

Pork adulteration in commercial meatballs determined by chemometric analysis of NIR Spectra

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Received: 28 October 2014 / Accepted: 9 March 2015 / Published online: 13 March 2015
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Abstract The use of chemometrics to analyse near infrared (NIR) spectra to determine pork adulteration in beef meatball was developed. Since, the adulteration of pork in beef meatball is frequently occurring. This study was aimed to develop a fast and simple technique for the determination and quantification of pork adulteration in beef meatball using NIR spectra and chemometrics. Both partial least-squares (PLS) calibration and linear discriminant analysis (LDA) model were developed to determine pork adulteration in beef meatballs. The models using the first derivative spectra, accurately classified 100 % of the pork adulterated beef meatballs samples using training set and test set. The PLS and LDA models were subsequently used for the determination of pork adulteration in real beef meatball samples. The results showed that the PLS and LDA models developed were in good agreement with the immunochromatographic method. Therefore, the potential of NIR spectra and chemometrics as a rapid method for halal authentication and identification of pork adulteration in beef meatball have been successfully developed.

Keywords NIR spectroscopy · Chemometrics · Meatball · Food adulteration · Halal authentication

Introduction

Currently, the consumers awareness towards consumer product are increasing, since consumers have to know accurate information of the product materials before they make any purchase decision. In line with this awareness, European Commission (EC) legislation (178/2002) on food safety states clearly that each stake holder in a food supply chain must be able to trace out all raw materials utilised in the manufacturing of food products [1]. A convenient method of verifying labelling of commercial food products is of great concern and value to ensure food safety and gain consumers' trust [2]. Today, as processed meats are being increasingly used in modern food, e.g. meatball is popular processed meats throughout the world. They can be formulated using any kind of meat, such as beef, chicken, pork, or even fish muscle. However, beef meatball is most popular and widely found in the markets [3]. In many case, substitution of beef in meatball formulations with lower valued meats such as pork frequently finds to take advantage with market competition and mostly to earn economic benefit. In the religious laws of Islam and Judaism, the presence of pork or its derivatives in food products is a serious religious concern as pork is prohibited [2].

A number of analytical methods have been proposed for the analysis of pork and/or lard, such as electronic nose coupled to gas chromatography–mass spectrometry [4], Fourier transform infrared spectroscopy [3], enzyme-linked immunosorbant assay [5], PCR(polymerase chain reaction)-electrophoresis [6], PCR–RFLP (Restriction fragment length polymorphism) [7], TaqMan[®] probe real time PCR [8], molecular beacon real time PCR [9], SYBR[®] green real-time PCR [10], and nanoparticle sensors coupled with optical or fluorescence spectroscopy [11, 12]. Near infrared spectroscopy (NIRS) has proven to be a rapid and effective

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tool in meat quality analysis for a wide variety of products and parameters [13]. In many literature, they can be found for the prediction of fatty acid profiles in a wide range of products, like vegetable oils [14], fish oil [15], rabbit meat [16], beef meat [17, 18] and egg yolk [19]. Nevertheless, this literature contains little information on the use of NIRS on analysis of pork adulteration in processed meats or minced meats.

A few reports were found in the literature in relation to the use of NIRS for species identification in meat. These reports account in the use of NIRS to discriminate between kangaroo and beef meat [20], chicken meat cuts [21], lamb and beef mixtures [22] and between beef, chicken and pork [23, 24]. One of the advantages of NIRS technology is not only to assess chemical structures through the analysis of the molecular bonds in the near infrared spectrum, but also to build a characteristic spectrum that represents the “finger print” of the sample. This opens the possibility of using spectra to determine complex attributes of organic structures, which are related to molecular chromophores, organoleptic scores and sensory characteristics [25, 26]. In addition, the application of statistical packages such as principal component or discriminant analysis provides the possibility to understand the optical properties of the sample and classify them without the need for chemical information. NIRS depends on chemometrics which involves multivariate analysis for interpreting large data sets [27, 28]. Currently, partial least squares (PLS) regression is probably the most widely applied regression method in chemometrics. The aim of this work was to study the potential of this technique for the analysis of pork adulteration in beef meatball without depends on chemical information.

Materials and methods

Beef and pork were obtained from different slaughter houses in Jember, Indonesia. Usually the slaughter house for pig is specified for pig only, this is due to different feeding of corresponding animals, while slaughter house for cow together with goat and sheep. The materials used for making beef meatball were purchased from local market at Jember. All solvents used for analysis were of pro analytical grade.

Beef meatball preparation

Meatball was prepared by emulsifying 90 % of fine ground meat (beef and or pork) with 10 % of starch and mixing it with salt and certain spices, and finally shaping it into balls. It is then cooked in boiling water for 10–20 min. Meatball was further cut into small pieces and blended for powdered

process using an electric blender (Philip 2115, Indonesia). Then the powder was sieved into 10 mesh. The powder yielded was further used for NIR analysis. The sample was powdered in order to reduce the effect of different beef meatball texture and mainly to homogenize the meatball sample. This is due to in the preliminary study; it was found that the texture and heterogeneity of the meatball sample will slightly affect to the NIR spectra. Therefore, for the better result of NIR spectra obtained, the powdered step is needed for homogenization of the all sample tested.

