopy using attenuated total reflectance (ATR) as sample technique for identification of porcine gelatin and bovine gelatin. The chemometrics of PCA was used using absorbancies at combined wavenumbers region of 3290–3280 cm^{-1} and 1660–1200 cm^{-1} . The Cooman's plot showed a clear distinction between gelatin samples of bovine and porcine origins.

Conclusion

Vibrational spectroscopy (infrared and Raman) is versatile technique to be used for characterization and quantification of pig derivatives. By selecting the optimum frequency region capable of offering the best predictive and descriptive modelling, analysis can be done fast with no sample preparation. Therefore, vibrational spectroscopy is ideal technique for quality control and halal authentication of consumer

products. However, vibrational spectroscopy has main drawback, namely, if the composition of the sample to be analyzed is different, the spectra of pig derivatives obtained are also be different, and as a consequence, the calibration model must be redeveloped.

References

- Bosque-Sendra, J. M., Cuadros-Rodríguez, L., Ruiz-Samblás, C. and de la Mata, A. P. 2012. Combining chromatography and chemometrics for the characterization and authentication of fats and oils from triacylglycerol compositional data—A review. *Analytica Chimica Acta* 724: 1– 11.
- Che Man, Y. B., Rohman, A. and Mansor, T. S. T. 2011. Differentiation of lard from other edible oils by means of Fourier transform infrared spectroscopy and chemometrics. *Journal of the American Oil Chemists' Society* 88: 187 – 192.
- Che Man, Y. B. and Sazili, A. Q. 2010. Food production from the halal perspective. In: Isabel, G. L., and Hui, Y. H. (Eds). *Handbook of Poultry Science and Technology*, p. 183 -215. New York: Wiley.
- Che Man, Y. B., Marina, A. M., Rohman, A., Al-Kahtani, H. A. and Norazura, O.A. 2014. FTIR Spectroscopy Method for Analysis of Palm Oil Adulterated with Lard in Pre-fried French fries. *International Journal of Food Properties* 17: 354–362
- Che Man, Y. B., Syahariza, Z. A., Mirghani, M. E. S., Jinap, S. and Bakar, J. 2005. Analysis of potential lard adulteration in chocolate and chocolate products using fourier transform infrared spectroscopy. *Food Chemistry* 90: 815 –819.
- Diem, M. 1993. *Introduction to Modern Vibrational Spectroscopy*. New York: Wiley.
- El-Gindy, A. and Hadad, G.M. 2012. Chemometrics in pharmaceutical analysis: an introduction, review, and future perspectives. *Journal AOAC International* 95(3): 609-623.
- Fan, Y., Cheng, F. and Xie, L. 2010. Quantitative analysis and detection of adulteration in pork using near-infrared spectroscopy. In Moon, S. K., Shu, I. T., and Kaunglin, C. (Eds). *Proceeding SPIE 7676. Sensing for Agriculture and Food Quality and Safety II*, p. 76. Florida: Orlando.
- Flåtten, A., Bryhni, E. A., Kohler, A., Egelandsdal, B. and Isaksson, T. 2005. Determination of C22:5 and C22:6 marine fatty acids in pork fat with Fourier transform mid-infrared spectroscopy. *Meat Science* 69: 433–440.
- Food and Drug Administration. (July 2006). *Alphabetical List of SCOGS Substances*. Retrieved on April 15, 2015 from website: www.cfsan.fda.gov/*dms/opascogc,
- Griffiths, P. and Chalmers, J.M. 1999. *Handbook of Vibrational Spectroscopy*. New York: John Wiley and Sons.
- Guntarti, A., Martono, S., Yuswanto, A. and Rohman, A. 2015. FTIR spectroscopy in combination with

