

Analysis of lard, chicken fat and beef fat in ternary mixture using FTIR spectroscopy and multivariate calibration for halal authentication

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Abstract

Animal fats including lard (LD), beef fat (BF) and chicken fat (CF) are commonly used in food products. However, they raised some health and religious issues, therefore the simultaneous analysis of animal fats is necessary. The objective of this study was to employ FTIR spectroscopy in combination with chemometrics of multivariate calibration for analysis of LD, CF, and BF in ternary systems simultaneously. Animal fats were prepared using the rendering process of adipose tissues from corresponding animals. The obtained fats were used to prepare calibration and validation samples. FTIR spectra scanned at the mid-infrared region (4000-650 cm^{-1}) were used as variables during calibration and validation modelling with the aid of multivariate calibrations. Based on optimization (wavenumbers region, FTIR spectral mode, and multivariate calibration types) using the parameter of the highest R^2 values and lowest values of RMSEC and RMSEP, PLSR using a variable of normal FTIR spectral at the combined wavenumber regions of 3100-2750 and 1800-660 cm^{-1} for prediction of animal fats (LD, CF and BF) simultaneously. FTIR spectroscopy in combination with multivariate calibrations could be a useful technique for analysis of animal fats in the ternary mixture, including analysis of lard for halal authentication.

1. Introduction

Animal fats are rendered tissue fats that can be obtained from a variety of animals. Typically, animal fats are the by-products of the meatpacking industry (Giriprasad and Goswami, 2013). Lard, chicken fat (CF) and beef fat (BF) are fats extracted from adipose tissues of corresponding animals. Edible animal fats are complex mixtures containing a large number of mainly composed of 95-98% of triacylglycerols (TAGs), glycerols esterified with fatty acids, and 2-5% of diacylglycerols (DGs), free fatty acids (FFAs), phospholipids, and other minor components including vitamins and sterols (Buchgraber *et al.*, 2004; van Ruth *et al.*, 2010). Animal fats are widely applied in numerous

applications including nutrition and dietary, food, cosmetics, pharmaceuticals, paints and varnishes, detergents food and pharmaceutical products (Alvarez and Rodríguez, 2000). Some issues are raised regarding negative perceptions in terms of food safety, environmental, religious and health implications of using lard and other animal fats in the products (Troy *et al.*, 2016). The followers of Islam and Jew are prohibited to consume lard because lard is a non-halal and non-kosher component (Che Man and Rohman, 2011). Therefore, analysis of animal fats was essential for evaluating the specific purpose such as the nutritional value of food products.

Some analytical methods have been reported for

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quality control and analysis of animal fats including high-performance liquid chromatography, especially combined with a mass spectrometer (LC-MS) through analysis of triacylglycerol compositions (Fauconnot *et al.*, 2004), two-dimensional gas chromatography combined with a mass spectrometer (GC x GC-MS) for analysis of fatty acid profiles (Chin *et al.*, 2009), nuclear magnetic resonance (NMR) spectroscopy through analysis of fatty acids (Mihai *et al.*, 2018), direct analysis in real-time (DART) ionization coupled to time-of-flight mass spectrometry (TOFMS) in combination with chemometrics for profiling of triacylglycerols (Vaclavik *et al.*, 2011), differential scanning calorimetry (Marikkar *et al.*, 2021), and Raman spectroscopy combined with chemometrics (Lee *et al.*, 2018). Some of these methods are rather complex and involve sophisticated instruments. Besides, the used methods are used for the analysis of animal fats from the chemical compositions not as a whole the matter. For this reason, spectroscopic-based methods, namely Fourier transform infrared spectroscopy are recently used for the analysis of animal fats.

