

# Application of class-modelling techniques to infrared spectra for analysis of pork adulteration in beef jerkys

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**Abstract** The use of chemometrics to analyse infrared spectra to predict pork adulteration in the beef jerky (dendeng) was explored. In the first step, the analysis of pork in the beef jerky formulation was conducted by blending the beef jerky with pork at 5–80 % levels. Then, they were powdered and classified into training set and test set. The second step, the spectra of the two sets was recorded by Fourier Transform Infrared (FTIR) spectroscopy using attenuated total reflection (ATR) cell on the basis of spectral data at frequency region 4000–700  $\text{cm}^{-1}$ . The spectra was categorised into four data sets, i.e. (a) spectra in the whole region as data set 1; (b) spectra in the fingerprint region (1500–600  $\text{cm}^{-1}$ ) as data set 2; (c) spectra in the whole region with treatment as data set 3; and (d) spectra in the fingerprint region with treatment as data set 4. The third step, the chemometric analysis were employed using three class-modelling techniques (i.e. LDA, SIMCA, and SVM) toward the data sets. Finally, the best result of the models towards the data sets on the adulteration analysis of the samples were selected and the best model was compared with the ELISA method. From the chemometric results, the LDA model on the data set 1 was found to be the best model, since it could classify and predict 100 % accuracy of the sample tested. The LDA model was applied toward the real samples of the beef jerky marketed in Jember, and the results showed that the LDA model developed was in good agreement with the ELISA method.

**Keywords** FTIR spectroscopy · Chemometrics · Beef jerky · Adulteration · Halal authentication

## Introduction

The analysis of food authenticity and the detection of adulteration are major issues in the food industry, and are attracting of concern among consumers. Adulteration of food has ranged from the simple addition of natural compounds to the much more serious case of contamination with harmful substances (Defernez and Wilson 1995). In meat and meat products, major authenticity and adulteration issues concern the substitution of high value raw materials with cheaper materials, such as less costly cuts, mechanically recovered meat, offal, blood, water, eggs, gluten or other proteins of animal or vegetable origin (Al-Jowder et al. 1997). For instance, in the some food manufacturers choose to blend beef with pork to reduce production cost due to pork is the cheapest meat commonly available for these food industries.

Food products containing pork are of great concern to followers of Islamic and Jewish religions. Both religions prohibit the consumption of pork in any products (Regenstein et al. 2003; Bonne and Verbeke 2008). Besides, diets rich in pork are known to associate with certain health risk, such as hypercholesterolemia and coronary heart disease with daily intake (Rashood et al. 1995). In view of the restrictions on their consumption by some religions and the biological complications associated with pork, a proper method is urgently needed to analyse the presence of pork in food.

Currently, the analytical methods used to analyse pork adulteration rely on either protein or DNA analysis. Protein based methods include; Fourier transform infrared (FTIR) spectroscopy (Rohman and Che Man 2010; Rohman et al. 2011), near-infrared spectroscopy (Cozzolino and Murray

