Original Article

Analysis of Lard in Sausage Using Fourier Transform Infrared Spectrophotometer Combined with Chemometrics

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Abstract

Sausage is one of foods which must be confirmed halal to consumers. Meat is commonly used in producing sausages, especially beef. However, due to high cost of meat producer usually mixes the ingredients with other cheaper meats, such as pork. This study aimed to analyze the differences in the spectral profile of lard and beef in the sausages using Fourier transform infrared (FTIR). Lard and beef tallow was extracted using Soxhlet apparatus at ±70°C for 6 h with *n*-hexane. After extraction, lard and beef tallow was evaporated. Then obtained fats were stored in eppendorf and analyzed using FTIR spectrophotometer. The results were then combined with chemometrics such as Partial least squares (PLS) for the quantitative analysis and principal component analysis (PCA) for classification. PLS and PCA analysis was performed on 1200–1000 cm^{-1}. The results of the analyzed PLS provided the linear regression equation $y = 0.921x + 4.623$ with $R^2 = 0.985$ and root-mean-square error of calibration (RMSEC) = 2.094%. External validation root-mean-square error of prediction (RMSEP) was 4.77% and internal validation root-mean-square-error cross-validation (RMSECV) was 5.12%. The results of the PCA analysis showed the classification of different quadrants between 100% pork sausage and 100% beef sausage. Thus, it can be concluded that FTIR spectroscopy method combined with chemometrics can be applied to identify the presence of pork in the sausage.

Keywords: *Beef fat, Fourier transform infrared spectrophotometer, lard, partial least squares, principal component analysis*

Received : 19-09-19.
Accepted : 01-11-19.
Published : 30-12-19.

Introduction

The halalness of a food product is critically taken into consideration in consuming food products. As Allah SWT says in Surah Al-Baqarah verse 173, "only forbidden to you are carrion, blood, pork, and animals slaughtered not by the name of Allah but by the name of idols. But whoever is forced to eat it, while he is no persecution, and he is not also exceeding the limits, then there is no sin for him. Again indeed, Allah is Forgiving, Most Merciful."

The sausage is one of the processed meat products, which is much appreciated by many people in Indonesia. These food products are made from meat that is mashed, seasoned, and wrapped in a casing so it has a distinctive taste and symmetrical size. So far, most of the sausage raw material is beef, although some are derived from pork or chicken.[1]

Halal food products, are closely related to raw materials, and processing.[2] Sausage is a food product made from meat. Usually chicken or beef. Beef or chicken can be replaced by pork.[3]

Access this article online	
Quick Response Code:	Website: www.jpbsonline.org
	DOI: 10.4103/jpbs.JPBS_209_19

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How to cite this article: Guntarti A, Ahda M, Kusbandari A, Prihandoko SW. Analysis of lard in sausage using Fourier transform infrared spectrophotometer combined with chemometrics. J Pharm Bioall Sci 2019;11:S594-600.

The existence of pork in the sausage can be detected by Fourier transform infrared (FTIR) spectrophotometer. FTIR spectroscopy is the latest method of infrared (IR), which is already widely used for the analysis of food oil.[4] The analysis can be done by looking at the spectral pattern of a fat sample using an FTIR spectrophotometer.[5] Furthermore, contaminants of pigs in "abon" products also can be detected using real time PCR, with primers that are specific in mitochondria.[6] The oil analysis method using FTIR has been developed because it is easier, faster, cheaper, and eco-friendly.[7] The development of analytical methods using FTIR has now been combined with chemometrics techniques. Chemometrics is a chemical discipline that uses mathematics, statistics, and formal logic to design or select optimal experimental procedures and provide maximum chemical information relevant to analyzing chemical data.[8] This combination makes analysis better, especially for testing oils and fats or a mixture of both.[9] This study aimed to analyze pork in processed food products in the form of sausages using FTIR spectroscopy method combined with chemometrics. The novelty of this research was to use different fat samples from different animals. Although the samples were sauces, the result will be different because different fat samples also had different wave numbers.

Materials and Methods

Materials

Beef, pork, and sausages were all purchased from the Godean market, the Kranggan market, and Sleman area, respectively. *n*-Hexane and Na_2SO_4 anhydrous were purchased from the City of Yogyakarta.