Training set and test set

The training sets were prepared by spiking pork to beef meatball in concentration range of 10.0–90.0 %. Meatball containing of 100 % beef and 100 % pork was also made to observe the spectral differentiation. In order to reduce the effect of other composition that usually added during meatball preparation (e.g. starch, salt and certain spices) towards NIR spectra obtained. The other composition and beef content were varying, and used for classification as pure. Similarly, this was also applied for the pork adulterated meatball, and used for classification as adulterated. This procedure is needed to make sure that the different in NIR spectra is due to the different in beef and pork content in meatball and not other compositional changes.

For test set, another series of meatball containing the mixture of pork and beef were prepared (20, 50 & 70 %). Meatball was further subjected to powder. The powders obtained were analyzed using NIR spectroscopy. The spectral regions where the variations were observed were chosen for developing PLS and classification model (i.e. linear discriminant analysis (LDA), soft independent modelling of class analogies (SIMCA) and support vector machine (SVM).

Near infrared analysis

Samples were scanned in reflectance mode (850–2000 nm) in a scanning monochromator NIRS Luminar 3070 (Model 920B, Brimrose corporation, MD, USA). Samples were scanned in a circular cup (50 mm diameter, 10 mm depth) (Part number IH-0307, NIRSystems, USA) sealed with disposal paper back. Samples were not rotated when spectra collection was made. Spectral data collected were recorded in the form of the logarithm of reciprocal of reflectance [$\log(1/R)$] with 5 nm interval (120 data points). Two pairs of InGaAs detectors collected the reflectance spectra and the readings were referenced using a ceramic disk. The spectrum of each sample was the average of 32 successive scans. Spectral data collection and manipulation were performed using NIRS Brimrose analytical software (Brimrose MACRO language, SNAP 32, MD, USA).

Chemometric analysis

Spectra were exported from the NIRS Brimrose software for chemometric analysis. Both dummy PLS analysis and LDA are performed by the UNSCRAMBLER software version 10.2 from CAMO (Computer Aided Modelling, Trondheim, Norway). PLS is used to develop calibration equations to predict dummy values of samples in the validation set from their spectral data. Three set of data were used: the original NIR spectra (850–700 nm), the first derivative of NIR spectra and the second derivative of NIR spectra. Cross validation was used in all cases as internal validation. Cross validation estimates the prediction error by splitting the calibration samples into two groups. One group was reserved for validation and the remaining groups were used for calibration. The process was repeated until all groups have been used for validation once [29, 30].

LDA is commonly used techniques for data classification and dimensionality reduction. LDA easily handles the case where the within-class frequencies are unequal and their performance has been examined on randomly generated test data. This method maximizes the ratio of between-class variance to the within-class variance in any particular data set thereby guaranteeing maximal separability [31]. In this case, the use of LDA for data classification is applied to classification between pure beef meatball (pure) and pork adulterated meatball (adulterated).

Real sample analysis

For real sample analysis, the PLS and LDA model based on selected data set was applied to the real samples of beef meatball collected from supermarket at Jember. The real samples were categorised into official halal logo (MUI, Indonesia), unofficial halal logo (by the producer) and without halal logo. The real sample analysis was compared with immunochromatography for pork as a strip test (Xematest Pork, Xema, Russia).

Results and discussions

NIR spectra characterisation

The typical spectra of beef meatball, pork meatball and pork adulterated meatball samples in NIR regions are shown in Fig. 1. Visual differences were observed between sample species in the near infrared region, where the intensity of the pork meatball was the highest, compared to pork adulterated meatball (mix) and beef meatball respectively. In the near infrared region absorption bands were observed at 980, at 1060, at 1250 and at 1650 nm. At

980 and 1060 nm related with OH third, second and first OH stretch overtones, mainly related with water content of the samples [32]. Since, water vapour could be reabsorbed during sample stored. Around 1250 nm, absorption bands related with CH second overtone. At 1650 nm with CH₂ stretch first overtone related with both fat and fatty acids and at 2000 nm with CH combinations associated with fat content and with saturated and unsaturated fatty acids, respectively [32, 33]. Most of the spectral information used for the discrimination analysis is contained in the NIR region around (950–2000 nm) due to both different pigments present in each specie and to matrix characteristics (intra-muscular fat, fatty acids, moisture) (Fig. 1). These results are similar with those reported by other authors [22, 34, 35].