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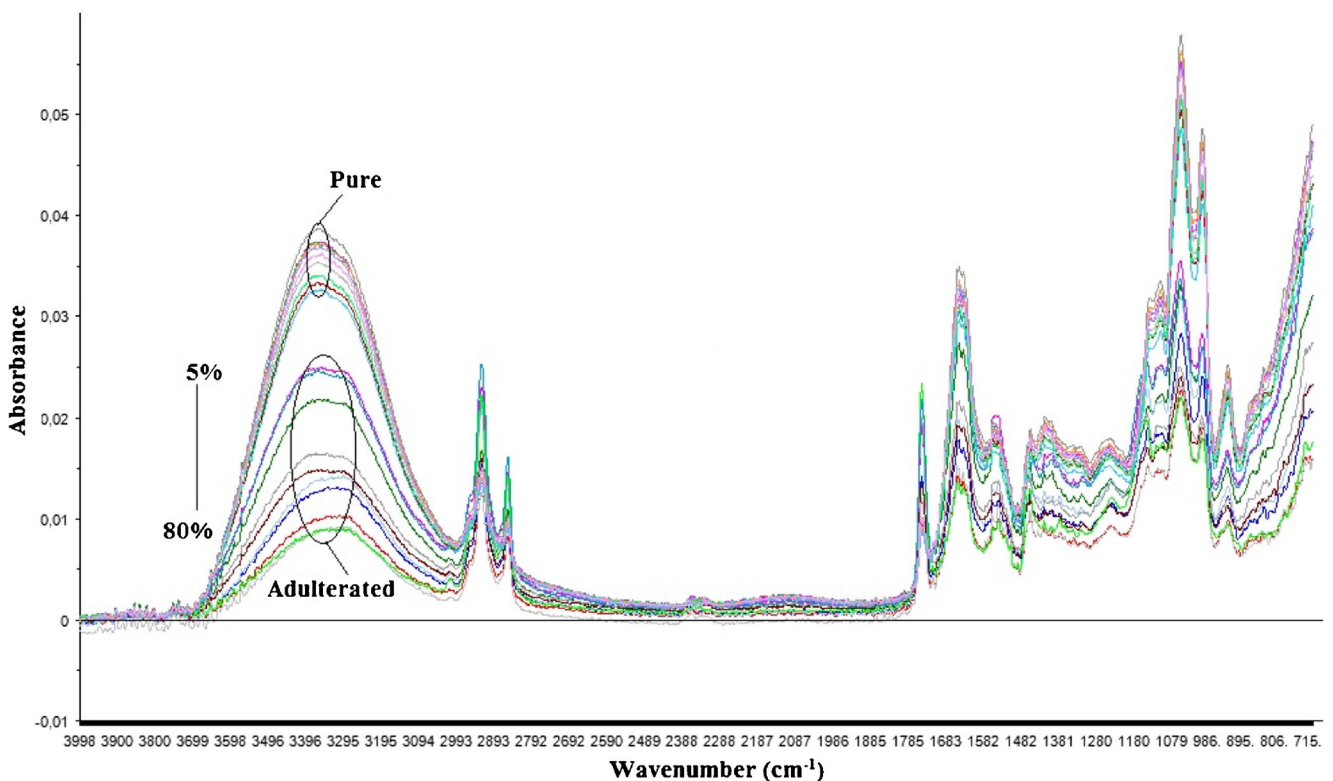
**Table 1** Composition of pork adulterated beef jerky as training set

Pork concentration (%)	Classification
0 %	Pure
5,05 %	Adulterated
10,14 %	Adulterated
14,18 %	Adulterated
20,03 %	Adulterated
30,10 %	Adulterated
40,00 %	Adulterated
50,00 %	Adulterated
60,01 %	Adulterated
69,93 %	Adulterated
80,00 %	Adulterated

2002), electronic nose (Che Man et al. 2005a, b; Tian et al. 2013), chromatography (Chou et al. 2007) and electrophoresis (Montowska and Pospiech 2007). Additionally, analyses of immunoassays, which rely on the use of antibodies raised against a specific protein, are also developed as ELISA (Chen and Hsieh 2000) and immunosensors (Fajardo et al. 2010). Protein based techniques have a number of limitations. They are limited when assaying heat treated products due to denaturation of proteins during thermal processing (Fajardo et al. 2010). DNA based methods include; polymerase chain reaction (PCR) amplification of mitochondrial DNA (Aida

et al. 2005, Che Man et al. 2007), PCR–restriction fragment length polymorphism (RFLP) analysis (Aida et al. 2007; Chen et al. 2010; Murugaiah et al. 2009) and PCR sequencing (Karlsson and Holmlund 2007; La Neve et al. 2008). DNA-based methods have led to a change from protein to DNA analysis, due to the advantages DNA in differentiating among different animal species (Ballin 2010). DNA is present in majority of cells and the information content of DNA, can also be extracted from all kinds of tissues (Lockley and Bardsley 2000), it is a stable molecule that allows analysis of processed and heat treated products (Aida et al. 2005), However, generally DNA analysis needs sample pretreatment and PCR amplification for their analysis, which in turn lengthening the procedure, laborious and time consuming.