FTIR spectroscopy is considered a fingerprint analytical technique with the main advantages of its simplicity and rapidity with minimum or without sample preparation. For minimum use of chemical reagents, FTIR spectroscopy is taken into account as a green analytical technique (Irnawati *et al.*, 2020). Combined with chemometrics, FTIR spectra have been reported to be successfully applied for analysis of animal fats of lard in a binary mixture with vegetable oils (Rohman *et al.*, 2012), analysis of chicken fat in a binary mixture with cod liver oil (CLO) (Rohman and Che Man, 2011a), and analysis of beef fat in CLO (Rohman and Che Man, 2011b). Most of the reported methods are intended for the analysis of individual animal fats. Using a detailed study, the application of FTIR spectroscopy for the analysis of animal fats simultaneously is very limited. Therefore, in this study, the combination of chemometrics and FTIR spectra was employed for simultaneous analysis of lard (LD), chicken fat (CF) and beef fat (BF) in ternary mixture systems.

2. Materials and methods

2.1 Materials

Animal fats (lard, chicken fat and beef fat) were prepared by rendering the corresponding adipose tissues of animals according to the procedure reported by Indrasti *et al.* (2010). The adipose tissues were purchased from several local markets in Yogyakarta, Indonesia. The rendering procedure of animal fats was performed at 105°C for 4 hrs in the conventional oven. The melted animal fats were strained using a triple-folded muslin

cloth, dried with the addition of anhydrous Na₂SO₄, and then centrifuged at 3,000 rpm for 20 mins. The layer of animal fats was decanted, shaken well, and subjected to centrifugation before being filtered using filter paper.

2.2 Preparation of calibration and validation samples

The calibration samples (25 samples) were prepared in a ternary mixture consisting of lard (LD), chicken fat (CF) and beef fat (BF). The composition of each animal fat was carried out in a random manner using Excel software (Microsoft Inc., USA) as in Table 1. After that, the validation samples were also prepared independently covering the concentration ranges as in calibration samples. The ternary mixture consisting of LD, CF, and BF was homogenized in a water bath to obtain the homogeneous mixture. All mixtures were subjected to an FTIR spectrophotometer.

Table 1. The composition of animal fats in the ternary mixture used for preparing calibration and validation samples

Samples	Concentration (% v/v)		
	Lard	Chicken fat	Beef fat
1	2	1	97
2	4	17	79
3	4	68	28
4	6	26	68
5	7	46	47
6	8	86	6
7	10	21	69
8	12	49	39
9	13	6	81
10	20	76	4
11	20	77	3
12	21	21	58
13	21	44	35
14	22	30	48
15	22	25	53
16	31	44	25
17	32	38	30
18	37	18	45
19	42	17	41
20	42	16	42
21	51	25	24
22	53	6	41
23	54	5	41
24	63	24	13
25	79	12	9

2.3 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 the OMNIC operating system software (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°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.

2.4 Data analysis

Data analysis for multivariate calibrations was carried out using chemometrics software (Thermo Scientific™ TQ Analyst™) included in the FTIR spectrophotometer instrument. For quantitative analysis of animal fats in a ternary mixture, 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 mean square error in calibration (RMSEC) and root mean square error of validation (RMSEP).

3. Results and discussion

Analysis of edible fats as a whole matter is very important for evaluating the contents of animal fats in the products. The most common method for analysis of animal fats is chromatographic-based methods through analysis of fatty acid composition using gas chromatography and triacylglycerol composition using liquid chromatography. Spectroscopic-based methods are ideal for the analysis of animal fat contents because these methods can analyze animal fats as a whole, not by analysis of specific components in animal fats. FTIR spectroscopy, as one of vibrational spectroscopy and considered as fingerprinting analytical technique, in combination with chemometrics is an ideal technique for

analysis of animal fats contents because of its rapidity and simplicity (Rohman and Fadzillah, 2018).