Theoretically, the differences in the spectra could be due to the fact that the differences in the chemical constituents of fatty acids and amino acids lead to possibility detection of adulteration using NIRS. Since, meat from animals of different species is characterized by specific composition, structure and odour [36–38]. For instance, beef has fatty acid higher than pork, e.g. lauric acid [37, 38]. Therefore, calculation of specific fatty acid ratios in fats from different animal species allows revealing the distinctive features [39]. There is also opinion that their amino acid composition, particularly myoglobin and myogen can be regarded as biomarkers of meat tissue [36]. Thus, this is the main reason of possibility to identify meat and processed meat from different animals due to difference in amino acids and fatty acids composition by NIR spectra followed by chemometrics analysis.

Training set

The composition of the training set used as the sample was consist of 10 samples, ranging from 0 % (pure or beef meatball) to 100 % (adulterated or pork meatball) as given in Table 1. The representative spectra of meatball obtained from beef and pork adulterated meatball as the training set are demonstrated in Fig. 2a. In order to have three set of data as a training for selection of the best data set, then the original NIR spectra was derivatised into the first derivative (Fig. 2b) and the second derivative (Fig. 2c) of NIR spectra. Here, all NIR spectra obtained in the first derivative (Fig. 2b) and the second derivative (Fig. 2c) have similar shape, and the different only in their spectral intensities, where higher pork content (higher adulteration) resulted higher spectra intensity.

At the training set, the lower level of adulteration was set at 10 %, since it is assumed if adulterated occurs, it must be higher than 10 %, since many adulteration occur due to economic benefit [3, 40]. Thus, it needs higher content of those adulterated material, as currently, the beef

Fig. 1 Spectra of powdered meatball (beef, mixed beef–pork in ratio 50 % and pork)

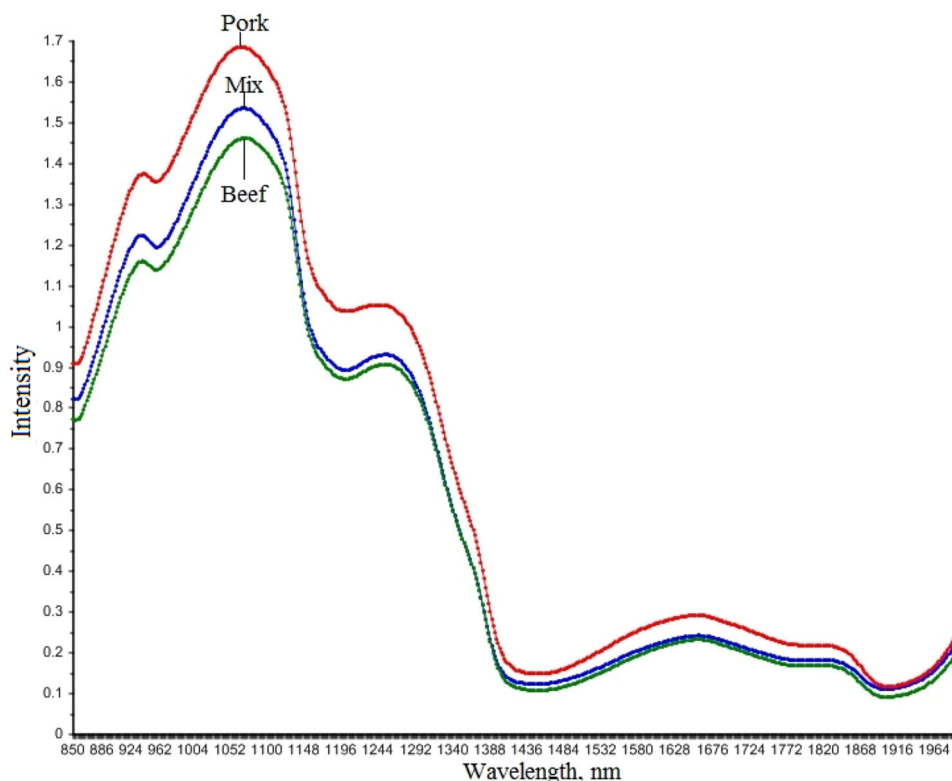


Table 1 Composition and classification of meatball (total weight of sample ± 10 g) as training set

Pork (g)	Beef (g)	Ratio of beef:pork (%) b/b	Classification
0	10.02	0	Pure
1.01	9.1	10.1	Adulterated
2.09	8.09	20.1	Adulterated
3.02	7.03	30.1	Adulterated
4.02	6.01	40.1	Adulterated
5.08	5.03	50.2	Adulterated
6.01	4.02	60.0	Adulterated
7.05	3.01	70.1	Adulterated
8.01	2.00	80.0	Adulterated
9.07	1.15	90.9	Adulterated
10.01	0	100.0	Adulterated

The classification into pure and adulterated of the sample was classified by experimenter

price was triple higher compared to the pork price in Jember. In addition, 10 % was selected for simple preparation of the pork adulterated beef meatball sample as we used adulterated sample in 10 % incremental value (i.e. 0, 10, 20, 30 till 100 %). However, this could also be the weakness of this proposed method as below 10 %, the error could be occur in determining pork adulteration in beef meatball.