Tools

The instruments used in the experiment included the following: Blenders, analytic scales, Eppendorf tubes, Soxhlet, FTIR spectrophotometer (Clairet Scientific, Northampton, UK) with deuterated triglycine sulfate detectors (deuterated triglycine sulfate) and The FTIR spectra were processed using FTIR software of Horizon MB version 3.013.1 (ABB, Canada).

Methods

Making reference sausages

Sausages were made by mixing beef, pork, and spices as per weight [Table 1].

Fat extraction

Fat was extracted with *n*-hexane solvents by Soxhlet apparatus at a temperature of ± 70°C for 6 h. Then, Na_2SO_4 anhydrous was added and shaken vigorously. The extracted fat then kept in an appendix at low temperatures.[10]

Fat analysis

Fats derived from sausages of various concentrations and market samples were analyzed using an FTIR spectrophotometer. Fat/oil were placed on ATR crystals at controlled temperatures (20°C).[11]

Data analysis

The data from FTIR analysis were processed using the chemometrics analysis program. The multivariate calibration model made with Horizon MB software uses principal component analysis (PCA) and partial least squares (PLS) techniques. An evaluation with parameters such as R^2 (coefficient of determination) and RMSEC (root-mean-square error of calibration) was carried out. The RMSEP and RMSECV can be calculated following equation:[12]

$$\text{RMSEP}: \sqrt{\frac{1}{N}\sum(\hat{y}_1, \text{pre} - y_i, \text{ref})^2}$$

where N is the number of data sets, $\hat{y}_i$, pre is the prediction value of the sample, and yi, ref is the reference value or the actual value.[12] Thus, we have

$$\text{RMSECV}: \sqrt{\frac{1}{N}\sum(\hat{X}_i\, i, \text{pre} - X_i, \text{ref})^2}$$

where N is the number of data sets, $\hat{X}_i$ is the prediction value of the sample, and $\hat{X}_i$, ref is the reference value or the actual value.

Table 1: Sausage formula mix beef and pork refinement

Concentration (%)	Cow (g)	Pig (g)	Seasoning (g)
Cow 100	225.00	–	25.00
Pig 100	–	225.00	25.00
$Pig_{(S–B)}$75	165.75	56.25	25.00
$Pig_{(S–B)}$65	146.25	78.75	25.00
$Pig_{(S–B)}$50	112.25	112.25	25.00
$Pig_{(S–B)}$35	78.75	146.25	25.00
$Pig_{(S–B)}$25	56.25	168.75	25.00

Result

Extraction results

Extraction was done at a temperature of ±70°C for 6h. The extraction process used n-hexane solvent because it has a non-polar solubility, low boiling point (easy to separate using evaporation), economical. After the final extraction, the oil solution was added with Na_2SO_4 anhydrate to remove water content. The water content in oil will affect the spectral reading. The oil color obtained was yellowish.

Fourier transform infrared spectrophotometer spectra analysis

Fat extracted with Soxhlet from 100% pork fat and 100% cow fat were analyzed by FTIR spectrophotometer at wave numbers 3000-600 cm^{-1}. Response Spectra is a functional group of fats [Figure 1].

Table 2 shows wave numbers and functional groups that explain the peaks in the spectra. Figure 2 presents the FTIR spectra of various sausages in various concentrations.

Quantitative analysis using partial least squares

Quantitative analysis of fat extracted using Partial Least Square (PLS) calibration.[2] Accuracy of PLS model was evaluated by coefficient of determination (R^2), while the precision of analytical method was assessed using root Mean Square Error of Calibration (RMSEC) and Root Mean Square Error of Prediction (RMSEP).[13] The classification among meatball samples was carried out using chemometrics of Principal Component Analysis (PCA) [Figure 3].

Fat grouping using principal component analysis

Sausage formula contains 100% pork and 100% beef classified using chemometrics from Principal Component Analysis (PCA). The wavenumber used is optimized and the wave number used for quantitative analysis, which is 1200-1000 cm^{-1}.[14] The discriminant of beef and lard are presented on [Figure 4]. Samples were obtained from the Sleman area and the City of Yogyakarta. The results of grouping with samples on the market are presented in Figure 5.