Figure 1 exhibits FTIR spectra of animal fats namely lard, chicken fat and beef fat scanned at a wavenumber region of 4000-650 cm^{-1} using an attenuated total reflectance accessory. Each peak and shoulders of FTIR spectra come from stretching and bending vibrations of functional groups in animal fats. The functional groups appearing in FTIR spectra represented triacylglycerols which are the main components of animal fats. All spectra of animal fats are similar in terms of the number of peaks/shoulders, however, using detailed investigation, the peak intensities among FTIR spectra are different as a nature of FTIR spectra as fingerprinting technique. These differences in peak intensities can be exploited as a basis for the differentiation of animal fats and optimized for quantitative analysis of animal fats. Some wavenumbers region was optimized, especially in fingerprint regions, along with multivariate calibrations of partial least square regression (PLSR) and principal component regression (PCR) to get the best prediction model of animal fats in the ternary mixture.

Table 2 compiles the statistical results for modelling the prediction levels of lard in ternary mixture with CF and BF during the optimization step exploring wavenumber regions, FTIR spectral mode (normal versus derivatives) and multivariate calibrations (PLSR versus PCR). The R^2 values were used for the evaluation of the accuracy of the analytical method either in calibration and validation models, while RMSEC and RMSEP values were used for the evaluation of precision. The higher R^2 values and lower RMSEC and RMSEP values indicated the reliable method (Rohman *et al.*, 2021). Based on the highest R^2 values and lowest values of RMSEC and RMSEP, PLSR using a variable of normal FTIR spectral absorbances using the combined wavenumber regions of 3100-2750 and 1800-660 cm^{-1}

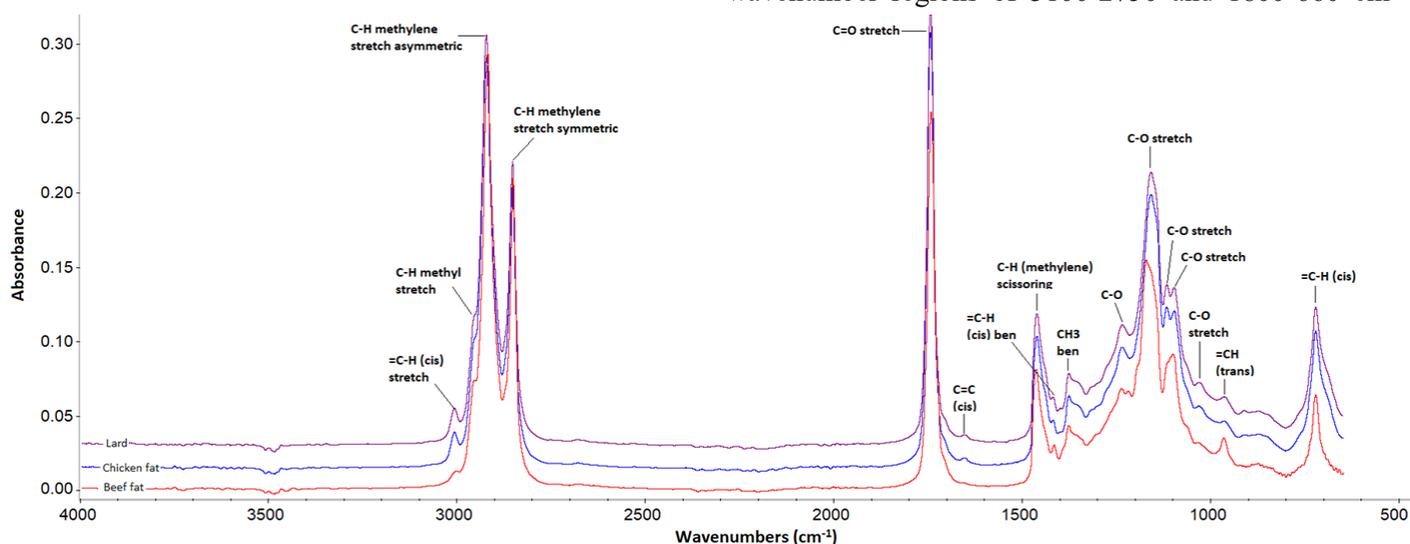


Figure 1. FTIR spectra of animal fats, namely lard, chicken fat and beef fat, scanned at mid infrared region (4000-650 cm^{-1}) using accessory of horizontal attenuated total reflectance.

