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FULL PAPER

Quantitative analysis and discrimination of lard in chicken fat using FTIR spectroscopy and chemometrics for halal authentication

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Abstract

Lard (LD) has a similarity with chicken fat (CF) in terms of physicochemical characteristics especially fatty acid composition and FTIR spectra, therefore, analysis of lard in a binary mixture with CF is challenging. This study aimed to employ FTIR spectra in normal and derivative modes in combination with multivariate calibrations for quantitative analysis of LD and CF in the binary mixture and to discriminate pure LD and LD in the mixture with CF using discriminant analysis (DA). LD and CF were prepared randomly at a certain concentration to be used as calibration and validation sets, respectively. All mixtures were scanned using an FTIR spectrophotometer at 4000-650 cm^{-1} in absorbance mode. Some wavenumbers, FTIR spectral modes and multivariate calibrations were optimized to provide high accuracy and precision as indicated by the high value of coefficient determination (R^2) and low values of errors in calibration and validation. The results revealed that FTIR spectra using normal spectra at combined wavenumbers of 3100-2750 and 1500-660 cm^{-1} with R^2 for the relationship between actual and FTIR predicted values of > 0.99 in calibration and validation with an error in calibration of 0.008% and error validation of 0.032%. DA could discriminate LD and LD in the mixture with CF with an accuracy level of 100%. FTIR spectroscopy in combination with chemometrics offered a reliable method for quantitative analysis and discrimination of LD in a binary mixture with CF.

1. Introduction

Lard, obtained from the rendering of adipose tissues of pigs, is one of the edible fats and is considered generally recognized as safe (GRAS) by Food and Drug Administration. However, lard is not allowed to be consumed by Muslims and Jews, because lard is a non-halal and non-kosher fat (Regenstein *et al*., 2003). Islam prohibits its followers to consume any products containing pig derivatives such as lard, pork and porcine gelatins (Mursyidi, 2013; Heidari *et al*., 2020). Lard (LD) is a good component to be used in cosmetics products (Cherian *et al*., 2020) and is typically used in food products such as in the baking industry (Man *et al*., 2011). Among animal fats, LD had close similarity in terms of fatty acid compositions and FTIR spectra with chicken fat (CF) (Rohman and Che Man, 2011), therefore the simultaneous analysis of LD and CF in the binary mixture was challenging.

Analysis of LD in the mixture with other edible fats and oils as well as in food and cosmetics products has been reported to be analyzed using several instrumental techniques. Among these methods is differential scanning calorimetry by differentiating thermal profile and triacylglycerol compositions of lard and others (Nurrulhidayah *et al*., 2015; Azir *et al*., 2017), electronic nose using surface acoustic wave detector (Mansor *et al*., 2011), proton nuclear magnetic resonance (NMR) spectroscopy in combination with chemometrics (Fadzillah *et al*., 2015), gas chromatography by analyzing specific fatty acids and fatty acid compositions

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(Indrasti *et al*., 2010), liquid chromatography in combination with mass spectrometer detector (Kallio *et al*., 2001), and polymerase chain reaction using a specific primer to pig DNA (Aida *et al*., 2005). Some of these methods involved extensive sample preparation and sophisticated instruments, therefore, rapid and reliable methods based on molecular spectroscopic techniques are used, recently, for the analysis of LD.

Molecular spectroscopy was based on the interaction of light at a certain wavelength or wavenumbers with samples (LD and CF). Fourier transform infrared (FTIR) spectroscopy in combination with chemometrics (Rohman *et al*., 2014), Raman spectroscopy (Taylan *et al*., 2020), and nuclear magnetic resonance (NMR) (Fang *et al*., 2013) have been reported in the use of analysis of lard. Due to its nature as a fingerprint analytical technique, FTIR spectra are widely used for the analysis of lard in binary mixtures with other edible fats and oils such as olive oil in cream and lotion cosmetics (Rohman *et al*., 2014), palm oil in cream (Lukitaningsih *et al*., 2012), and in other vegetable oils (Che Man and Rohman, 2011). In this study, FTIR spectroscopy combined with multivariate calibrations was employed for simultaneous analysis of LD in CF in binary mixtures.

2. Materials and methods

2.1 Materials

Adipose tissues of chicken and pork were bought from a local market in Yogyakarta, Indonesia. The animal fats (lard and chicken fat) were prepared according to Rohman and Che Man, (2009) by rendering processes of adipose tissues. The rendering was undertaken at a temperature of 90–100^{o}C for 3 hrs in a conventional oven. The melted fat was strained through a triple-folded muslin cloth, dried by the addition of anhydrous Na_2SO_4, and then centrifuged at 3,000 rpm for 20 mins. The fat layer was decanted, shaken well, and centrifuged again before being filtered through Whatman filter paper. The filtered samples were used for preparation for calibration and validation samples. The solvents of hexane and acetone were of pro-analytical grade.