Infrared spectroscopy has recently been applied to this problem using some experimental and statistical approaches. FTIR spectroscopy represents an important tool used for quality control and monitoring process in the food industry because it is less expensive, better in performance and easier to use than other method (Van de Voort et al. 1993). FTIR spectroscopy offers a fast and non-destructive alternative to chemical measurement techniques for qualitative characterization and quantitative measurements. Infrared spectroscopy has been used to provide information on the molecular composition and structure of a diverse range of materials (Cronin and McKenzie 1990; Guillen and Cabo 1997). In comparison with



**Fig. 1** IR Spektrum of training set, where the pure beef jerky has the highest absorbance intensity, while the lower intensity belong to pork adulterated beef jerky (80 %)

**Table 2** Composition of pork adulterated beef jerky as test set

Pork concentration (%)	Classification
0 %	Pure
1,00 %	Adulterated
5,05 %	Adulterated
69,93 %	Adulterated
100 %	Adulterated

conventional instruments, FTIR has a higher energy throughput, excellent reproducibility and accuracy from the laser source. With the increasing use of computers, FTIR can easily manipulate spectral information, and its advance chemometric software is equipped to handle the calibration.

Currently, FTIR spectroscopy has received great attention to be used in the analysis of edible fats and oils. Previous research has also shown the potential of FTIR spectroscopy for analysis of lard in cake formulation (Syahariza et al. 2005), chocolate products (Che Man et al. 2005a, b), edible oils (Guillen and Cabo 1997), animal fats (Che Man and Mirghani 2001; Jaswir et al. 2003; Rohman and Che Man 2010; Rohman et al. 2011). The main objective of this study was to develop procedures for rapid analysis of pork adulteration in processed meat products, particularly in beef jerky. Since in Islamic rule, if the processed meat products already adulterated with pork, the products can be classified as *haram* or prohibited, which in turn can not be consumed any more by

the muslim, even if the amount of pork adulterated is very small amount.

## Materials and methods

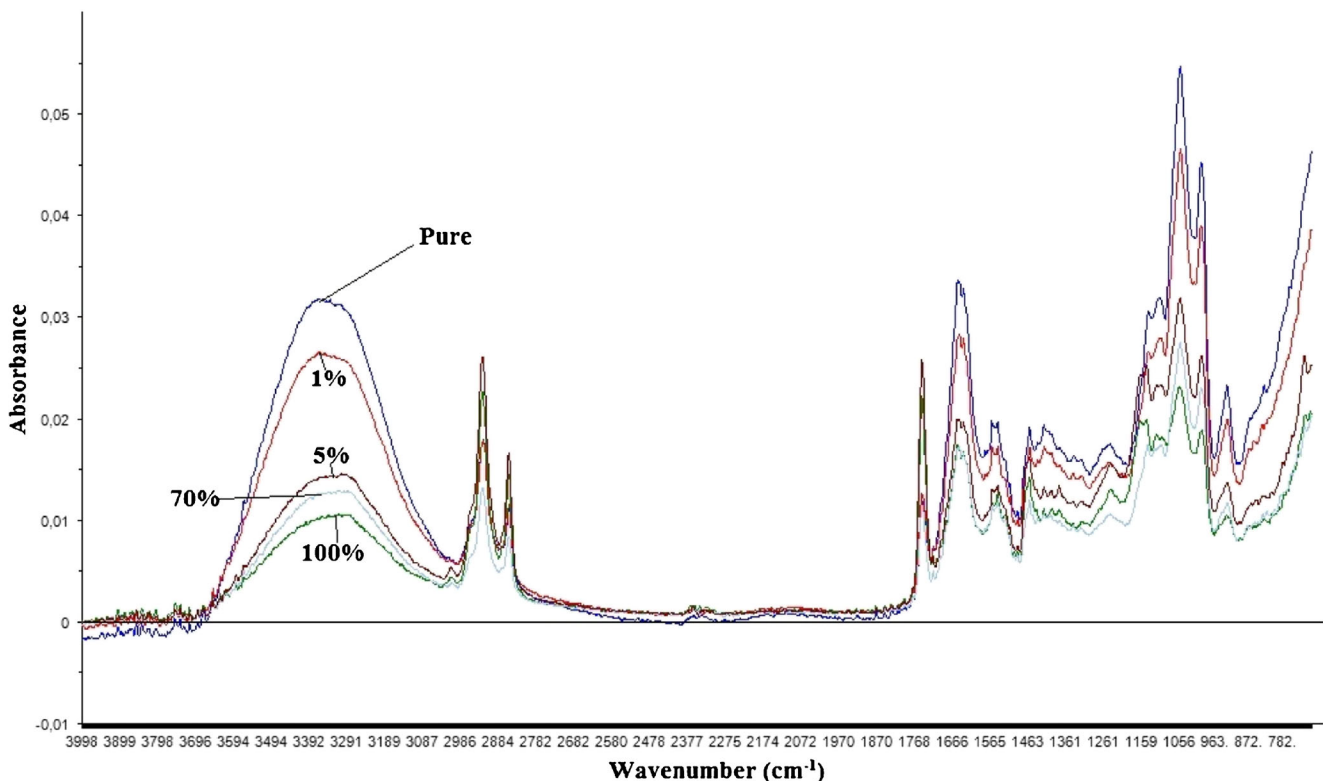
Beef and pork were obtained from different slaughter houses in Jember, Indonesia (Usually the slaughter house for pig is specified for pig only) by taking into account the different feeding of corresponding animals. The materials used for making beef jerky were purchased from local market. All solvents used for analysis were of pro-analytical grade.