Table 2. The statistical results during the optimization of lard in ternary mixture with chicken fat and beef fat.

Multivariate calibrations	Wavenumber (cm ⁻¹)	Spectra	Calibration		Validation	
			R ²	RMSEC	R ²	RMSEP
PLS	3100-660	normal	0.9832	0.0374	0.9660	0.0522
		derivative 1	0.9942	0.0220	0.9661	0.0552
		derivative 2	0.5397	0.1720	0.4612	0.1810
	1800-660	normal	0.9920	0.0259	0.9842	0.0361
		derivative 1	0.9984	0.0117	0.9658	0.0530
		derivative 2	0.9959	0.0185	0.9026	0.0884
	1500-1000	normal	0.9783	0.0425	0.9668	0.0521
		derivative 1	0.9956	0.0192	0.9734	0.0485
		derivative 2	0.9944	0.0217	0.9249	0.0809
	3100-2750 and 1800-660	normal	0.9971	0.0156	0.9874	0.0328
		derivative 1	0.9979	0.0134	0.9785	0.0447
		derivative 2	0.9977	0.0050	0.9425	0.0722
	3100-2750 and 1500-660	normal	0.9911	0.0273	0.9869	0.0373
		derivative 1	0.9974	0.0147	0.9696	0.0518
		derivative 2	0.9948	0.0208	0.9322	0.0782
PCR	3100-660	normal	0.9539	0.0615	0.9426	0.0677
		derivative 1	0.8163	0.1180	0.8245	0.1150
		derivative 2	0.7350	0.1390	0.6793	0.1480
	1800-660	normal	0.9734	0.0469	0.9653	0.0529
		derivative 1	0.9664	0.0526	9263.0000	0.0761
		derivative 2	0.6924	0.1480	0.5658	0.1690
	1500-1000	normal	0.9730	0.0472	0.9673	0.0516
		derivative 1	0.9731	0.0472	0.9533	0.0637
		derivative 2	0.9265	0.0770	0.8755	0.0999
	3100-2750 and 1800-660	normal	0.9762	0.0444	0.9658	0.0524
		derivative 1	0.9746	0.0459	0.9607	0.0599
		derivative 2	0.7016	0.1460	0.6433	0.1540
	3100-2750 and 1500-660	normal	0.9593	0.0578	0.9810	0.09810
		derivative 1	0.9638	0.0546	0.9432	0.0713
		derivative 2	0.7721	0.1300	0.6499	0.1530