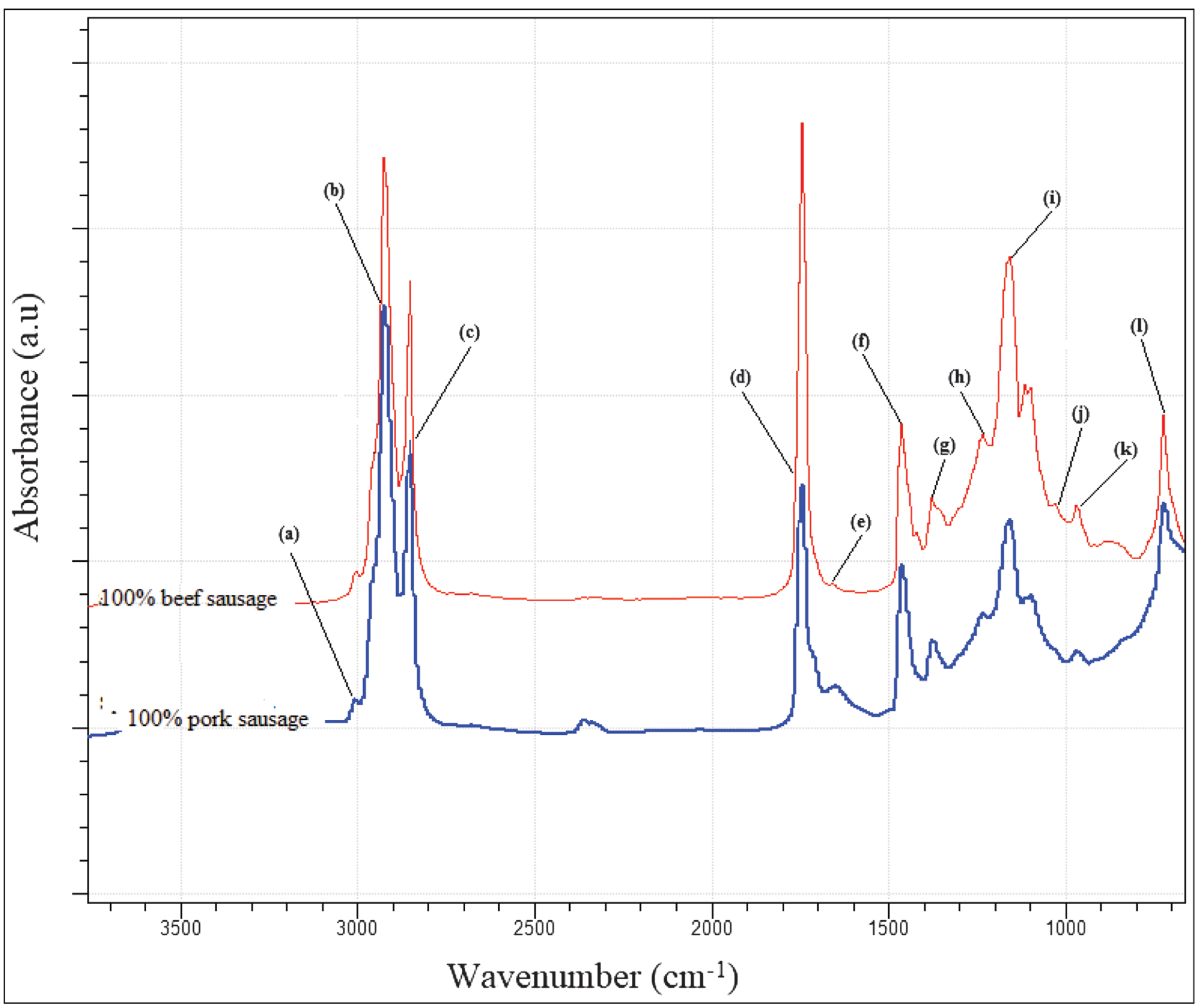

Figure 1: FTIR spectrum from pure beef sausage (100% beef sausage) and pure pork sausage (100% pork sausage)