2.2 Preparation of calibration and validation samples

For modelling using multivariate calibration models of PLS and PCR, a set calibration (25 samples) and validation (25 samples) were prepared independently by mixing LD and CF in reaction tubes using a calibrated pipette. The concentration ranges of LD and CF used in those samples were compiled in Table 1. The mixture was homogenized in a water bath to obtain the homogeneous mixture. All mixtures were subjected to an FTIR spectrophotometer.

Table 1. The composition (%) of lard and chicken fat as binary mixtures used as calibration and validation sample sets.

Sample No.	Lard (%)	Chicken fat (%)
1	4	96
2	12	88
3	25	75
4	31	69
5	32	68
6	35	65
7	42	58
8	45	55
9	51	49
10	54	46
11	56	44
12	57	43
13	59	41
14	62	38
15	64	36
16	67	33
17	70	30
18	72	28
19	74	26
20	83	17
21	85	15
22	86	14
23	90	10
24	95	5
25	96	4

2.3 Discriminant analysis

Discriminant analysis (DA) is one of supervised pattern recognition, in this study, used for discrimination between pure LD and LD in the mixture with CF. Lard (LD) and CF were mixed to obtain a series of training sets of pure LD and LD in a binary mixture with CF. The samples with pure LD were assigned as “pure”, while a series of LD in a binary mixture with CF was assigned as “mixture”. All samples were subjected to FTIR spectral measurement and were discriminated against using DA.

2.4 FTIR spectra measurement

Thermo Scientific™ Nicolet™ iS10 FTIR spectrometer (Thermo Fisher Scientific, USA) equipped with a detector of deuterated triglycine sulphate (DTGS) and connected with a software of OMNIC operating system (Version 7.0 Thermo Nicolet) was applied for scanning FTIR spectra of all evaluated samples. Drops of oil samples were placed in contact with horizontal attenuated total reflectance (HATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25^{o}C). FTIR spectra were collected at wavenumbers of 4,000–650 cm^{-1} at a resolution of 8 cm^{-1} using 32

scannings. All spectra were corrected background of an air spectrum. After every scan, a new reference air background spectrum was taken. The plate was carefully cleaned by wiping with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as absorbance values at each data point in triplicate.

2.5 Chemometrics analysis

FTIR spectra of all samples were subjected to data analysis using chemometrics software of Thermo Scientific™ TQ Analyst™ included in the FTIR spectrophotometer. For quantitative analysis of LD in a binary mixture with CF, two multivariate calibrations (PLS and PCR) were used. The statistical parameters evaluated for multivariate calibration included R^2 values in calibration and validation models, root means a square error in calibration (RMSEC) and root mean square error of validation (RMSEP) (Rohman *et al.*, 2011). Discrimination between LD and LD in CF was performed using DA employing the same chemometrics software. The variable used for multivariate calibration (PLS and PCR) and DA were absorbances of FTIR spectra at wavenumbers 4000-650 cm^{-1} with a resolution of 8 cm^{-1}.

3. Results and discussion

Analysis of edible fats and oils is typically carried out using chromatographic-based techniques such as liquid chromatography by analyzing the specific components of triacylglycerol (TAG) compositions and gas chromatography by determining fatty acid (FA) compositions (Rohman and Man, 2011). Chromatography needs extensive sample preparation such as derivatization, therefore, rapid methods such as FTIR spectroscopy can be developed as an alternative method for analysis of edible fats and oils intended for specific purposes like monitoring the quality control of fats and oils and for authenticating high-price fats and oils from lower-priced ones. FTIR spectroscopy is a fingerprint analytical technique that can be used for the analysis of edible fats and oils as a whole matter rather than as specific components as in chromatographic methods (Singh *et al.*, 2010).

Figure 1 exhibits FTIR spectra of pure LD and CF scanned at the mid-infrared region, corresponding to wavenumbers of 4000–650 cm^{-1}. Each peak and shoulder in FTIR spectra related to functional groups present in the evaluated samples (LD and CF). Table 2 compiles the functional groups responsible for IR absorption as a result of the stretching and bending vibrations of functional groups. Edible fats and oils are mainly composed of TAG, therefore, both spectra look very similar indicating similar functional groups present in LD and CF. However, based on the fact that FTIR spectra are fingerprint nature, both spectra can be differentiated by detailed investigation in peak intensities (absorbance), especially in fingerprint regions (1500-650 cm^{-1}). The regions where peak intensities are a bit different can be further optimized for quantitative analysis of LD in a binary mixture with CF.