The selected condition was marked with bold

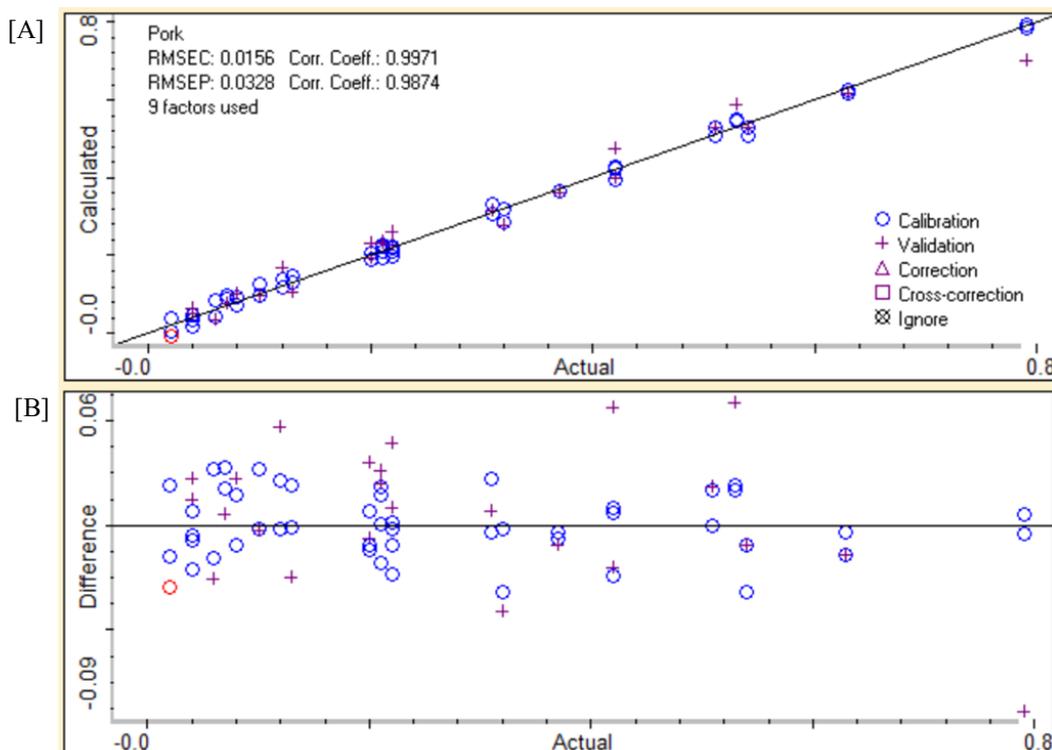


Figure 2. Partial least square regression for the correlation between actual values of lard (x-axis) and FTIR predicted values of lard in ternary mixture with chicken fat and beef fat [A] along with residual analysis [B].

Table 3. The statistical results during the optimization of chicken fat in ternary mixture with lard and beef fat.

Multivariate calibrations	Wavenumber (cm ⁻¹)	Spectra	Calibration		Validation	
			R ²	RMSEC	R ²	RMSEP
PLS	3100-660	normal	0.9830	0.0433	0.9754	0.0517
		derivative 1	0.9981	0.0144	0.9672	0.0587
		derivative 2	0.9460	0.0766	0.9074	0.0971
	1800-660	normal	0.9983	0.0136	0.9902	0.0327
		derivative 1	0.9984	0.0135	0.9729	0.0543
		derivative 2	0.9990	0.0104	0.9308	0.0861
	1500-1000	normal	0.9552	0.0699	0.9548	0.0688
		derivative 1	0.9529	0.0716	0.9436	0.0784
		derivative 2	0.9775	0.0499	0.9366	0.0826
	3100-2750 and 1800-660	normal	0.9972	0.0178	0.9875	0.0373
		derivative 1	0.9998	0.0047	0.9809	0.0463
		derivative 2	0.9997	0.0055	0.9563	0.0693
	3100-2750 and 1500-660	normal	0.9971	0.0179	0.9816	0.0442
		derivative 1	0.9995	0.0077	0.9821	0.0462
		derivative 2	0.9996	0.0066	0.9626	0.0652
PCR	3100-660	normal	0.9290	0.0874	0.9070	0.0973
		derivative 1	0.8501	0.1240	0.8580	0.1190
		derivative 2	0.8055	0.1400	0.7814	0.1440
	1800-660	normal	0.9580	0.0678	0.9550	0.0706
		derivative 1	0.9457	0.0768	0.9298	0.0870
		derivative 2	0.7786	0.1480	0.7274	0.1600
	1500-1000	normal	0.9655	0.0616	0.9718	0.0550
		derivative 1	0.9656	0.0614	0.9529	0.0736
		derivative 2	0.9484	0.0749	0.9161	0.0966
	3100-2750 and 1800-660	normal	0.9448	0.0774	0.9144	0.0954
		derivative 1	0.9531	0.0715	0.9417	0.0784
		derivative 2	0.8028	0.1410	0.7677	0.1480
	3100-2750 and 1500-660	normal	0.9646	0.0623	0.9692	0.0571
		derivative 1	0.9476	0.0755	0.9387	0.0799
		derivative 2	0.8449	0.1260	0.7763	0.1460

The selected condition was marked with bold

for prediction of lard in ternary mixture with CF and BF. This condition was also used for the prediction of CF and BF, with the results obtained during optimization compiled in Table 3 and Table 4, respectively.