Test set

In order to test, the reliability of the training set used, the data set as the original (Fig. 2a), the first derivative (Fig. 2b) and the second derivative (Fig. 2c) of NIR spectra were test with the test set. The composition of the test set used was consisting of five samples, and their composition was given in Table 2. The representative spectra of the test set are given in Fig. 3. The original spectra of the test set was also has similar shape and the different only in its intensity according to the degree of pork adulterated in beef meatball, where the pure meatball has the lowest spectral intensity and the pork meatball has the highest spectral intensity. Therefore, these wavelength regions, which NIR spectra intensity variations were observed, are used as a basis for the analysis of pork adulterated in beef meatball samples [20, 34].

PLS regression models

The spectra of training set for each data set (original spectra, first derivative and second derivative spectra) were used to construct correlation between amounts of pork adulterated versus the intensity of spectra using PLS regression equation. The results of PLS are given in Table 3. Based on the PLS regression value (R^2), it can be seen correlation between the real value versus the predicted

Fig. 2 Spectra of meatball as training set (0–100 %); **a** original spectra, **b** first derivative, dan **c** second derivative

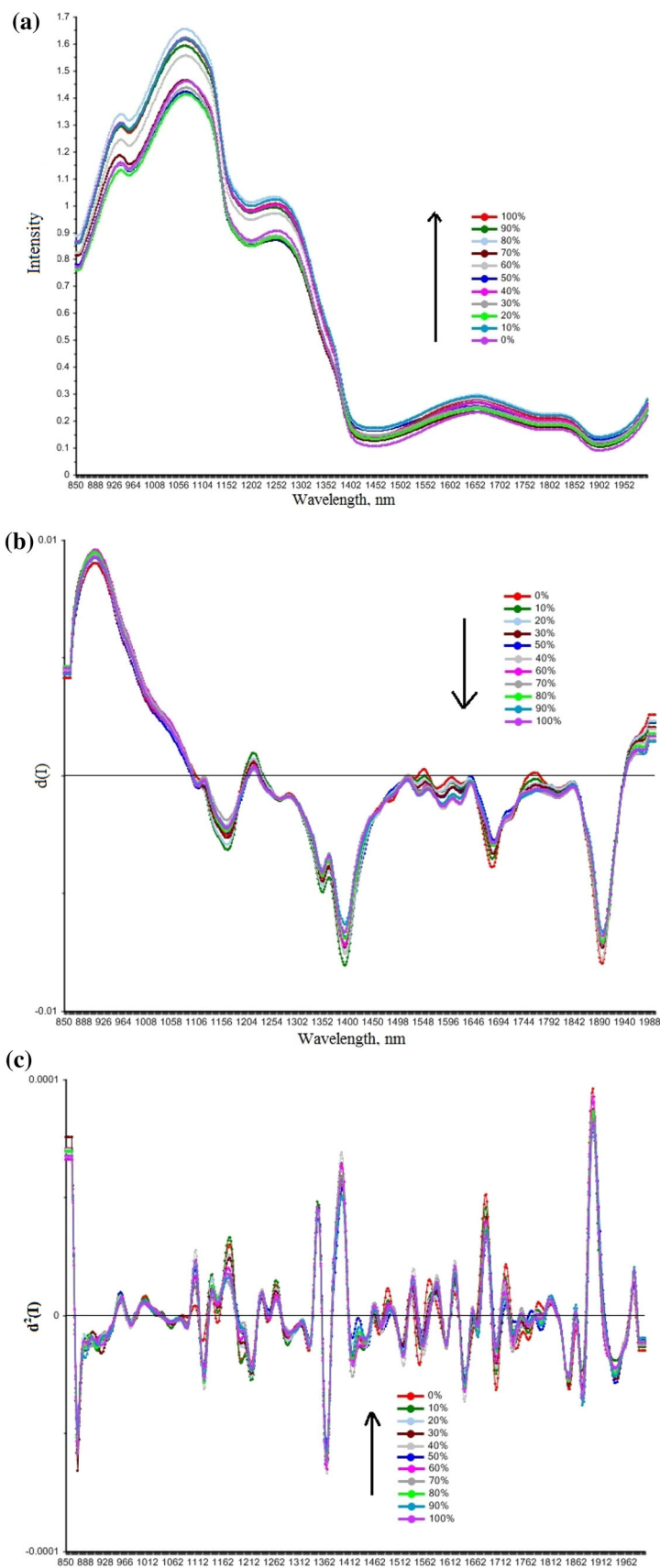


Table 2 Composition and classification of meatball (total weight of sample ± 10 g) as test set

Pork (g)	Beef (g)	Ratio of beef:pork (%) b/b	Classification
0	10.02	0	Pure
2.00	8.00	20.0	Adulterated
5.30	5.30	50.1	Adulterated
7.00	3.00	70.0	Adulterated
10.05	0	100.0	Adulterated

The classification into pure and adulterated of the sample was classified by experimenter

value for each data set [3, 15]. In this case, the data set 2 (the first derivative) has the highest R^2 and the lowest root mean square error (RMSE) value compared to the data set 1 (the original spectra) and the data set 3 (the second derivative). The data set 2 has R^2 value of 0.971 and RMSE value of 5.384, while the data set 1 and 3 have R^2 values of 0.944 and 0.950 with RMSE values of 7.475 and 7.055 respectively.