Table 2: Function clusters and vibration models on beef fat and lard

No.	Vibration wave number cm^{-1}			Function clusters vibration	Absorbance	
	Pig	Cow	Reference		Pig	Cow
(a)	3009	3005	3006	*Cis* C = CH	0.04	0.002
(b)	2920	2920	2924	Asymmetric and symmetric stretching vibration of methylene groups ($–CH_2$)	0.52	0.52
(c)	2847	2851	2852	Asymmetric and symmetric stretching vibration of methylene groups ($–CH_2$)	0.35	0.35
(d)	1747	1744	1743	Carbonyl functional group (C = O) from esters that connected to triacylglycerol	0.30	0.52
(e)	1651	1656	1654	*cis* C = C stretching	0.06	0.02
(f)	1462	1462	1465	Bending vibration of the aliphatic CH_2 and CH_3 clusters	0.20	0.20
(g)	1377	1377	1377	Symmetric bending vibration in CH_3 clusters	0.11	0.11
(h)	1234	1234	1228	Vibration of stretching of C–O clusters in esters	0.14	0.19
(i)	1161	1161	1155	Group vibration C–O in esters	0.25	0.40
(j)	1095	1099	1097	Bended vibration –CH and the changes of vibration shape –CH in fatty acids	0.11	0.11
(k)	968	969	962	Bended vibration of function cluster –CH in trans-olefins is isolated	0.09	0.10
(l)	721	721	721	Overlapping vibrations of the methylene group ($–CH_2$) with vibrations out of the field on *cis*-substituted olefins	0.27	0.20

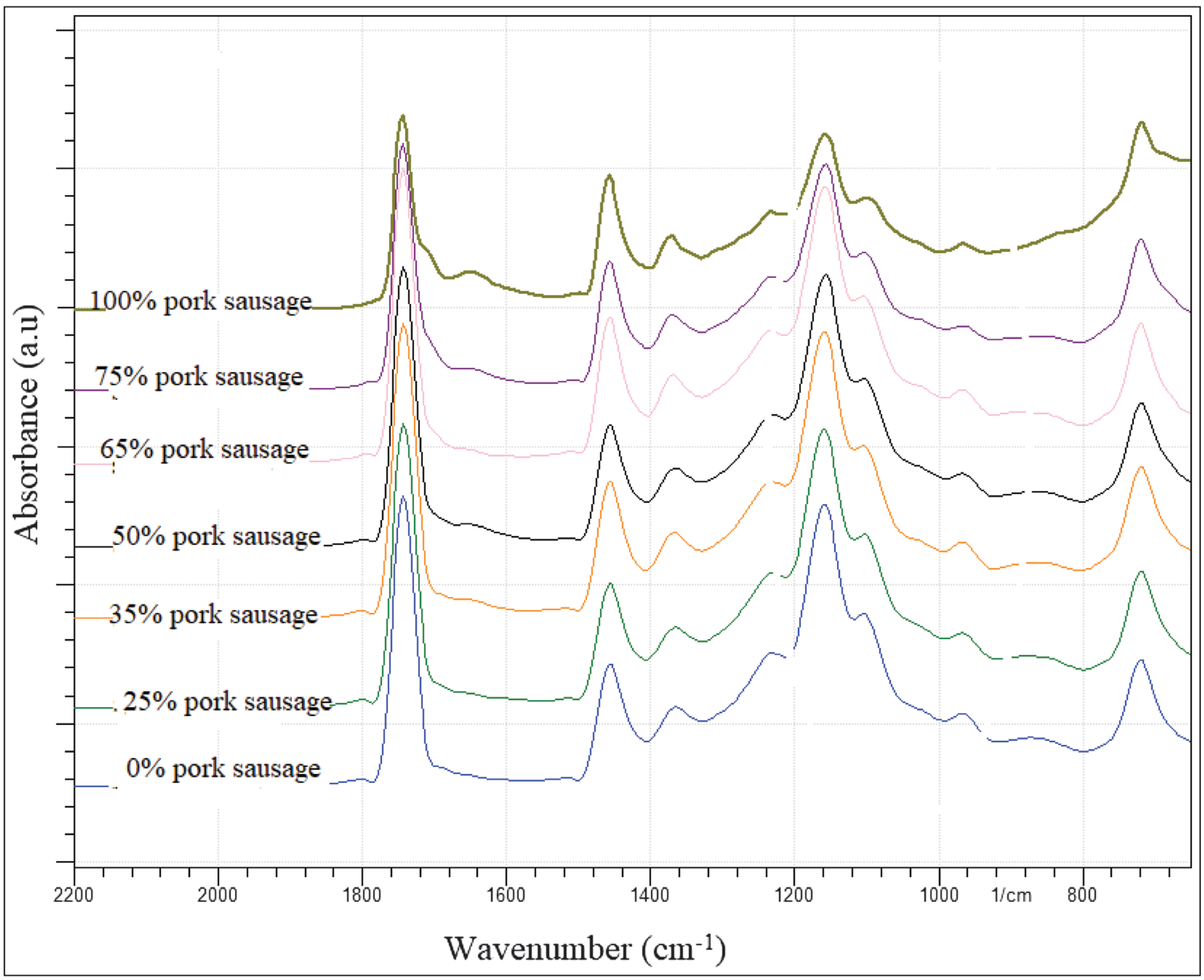

Figure 2: Spectrum of FTIR sausage various concentrations reference (0%–100%)