Figure 2A reveals an example of the relationship between actual values of lard (x-axis) and FTIR predicted values of lard having R² of 0.9971 in calibration and 0.9874 for validation, with RMSEC of 0.0156 and RMSEP of 0.0328. In addition, the errors are randomly occurring during the calibration and prediction as indicated in residual analysis. The difference between actual and predicted values falls around zero (0) indicating that systematic error is negligible (Jamwal *et al.*, 2020). Therefore, based on R², RMSEC and RMSEP values, the developed method is accurate and precise enough for the analysis of animal fats in a ternary mixture simultaneously.

4. Conclusion

FTIR spectroscopy in combination with partial least square regression is an accurate and precise analytical tool for quantitative analysis of animal fats (lard, chicken fat and beef fat) in a ternary mixture simultaneously.

Using the optimization step based on the selection of wavenumber regions, multivariate calibration types and FTIR spectral mode, FTIR spectroscopy is a reliable method for analysis of lard with the main advantage of its simplicity and rapidity without or with minimum sample preparation.

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Table 4. The statistical results during the optimization of beef fat in ternary mixture with lard and chicken fat.

Multivariate calibrations	Wavenumber (cm ⁻¹)	Spectra	Calibration		Validation	
			R ²	RMSEC	R ²	RMSEP
PLS	3100-660	normal	0.9574	0.0734	0.9546	0.0674
		derivative 1	0.9581	0.0729	0.9546	0.0677
		derivative 2	0.9635	0.0680	0.9558	0.0671
	1800-660	normal	0.9593	0.0718	0.9549	0.0676
		derivative 1	0.9655	0.0662	0.9575	0.0663
		derivative 2	0.9691	0.0627	0.9565	0.0670
	1500-1000	normal	0.9555	0.0750	0.9432	0.0758
		derivative 1	0.9596	0.0715	0.9556	0.0677
		derivative 2	0.9621	0.0693	0.9583	0.0651
	3100-2750 and 1800-660	normal	0.9566	0.0741	0.9552	0.0670
		derivative 1	0.9792	0.0516	0.9645	0.0612
		derivative 2	0.9589	0.0721	0.9532	0.0691
	3100-2750 and 1500-660	normal	0.9561	0.0745	0.9550	0.0672
		derivative 1	0.9969	0.0199	0.9908	0.0314
		derivative 2	0.9563	0.0744	0.9521	0.0701
PCR	3100-660	normal	0.9701	0.0617	0.9537	0.0697
		derivative 1	0.9766	0.0547	0.9717	0.0535
		derivative 2	0.9739	0.0577	0.9679	0.0559
	1800-660	normal	0.9788	0.0520	0.9740	0.0517
		derivative 1	0.9783	0.0526	0.9674	0.0574
		derivative 2	0.7786	0.1480	0.7274	0.1600
	1500-1000	normal	0.9757	0.0557	0.9691	0.0562
		derivative 1	0.9811	0.0492	0.9827	0.0414
		derivative 2	0.9748	0.0568	0.9687	0.0555
	3100-2750 and 1800-660	normal	0.9717	0.0601	0.9574	0.0660
		derivative 1	0.9531	0.0715	0.9417	0.0784
		derivative 2	0.9702	0.0616	0.9621	0.0617
	3100-2750 and 1500-660	normal	0.9740	0.0576	0.9583	0.0657
		derivative 1	0.9833	0.0462	0.9814	0.0437
		derivative 2	0.9683	0.0635	0.9654	0.0604

The selected condition was marked with bold

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