In order to test the accuracy of PLS model for quantitative analysis of pork adulterated on beef meatball, the model was tested using the test set as described above and the results are given in Fig. 4. Based on this result, it also shows that the data set 2 gave the best result compared to the data set 1 and 3. The R^2 value of the data set 2 was

Table 3 PLS regression of training set

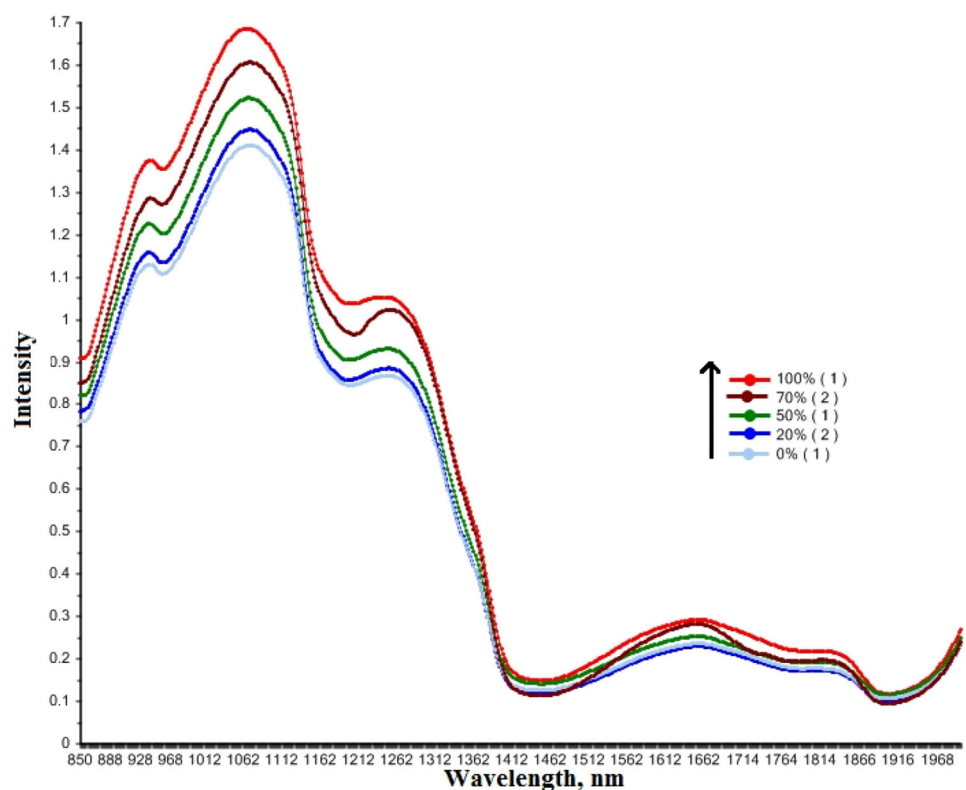
Data set	R^2	RMSE
Original spectra	0.944	7.475
First derivative	0.971	5.384
Second derivative	0.950	7.055

R^2 regression value, $RMSE$ root mean square error

0.971 with RMSE value was 5.384, while the R^2 value of the data set 1 was 0.911 and RMSE value was 9.586. However, as the lower level of pork adulterated on meatball was set at 10 %, meaning that the PLS model only good if work in this level, below this value the error in quantitative analysis could be occur.

LDA models for classification analysis

The spectra of training set were then used to construct the classification model based on LDA model [23, 33, 35]. In this model, the samples were classified into two categories, i.e. pure (pure beef meatball) and adulterated (pork adulterated beef meatball). The pure one could describe the halalness of meatball, while the adulterated meaning the *haramness* (prohibited) of meatball, that cannot be consumed based on Islamic regulation. In the LDA model, the classification is presented as graphical mapping and

Fig. 3 Spectra of meatball as test set (0, 20, 50, 70, 100 %)