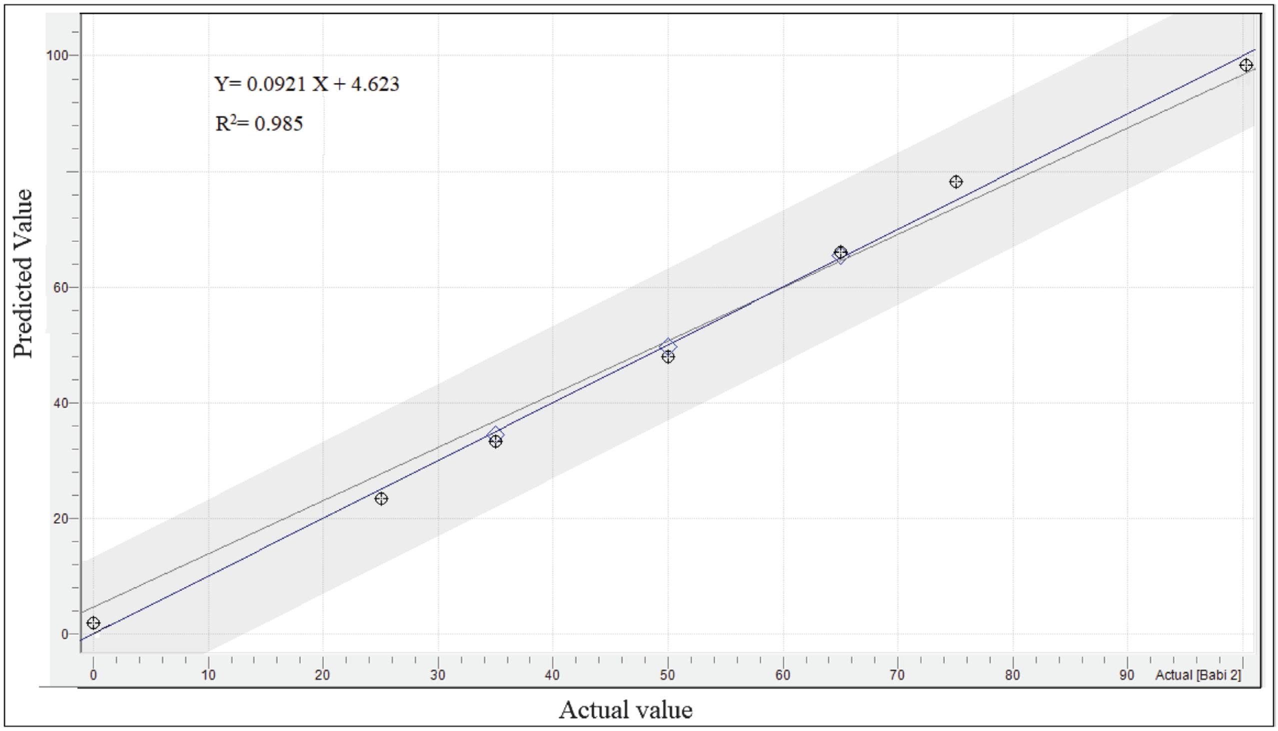

Figure 3: The results of PLS analysis, curve of the relationship between actual value (*x*-axis) and prediction value (*y*-axis) in reference sausage

Discussion

Fourier transform infrared spectrophotometer spectra analysis

Figure 1 shows the function clusters and vibrational models found in beef fat and lard. Table 2 shows the peak area of the wave numbers 1747 and 1744 cm^{-1}. There is a significant difference in absorbance between the two types of fat. In the region of the wave number 1750-1717 cm^{-1} is the carbonyl group (C=O) ester of triacylglycerol.[7] The wave number peak 1161 cm^{-1} is the stretching vibration of the C–O cluster in the ester, and shows a significant difference between lard and beef fat so that in this area analysis of differences in the profile of lard with cow fat is done. The FTIR spectra showed that the differences both pork and beef are 1200–650 cm^{-1}. Figure 2 presents the FTIR spectra of various sausages in various concentrations.