### Beef jerky preparation and powdered of the product

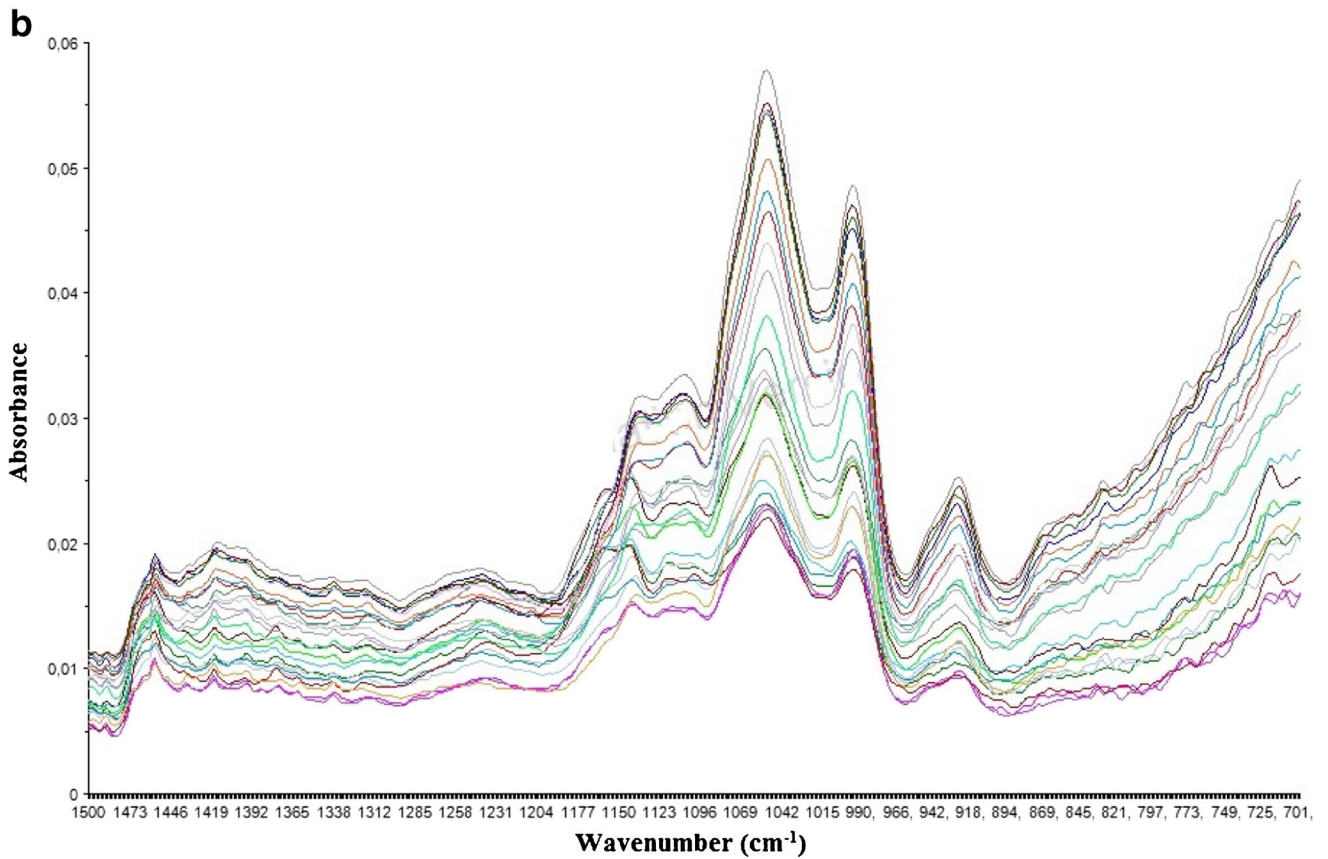
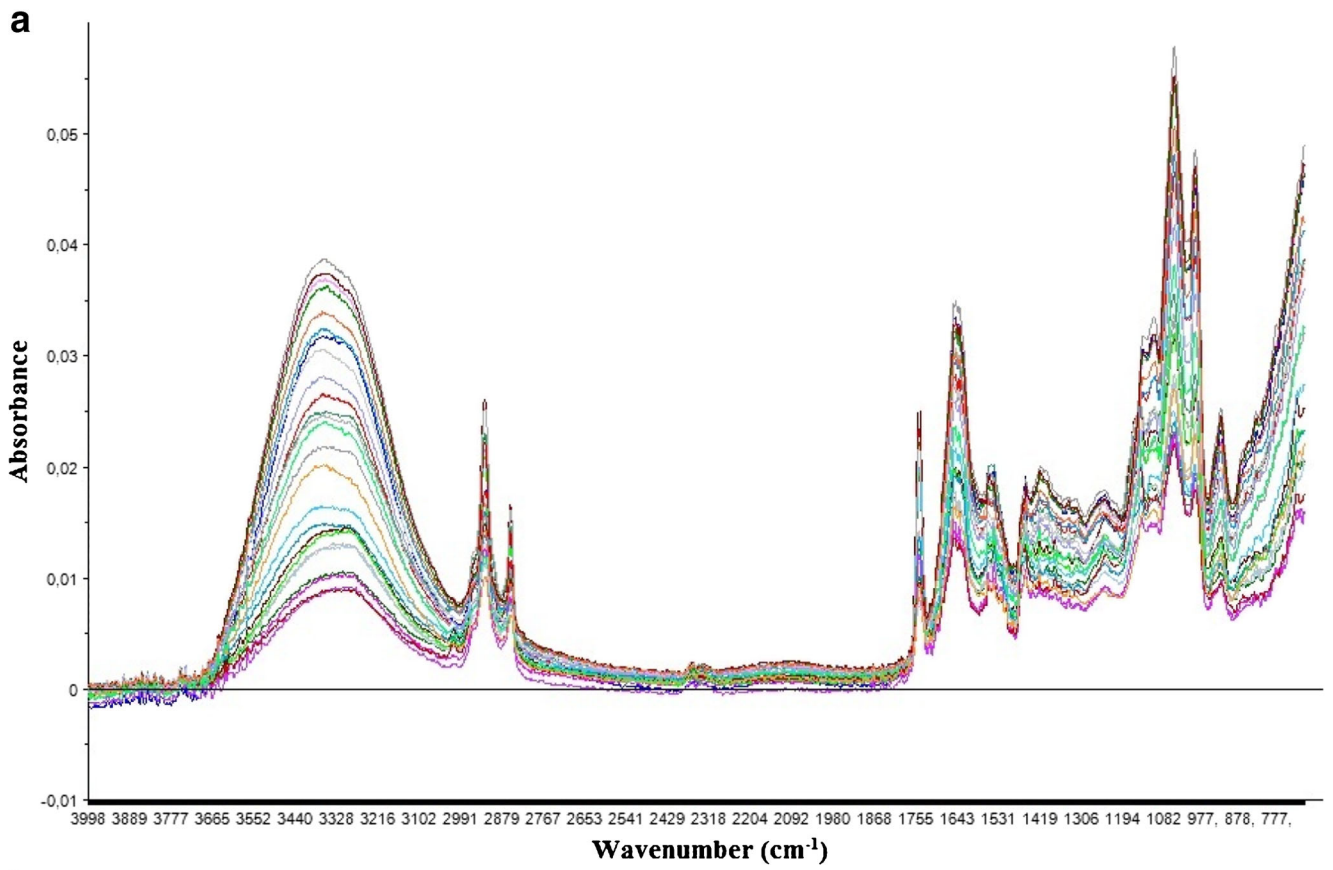
Beef Jerky was prepared by emulsifying 57.5 % of fine meat (beef or pork) with brawn sugar (25 %), salt (5 %) and certain spices (12.5 %), and finally shaping it into jerkys. It is then left for 6 h and dried through oven (75 °C) for 6 h. Jerky was further cut into small pieces and blended for powdered process. Then the powder was sieved into 10 mesh. The powder yielded were further used for FTIR analysis.