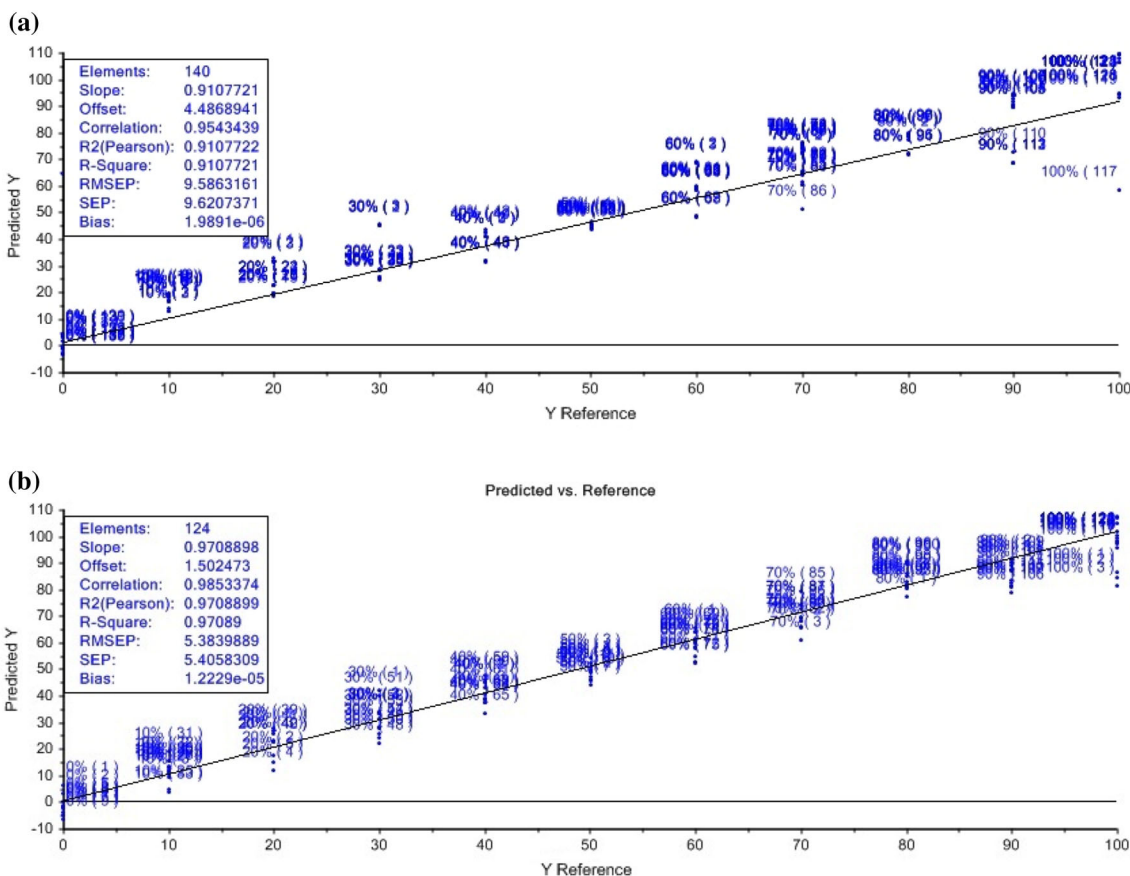


Fig. 4 PLS Model for Test set; **a** data set 1 (original data); and **b** data set 2 (first derivative)

predicted table [23, 33, 35]. The results of the LDA model are given in Fig. 5. Figure 5a–c show the LDA model for the original data, the first derivative and the second derivative, where in each figure show accuracy of model in classification of the data based on the training set. In this case, the data set 2 (the first derivative) has accuracy a value of 100 %, meaning that the model could classify correctly 100 % of the data. While the data set 1 (the original data) and the data set 3 (the second derivative) has accuracy values are 90.24 and 86.67 % respectively. Therefore, the LDA model based on the data set 2 (first derivative) was selected and used for further application in classification analysis.

In order to evaluate the reliability of the LDA model used, it was tested for the classification of the spectral data into pure and adulterated category. The LDA model used was tested with the test set. The results of the LDA model used toward the test set are given in Table 4. Based on Table 4, it was shown that the data set 2 (the first derivative) has accuracy a value of 100 % in the classification prediction of the test set. While both the data set 1 (the original spectra) and the data set 3 (the second derivative) only have accuracy value of 80 % in the classification

prediction of the test set. Thus, the data set 2 (the first derivative) has the highest and the best accuracy (100 %) in this LDA model.

In order to show the capability of the LDA model for classification of the data into pure and adulterated category, it was used for both the training set as a recognition model and the test set as a prediction model. Ideally, the LDA model should be able to classify the data set into both capabilities as recognition and prediction models in accuracy value of 100 % [31]. The results of the LDA model of the data sets (1, 2 and 3) are given in Table 5.

Based on Table 5, it can be stated that the best LDA classification model was the data set 2 (the first derivative), since it has capability to recognise and predict the data given in an accuracy value of 100 %, meaning that the LDA model using the data set 2 could classify correctly 100 % of all the data given, for both as recognition model of the training set (11 samples) and as the prediction model of the test set (5 samples) in classification of the data into pure and adulterated category. Thus, the LDA model used based on the data set 2 (the first derivative) was selected and used for further application.