Quantitative analysis using partial least squares

The linear regression between the actual concentration and the results of the FTIR-PLS prediction shows quite good results, namely the linear regression equation $y = 0.921x + 4.623$ with $R^2 = 0.985$. The R^2 value indicates the ability of a method to produce a rate proportional analysis to the concentration sample. The value of R2 approaches 1 showed that the linear relationship between the actual value and prediction Value is good and RMSEC, RMSEP, and RMSECV values are low, indicate an error that occurred in the analysis is low.[13] The RMSEC value is 2.094%. The RMSEC value is used to evaluate errors in the calibration model.[15]

RMSEP value of 4.77% RMSECV value 5.12%. The smaller RMSEP and RMSECV values indicate a smaller error so that the model built has an ability that is getting better in the analysis.[16]

Fat grouping using principal component analysis

The results of the analysis on both types of fats, namely 100% beef (A) and 100% lard (B), suggest that the two fats are in different quadrants and are separated by great distances. At wave numbers 1200-1000 cm-1 can be used for the analysis of a mixture of lard and beef fat. The distance between pork fat and cow fat is very far.[17] This shows that there is a difference between pork fat and beef fat [Figure 4].

Then an analysis of the sausage samples sold in the community was carried out. Samples were obtained from the Sleman area and the City of Yogyakarta. The sample is S (1, 2, 3, 4, and 5) [Figure 5]. It is known that all samples are in the 100% cow quadrant. This means that there is no counterfeiting of the market sausage sample by using non-halal meat or fat (pork).

Conclusion

The coefficient of determination (R^2) of 0.985 and the RMSEC value of 2.094% can be determined by