### Training set and test set

The training sets was prepared by spiking pork to beef jerky in concentration range of 5.0–80.0 %. Jerky containing of 100 %



**Fig. 2** IR Spektrum of test set, where the pure beef jerky has the highest absorbance intensity, while the lower intensity belong to pork jerky (100 %)



◀ **Fig. 3** IR Spektra of data sets, **a** spectra in the whole region as data set 1; **b** spectra in the fingerprint region ( $1500\text{--}600\text{ cm}^{-1}$ ) as data set 2; **c** spectra in the whole region with treatment as data set 3; and **d** spectra in the fingerprint region with treatment as data set 4

beef and 100 % pork was also made to observe the spectral differentiation. For test set, another series of jerky containing the mixture of pork and beef were prepared (1, 5 & 70 %). The jerky was further subjected to powder. The powder obtained were analyzed using FTIR spectroscopy. The spectral regions where the variations were observed, they were chosen for developing class-model techniques (i.e. Linear Discriminant Analysis/LDA, Soft Independent Modelling of Class Analogy/SIMCA, and Support Vector Machines/SVM).

### FTIR spectroscopy analysis

A dedicated transparent glass container was used; the powdered samples were placed in direct contact with attenuated total reflectance (ATR) crystal (platinum diamond) on a multibounce plate at controlled ambient temperature ( $25\text{ }^{\circ}\text{C}$ ). An Alpha FTIR spectrometer (Bruker Optics, Bellerika, MA) equipped with a detector of deuterated triglycine sulphate (DTGS), a  $\text{CaF}_2$  window, and connected to software of the OPUS software, were used during FTIR data collection. In order to minimize water vapor interference, the instrument was maintained with dehumidifier of silica gel. FTIR spectra were recorded from 32 scans at a resolution of  $4\text{ cm}^{-1}$  at  $4000\text{--}700\text{ cm}^{-1}$ . These spectra were subtracted against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned in situ by scrubbing with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. Cleanliness was verified by collecting a background spectrum and compare to the previous one. These spectra were recorded as the average of the absorbance values at each data point in triplicate. The software *The Unscrambler X 10.2* (Camo Software, Madison, WI) was used for chemometrics analysis of class-models (i.e. LDA, SIMCA, and SVM). For the treated spectra, the original spectra were treated by normalised in order to achieved the lowest spectra became 0 and the highest one became 2. Then the normalised result were corrected with the baseline and smoothed for 13 times.

### Class-modelling techniques

The modelling techniques used in this work were LDA (Linear Discriminant Analysis), SIMCA (soft independent modelling of class analogy), and SVM (Support Vector Machines).

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 performances has been examined on randomly generated test data. This method maximises the ratio of between-class variance to the within-class variance in any particular data set thereby guaranteeing maximal separability (Miller and Miller 2010). In this case, the use of LDA for data classification is applied to classification between pure beef jerkys (pure) and pork adulterated jerky (adulterated).

SIMCA is a very flexible technique since it allows variation in a large number of parameters such as scaling or weighting of the original variables, number of components, expanded or contracted scores range, different weights for the distances from the model in the inner space and in the outer space and confidence level applied. The central feature of this method is the application of principal component analysis (PCA) to the sample category studied (e.g. authentication of product), generally after within-class autoscaling or centring. SIMCA models are defined by the range of the sample scores on a selected number of low-order principal components (PCs) and models. The scores range can be enlarged or reduced, depending mainly on the number of samples, to avoid the possibility of under or over-estimation (Forina et al. 2008).

SVM is supervised learning models with associated learning algorithms that analyze data and recognize patterns, used for classification and regression analysis. Given a set of training examples, each marked as belonging to one of two categories (e.g. pure and adulterated), an SVM training algorithm builds a model that assigns new examples into one category or the other, making it a non-probabilistic binary linear classifier. An SVM model is a representation of the examples as points in space, mapped so that the examples of the separate categories are divided by a clear gap that is as wide as possible. New examples are then mapped into that same space and predicted to belong to a category based on which side of the gap they fall on (Lee et al. 2004).

### Real sample analysis

For real sample analysis, the classification model selected was tested to the real samples of beef jerky collected from supermarket in 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 were compared with ELISA test kit (Xema, Russia).