Fig. 5 The LDA model; **a** data set 1 (original data); **b** data set 2 (first derivative); & **c** data set 3 (second derivative)

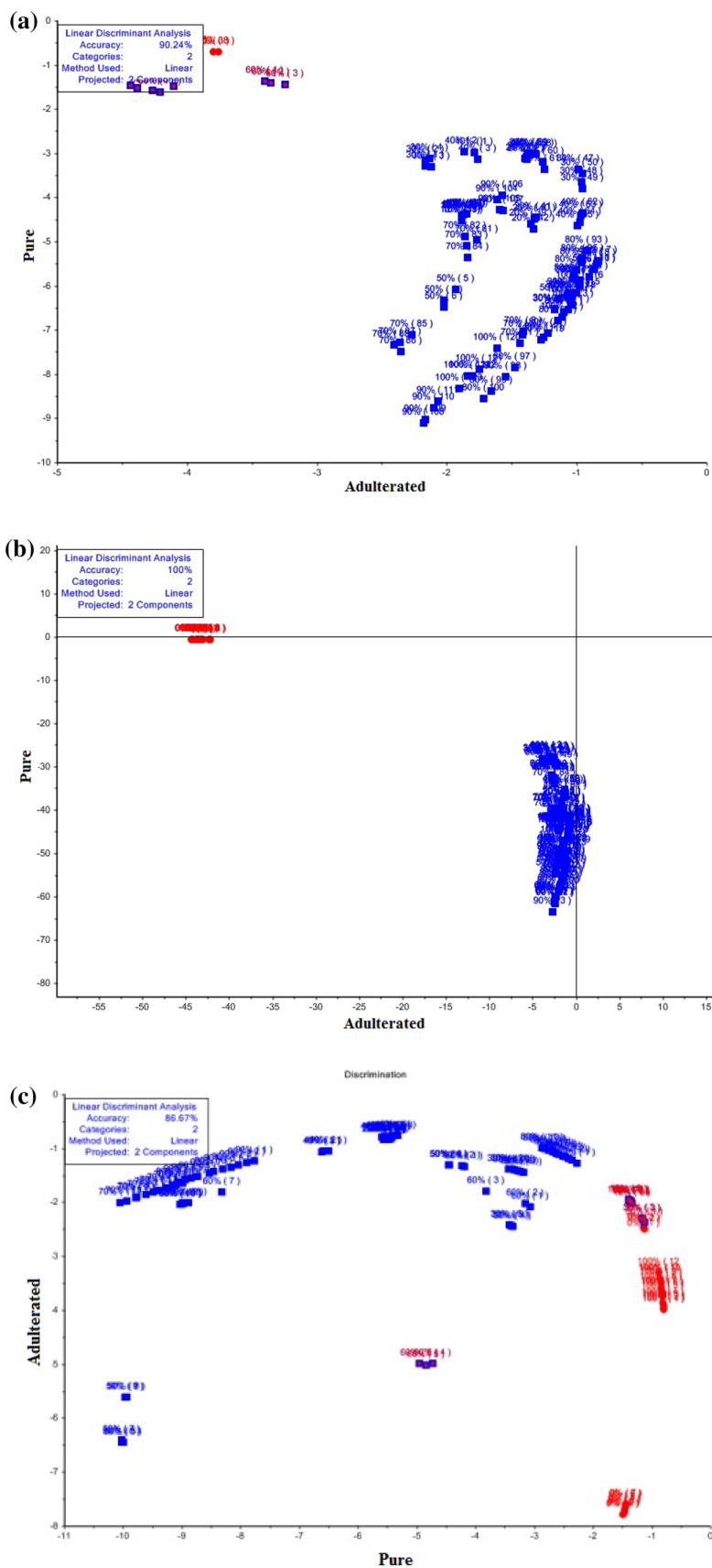


Table 4 The results of LDA model for classification of test set

Data set	Percentage of pork adulterated (%)	Predicted Classification Category		Prediction accuracy (%)
		LDA		
		Pure	Adulterated	
Original (1)	Pure (0)	√		80
	Adult (20.00)	√		
	Adult (50.05)		√	
	Adult (69.93)		√	
	Adult (100)		√	
First derivative (2)	Pure (0)	√		100
	Adult (20.00)		√	
	Adult (50.05)		√	
	Adult (69.93)		√	
	Adult (100)		√	
Second derivative (3)	Pure (0)		√	80
	Adult (20.00)		√	
	Adult (50.05)		√	
	Adult (69.93)		√	
	Adult (100)		√	

Adult adulterated

Table 5 The results of LDA classification model for recognition and prediction of the data given

Data set	LDA	
	Recognition (%)	Prediction (%)
1	90	85
2	100	100
3	85	85

% Recognition = the ability to recognise the training set

% Prediction = the ability to predict the test set

Application for the real samples

The PLS and LDA models were constructed using the data set 2 (the first derivative), have been applied toward the real samples, which consist six samples of beef meatballs (sample no. 1–3 with official halal logo, MUI, Indonesia), no. 4 and 5 with unofficial halal logo, by the producer and no. 6 without halal logo), and 1 samples of pork meatball (sample no. 7). All of the meatball samples used was purchased from supermarket in Jember. Before the real samples were measured using NIRS, all the real samples are powdered according to the procedure given above. Based the PLS model applied, it can be stated that only 1 sample (sample no. 7) accurately can be classified as pork meatball, since it give prediction value of 100 %, while the

other samples show prediction value of 0 % as given in Fig. 6, it means that the rest of the samples were classified as pure beef meatball.