Quantitative analysis of LD in a binary mixture with CF was facilitated by two multivariate calibrations of PLS and PCR. The first step is to optimize FTIR spectra modes (normal versus derivative), the wavenumbers region revealing the difference in peak intensities between LD and chicken, as well as multivariate calibrations (PLS and PCR). The aim and advantages of the derivatization method were to obtain better spectra resolution so that could resolve the crowded spectra to be easier to analyze (Windarsih *et al.*, 2020). Table 3 compiles the analytical performance of statistical parameters used to evaluate the developed model. R^2-values for the correlation between actual values of LD and FTIR predicted values were used for the accurate

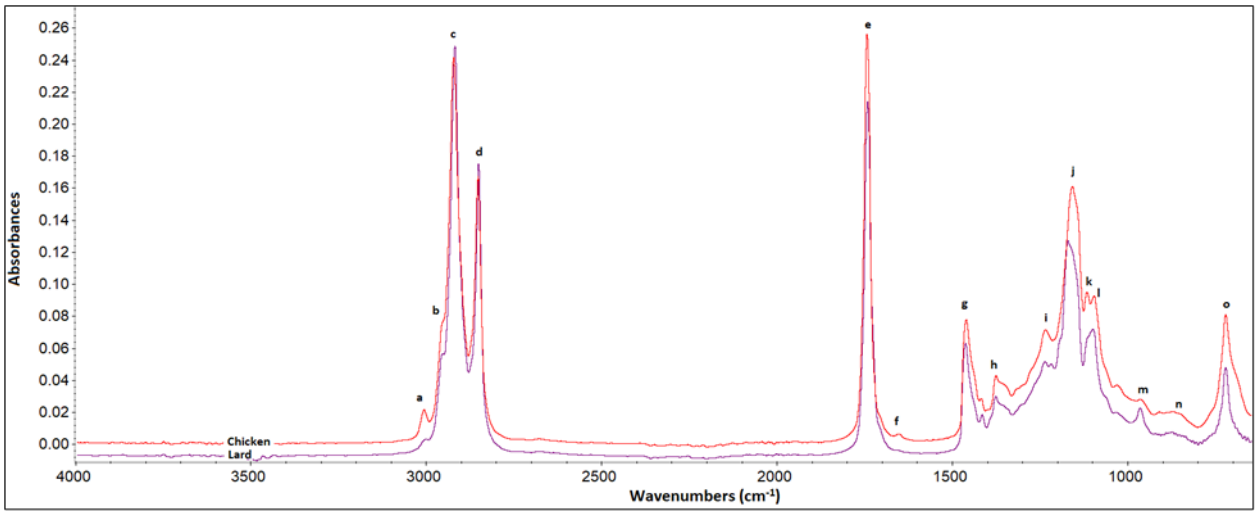

Figure 1. FTIR-attenuated total reflectance spectra of lard and chicken fat at mid infrared region (4000-650 cm^{-1}) scanned with resolution of 8 cm^{-1} and number of scanning of 32.

Table 2. Functional groups responsible for absorption of peaks and shoulders in lard and chicken fat

Code	Wavenumber region (cm^{-1})	Functional groups	Vibration modes
a	3007	*cis C=CH*	stretching vibration
b	2953	methylene ($-CH_2$)	asymmetric stretching vibration
c and d	2922 and 2852	methylene ($-CH_2$)	asymmetric and symmetric stretching vibrations
e	1744	carbonyl (C=O)	stretching vibration
f	1654	C=C	stretching vibration
g	1460	methylene ($-CH_2$)	bending vibration (scissoring)
h	1376	methyl ($-CH_3$)	symmetric bending vibration
i, j	1234, 1160	methylene ($-CH_2$)	bending vibration
k, l	1118, 1097	C-O	stretching vibrations
m	996	–HC=CH–(*trans)*	bending out of plane
n	850	0	wagging
o	721	$-(CH_2)_n$	rocking

Table 3. The performance of multivariate calibrations of partial least square (PLS) and principal component regression (PCR) for quantitative analysis of Lard (LD) in binary mixture with chicken fat