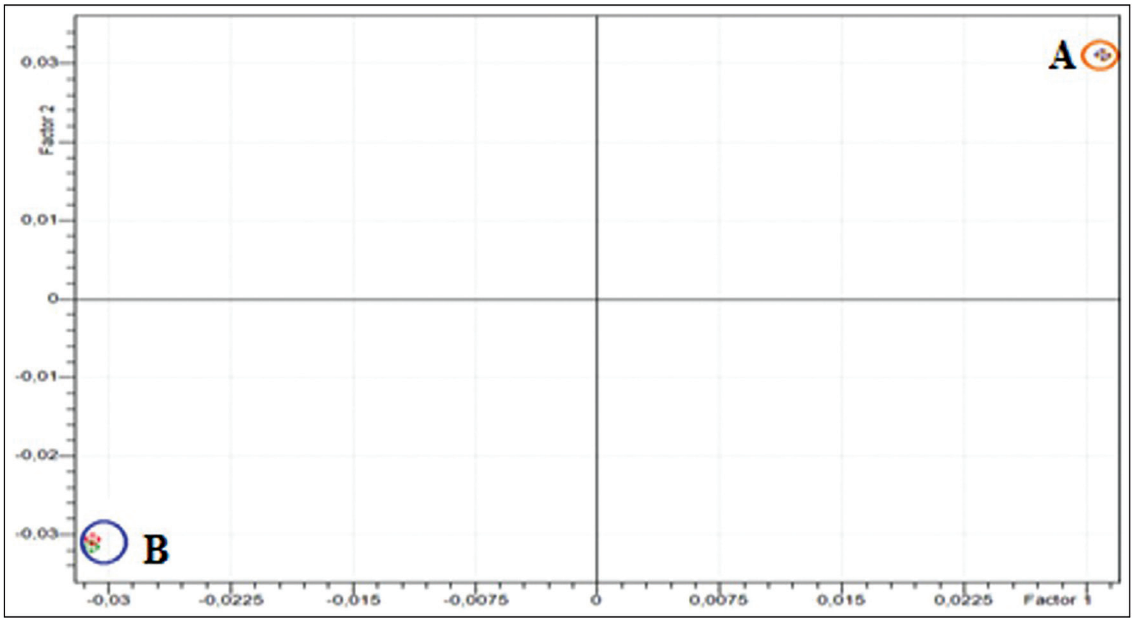

Figure 4: The results of PCA analysis of (A) 100% beef fat and (B) 100% lard

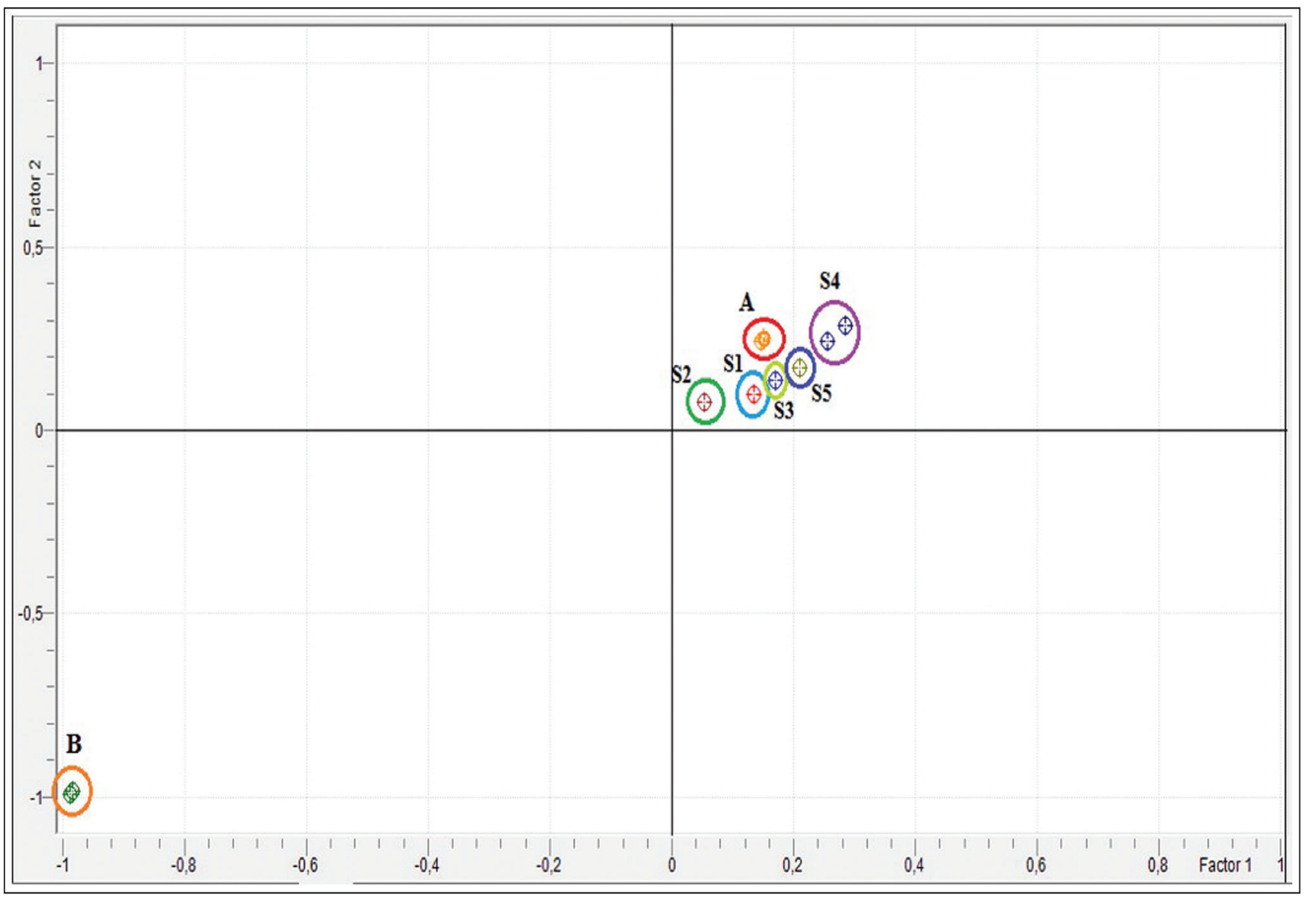

Figure 5: The results of PCA analysis (A) 100% beef sausage and (B) 100% pork sausage, and S (1, 2, 3, 4, and 5) market sausage samples

using the FTIR spectrophotometry combined with multivariate PLS calibration at 1200–1000 cm^{-1} wave number. FTIR spectrophotometry combined with PCA multivariate calibration can serve as an accurate and reliable method for the classification of lard and beef fat in the market.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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