The real samples were also classified using the LDA model constructed to categorise into pure and adulterated samples. The results are given in Table 6, where it also confirmed that only sample no. 7 can be categorised as adulterated sample, while the rest of the meatball samples were categorised as pure or pure beef meatballs. The real sample of meatball have also been tested using the immuno-chromatographic method as strip test (Xema pork test), and the results are summarised in Table 7, it was shown that the PLS and LDA model developed was in good agreement with the immuno method used as comparison method.

Based on this result, it can be stated that all beef meatball samples tested using the PLS and LDA models based on the data set 2 (the first derivative) can be classified as pure (beef meatball), it means that they are verified as halal and can be consumed according to Islamic law. While only one sample (no. 7) of meatball that made from pork meatball was quantified (100 %) and classified as adulterated (pork adulterated meatballs) using PLS and LDA classification models, respectively. Therefore, the proposed method based on NIR spectra and chemometric techniques (PLS–LDA) as a rapid analytical tool for the analysis of pork adulteration in beef meatball as halal authentication has been achieved.

Fig. 6 The PLS model toward real samples, where pork meat ball categorised as adulterated sample as indicated 100 % and other beef meatballs as pure as indicated 0 %

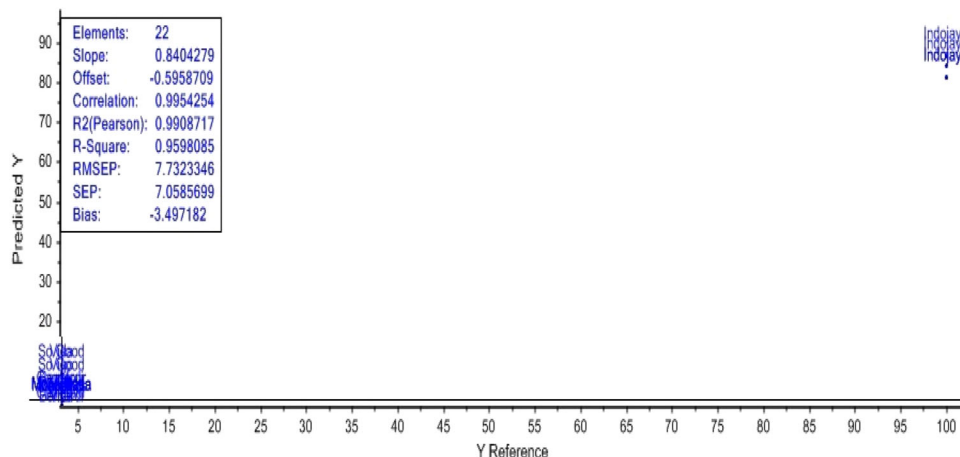


Table 6 The LDA classification model towards real samples

Sample ^a	Category	
	Pure	Adulterated
1	✓	
2	✓	
3	✓	
4	✓	
5	✓	
6	✓	
7		✓

^a Sample no. 1–6 are labelled as beef meatballs, no. 1–3 with official halal logo (MUI, Indonesia), no. 4 and 5 with unofficial halal logo (by the producer) and no. 6 without halal logo, and sample no. 7 is labelled as pork meatball

Table 7 Comparison result of NIR-chemometric versus immuno method

Sample ^a	Analysis of meat ball classification			
	NIR-chemometrics		Immuno method	
	Pure	Adulterated	Pure	Adulterated
1	✓		✓	
2	✓		✓	
3	✓		✓	
4	✓		✓	
5	✓		✓	
6	✓		✓	
7		✓		✓

^a Sample no. 1–6 are labelled as beef meatballs, no. 1–3 with official halal logo (MUI, Indonesia), no. 4 and 5 with unofficial halal logo (by the producer) and no. 6 without halal logo, and sample no. 7 is labelled as pork meatball

Conclusion

The pork adulteration of beef meatball can be determined by means of NIR spectra coupled with chemometric techniques (PLS and LDA). The PLS model constructed based on the first derivative spectra can be used to predict pork adulteration on beef meatball quantitatively. While, the LDA model constructed can be used to classify pure and pork adulterated beef meatballs. The PLS and LDA models have also been successfully tested to real beef meatball samples, and the results showed in good agreement with the immuno method. Thus, the potential of NIRS and chemometrics as a rapid analytical tool for the quantification and classification of pork adulteration in beef meatballs were demonstrated. The finding from this study will serve as a basis in developing a database for monitoring food adulteration, especially for Halal authentication purposes in processed beef meat.

Acknowledgments The authors thank the DitLitabMas, Higher Education, Ministry of Education and Culture, Republic of Indonesia, for supporting this work via the International Research Collaboration & Publication Program 2014 (No. 2303/UN25/LT/2014).

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