Multivariate calibrations	Wavenumber (cm^{-1})	Spectra	Calibration		Validation	
			R^2	RMSEC	R^2	RMSEP
PLS	3100-660	normal	0.9996	0.0066	0.9847	0.0433
		derivative 1	1.0000	0.0012	0.9803	0.0529
		derivative 2	0.9965	0.0206	0.9565	0.0795
	1800-660	normal	0.9984	0.0141	0.9844	0.0441
		derivative 1	0.9973	0.018	0.9862	0.0416
		derivative 2	0.9932	0.0287	0.965	0.0664
	1500-1000	normal	0.9945	0.0258	0.9892	0.0368
		derivative 1	0.9893	0.0361	0.9881	0.0398
		derivative 2	0.9934	0.0282	0.9728	0.0577
	3100-2750 and 1800-660	normal	0.999	0.0113	0.9897	0.0358
		derivative 1	0.9972	0.0186	0.9874	0.0411
		derivative 2	0.9953	0.0238	0.9658	0.0670
	3100-2750 and 1500-660	**normal**	**0.9994**	**0.0083**	**0.9916**	**0.0322**
		derivative 1	0.9967	0.02	0.9852	0.0449
		derivative 2	0.9992	0.0097	0.9586	0.0728
PCR	3100-660	normal	0.9865	0.0405	0.9682	0.0619
		derivative 1	0.966	0.0639	0.9715	0.0745
		derivative 2	0.8861	0.115	0.8714	0.133
	1800-660	normal	0.9935	0.0281	0.9831	0.046
		derivative 1	0.9877	0.0386	0.9711	0.0604
		derivative 2	0.9321	0.0895	0.9262	0.0935
	1500-1000	normal	0.9921	0.0309	0.9892	0.0372
		derivative 1	0.9878	0.0385	0.9889	0.039
		derivative 2	0.9667	0.0633	0.9699	0.0625
	3100-2750 and 1800-660	normal	0.9955	0.0233	0.9824	0.0465
		derivative 1	0.989	0.0365	0.9839	0.0468
		derivative 2	0.9418	0.0831	0.9386	0.0868
	3100-2750 and 1500-660	normal	0.992	0.0311	0.9826	0.0465
		derivative 1	0.9861	0.041	0.9702	0.0633
		derivative 2	0.9265	0.0929	0.9301	0.0933

Bold values are selected parameters.

evaluation of the analytical method, while RMSEC and RMSEP expressed the precision of the analytical method. In addition, the value of RMSEC and RMSEP static to zero, the better the predictive power of the model. The higher R^2 values and the lower RMSEC and RMSEP, the more accurate and more precise the analytical method. Based on statistical parameters of R^2 and RMSEC, PLS using absorbance values at combined wavenumbers of 3100-2750 and 1500-660 cm^{-1} offered the best calibration model with R^2 of > 0.99 and RMSEC of 0.008%. The second step is to validate the preferred calibration model using independent samples known as validation samples. Based on R^2 values for the correlation between actual values and FTIR predicted values in validation samples of > 0.99 and low value of RMSEP (0.032%), it can be deduced that the calibration model is a reliable method for prediction of LD in CF. Figure 2 exhibits the correlation between actual values (x -axis) of lard and FTIR calculated or predicted values (y-axis) exhibiting a close relationship between actual and predicted values.

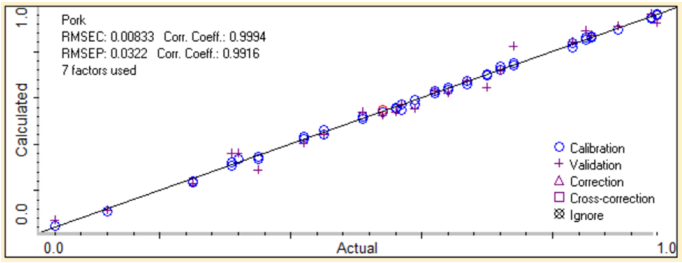

Figure 2. The correlation between actual values (x-axis) of lard and FTIR calculated or predicted values (y-axis).

Both PLS and PCR use a linear combination. However, how these linear combinations are chosen is different, in PLS, the linear combination was chosen based on the highest correlation between predictor variables and response variables. While in PCR, the chosen linear combination is based on principal components, irrespective of the strength of the relationships between the predictor and the response variables (Miller and Miller, 2010).

In order to discriminate LD and LD in a binary mixture with CF, supervised pattern recognition of discriminant analysis (DA) was used. DA was performed by calculating the distance from the centre of each class (pure LD and LD mixed with CF) in Mahalanobis distance units (Brereton, 2015). Using the same wavenumbers used for quantitative analysis, DA could discriminate LD and LD in the mixture with CF with an accuracy level of 100%. FTIR spectroscopy in combination with chemometrics offered a reliable method for quantitative analysis and discrimination of LD in a binary mixture with CF.

4. Conclusion

FTIR spectroscopy coupled with chemometrics of multivariate calibration and discriminant analysis has been successfully employed for quantitative analysis and discrimination of LD in a binary mixture with CF. By optimizing FTIR spectral mode, multivariate calibration types, and wavenumber regions, this method offered a reliable technique for the analysis of LD. The combination of FTIR spectra and chemometrics offered fast, green analytical techniques, and simple analytical techniques for the analysis of LD. Next, this method can be standardized to support the implementation of Indonesian Act No. 33 year 2014 on Halal Products Assurance.

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