- chemometrics for analysis of wild boar meat in meatball formulation. *Asian Journal of Biochemistry* 10(4): 165-172.
- Harz, M., Rösch, P. and Popp, J. 2009. Vibrational spectroscopy—A powerful tool for the rapid identification of microbial cells at the single-cell level. *Cytometry Part A* 75A: 104–113.
- Hashin, D. M., Che Man, Y. B., Norakasha, R., Shuhaimi, M., Salmah, Y. and Syahariza, Z.A. 2010. Potential use of Fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatins. *Food Chemistry* 118: 856–860.
- Jaswir, I., Mirghani, M. E. S., Hassan, T. H. and Mohd Said, M. Z. 2003. Determination of lard in mixtures of body fats of mutton and cow by Fourier transform-Infrared (FTIR) spectroscopy. *Journal of Oleo Science* 52: 633 – 638.
- Khanmohammadi, M. and Garmarudi, A. B. 2011. Infrared spectroscopy provides a green analytical chemistry tool for direct diagnosis of cancer. *Trends in Analytical Chemistry* 30(6): 864 – 874.
- Kuswandi, B., Putri, F. K., Gani, A. A. and Ahmad, M. 2015. Application of class-modelling techniques to infrared spectra for analysis of pork adulteration in beef Jerkys. *Journal of Food and Technology* 52(12): 7655-7668.
- Lamyaa, M. A. 2013. Discrimination of pork content in mixtures with raw minced camel and buffalo meat using FTIR spectroscopic technique. *International Food Research Journal* 20(3): 1389-1394.
- Lewis, I.R. and Edwards, H. G. M. 2001. *Handbook of Raman Spectroscopy*. New York: CRC Press.
- Lukitaningsih, E., Saadah, M., Purwanto, P. and Rohman, A. 2012. Quantitative analysis of lard in lotion cosmetics formulation using FTIR spectroscopy and partial least square calibration. *Journal of the American Oil Chemists' Society*. 89: 1537–1543.
- Mansor, T. S. T., Che Man, Y. B. and Rohman, A. 2011. Application of Fast Chromatography and Fourier transform infrared spectroscopy for analysis of lard adulteration in virgin coconut oil. *Food Analytical Methods* 4(3): 365-372.
- Moros, J., Garrigues, S. and de la Guardia, M. 2010. Vibrational spectroscopy provides a green tool for multi-component analysis. *Trends in Analytical Chemistry* 29: 578–591.
- Mursyidi, A. 2013. The Role of Chemical Analysis in the Halal Authentication of Food and Pharmaceutical Products. *Journal of Food and Pharmaceutical Sciences* 1: 1-4.
- Olsen, E. F., Rukke, E-O., Fla^otten, A. and Isaksson, T. 2005. Quantitative determination of saturated, monounsaturated and polyunsaturated fatty acids in pork adipose tissue with non-destructive Raman spectroscopy. *Meat Science* 76: 628–634.
- Rahmania, R., Sudjadi, S. and Rohman, A. 2015. The employment of FTIR spectroscopy in combination with chemometrics for analysis of rat meat in meatball formulation. *Meat Science* 100: 301–305.
- Riaz, M.N. and Chaudry, M.M. 2003. *Halal Food Production*. New York: CRC Press.
- Ripoche, A. and Guillard, A.S. 2001. Determination of fatty acid composition of pork fat by Fourier transform infrared spectroscopy. *Meat Science* 58: 299-304.
- Rohman, A. and Che Man, Y. B. 2008. Review article: Analysis of lard in food products for halal authentication study. *Agritech* 28(4): 192 – 201.
- Rohman, A. and Che Man, Y. B. 2009. Analysis of cod-liver oil adulteration using Fourier transform infrared (FTIR) spectroscopy. *Journal of the American Oil Chemists' Society* 86: 1149 - 1153.
- Rohman, A. and Che Man, Y. B. 2010. FTIR spectroscopy combined with chemometrics for analysis of lard in the mixtures with body fats of lamb, cow, and chicken. *International Food Research Journal* 17: 519 – 526.
- Rohman, A. and Che Man, Y. B. 2011. Analysis of Lard in Cream Cosmetics Formulations using FT-IR Spectroscopy and Chemometrics. *Middle-East Journal of Scientific Research* 7(5): 726-732.
- Rohman, A., Sisindari, S., Erwanto, Y. and Che Man, Y. B. 2011a. Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy. *Meat Science* 88: 91 – 95.
- Rohman, A., Che Man, Y. B., Ismail, A. and Puziah, H. 2011b. FTIR spectroscopy combined with chemometrics for analysis of lard adulteration in some vegetable oils. *Cyta-Journal of Food* 9(2): 96 - 101.
- Rohman, A., Nugroho, A., Lukitaningsih, E. and Sudjadi. 2014a. Application of vibrational spectroscopy in combination with chemometrics techniques for authentication of herbal medicine. *Applied Spectroscopy Reviews*. 49: 603-613.
- Rohman, A., Gupitasari, I., Purwanto, Triyana, K., Rosman, A. S., Ahmad, S. A. S. and Yusuf, F. M. 2014b. Quantification of lard in the mixture with olive oil in cream cosmetics based on FTIR spectra and chemometrics. *Jurnal Teknologi* 69(1): 113–119.
- Schmutzler, M., Beganovic, A., Bohler, G. and Huck, C.W. 2015. Methods for detection of pork adulteration in veal product based on FT-NIR spectroscopy for laboratory, industrial and on-site analysis. *Food Control* 57: 258 – 267.
- Singh, I., Juneja, P., Kaur, B. and Kumar, P. 2013. Review Article: Pharmaceutical applications of chemometric techniques. *ISRN Analytical Chemistry* Volume 2013, Article ID 795178, 13 pages, doi.org/10.1155/2013/795178.
- Syhariza, Z. A., Che Man, Y. B., Selamat, J. and Bakar, J. 2005. Detection of lard adulteration in cake formulation by Fourier transform infrared (FTIR) spectroscopy. *Food Chemistry* 92: 365 – 370.
- Syhariza, Z.A. 2006. *Detection of lard in selected food model systems using Fourier Transform Infrared (FTIR) Spectroscopy*. Selangor, Malaysia: Universiti Putra Malaysia, M.Sc Thesis.
- Xu, L., Cai, C. B., Cui, H. F., Ye, Z. H. and Yu, X. P. 2012. Rapid discrimination of pork in Halal and non-Halal Chinese ham sausages by Fourier transform infrared (FTIR) spectroscopy and chemometrics. *Meat Science* 92: 506 – 510.

- Yang, H. and Irudayaraj, J. 2000. Characterization of Semisolid Fats and Edible Oils by Fourier Transform Infrared Photoacoustic Spectroscopy. *Journal of the American Oil Chemists' Society* 77(3): 291 – 295.
- Yap, K. Y.-L., Chan, S. Y. and Lim, C. S. 2007. Authentication of traditional Chinese medicine using infrared spectroscopy: Distinguishing between ginseng and its morphological fakes. *Journal of Biomedical Sciences* 14: 265–273.