## Results and discussions

### Training set and IR spectra analysis

Meat powders obtained from the beef or pork jerkys were analysed using FTIR spectroscopy in the mid infrared region

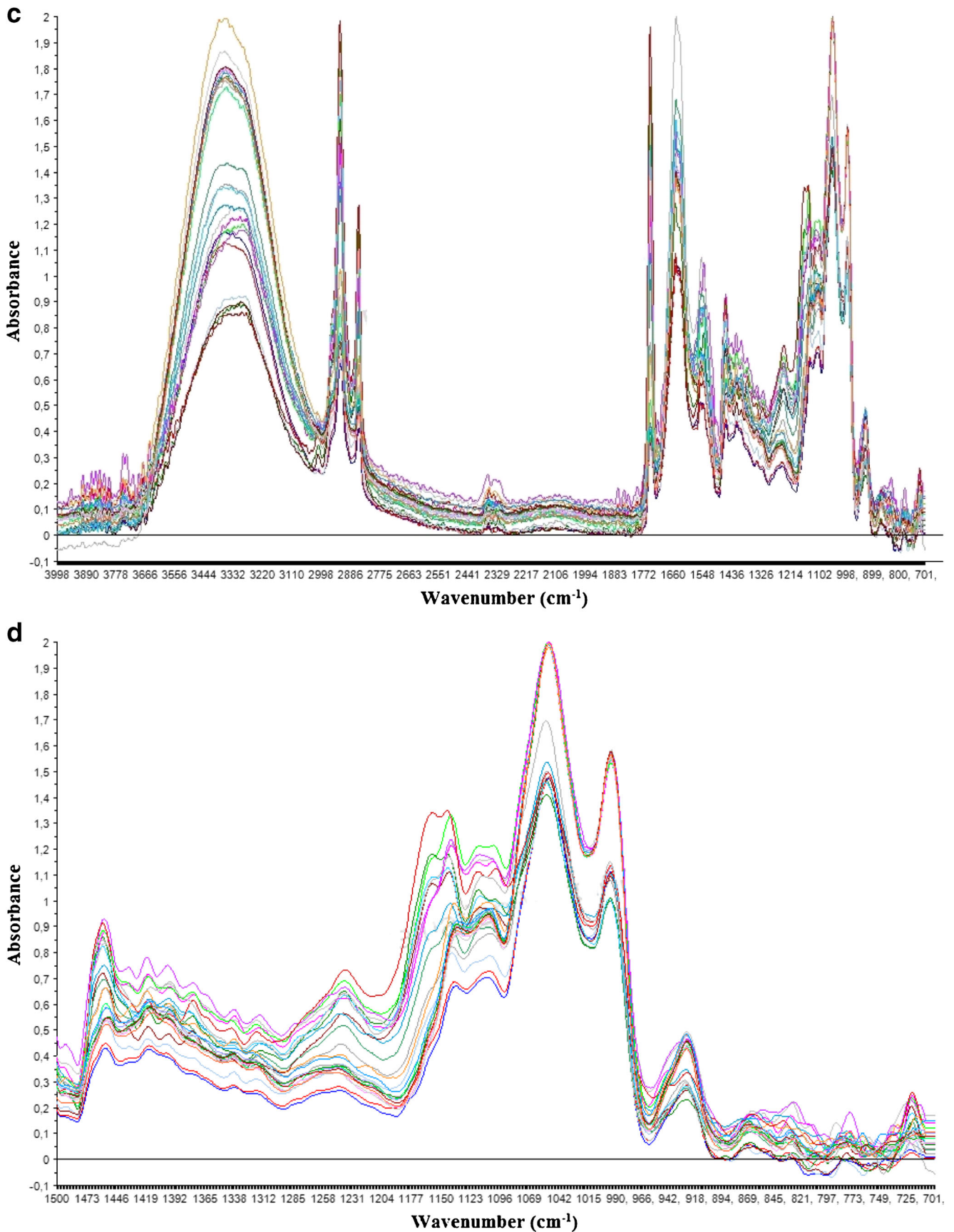


Fig. 3 (continued)

(4000–700  $\text{cm}^{-1}$ ). The advantage of IR spectroscopy for the qualitative analysis originates from the much information contents obtained and the possibility to assign certain absorption bands related to functional groups. Therefore, most of the

peaks and shoulders of the spectrum are attributable to specific functional groups (Bendini et al. 2007).

The composition of the training set used as the sample was consist of 10 pure beef jerky samples and 10 pork

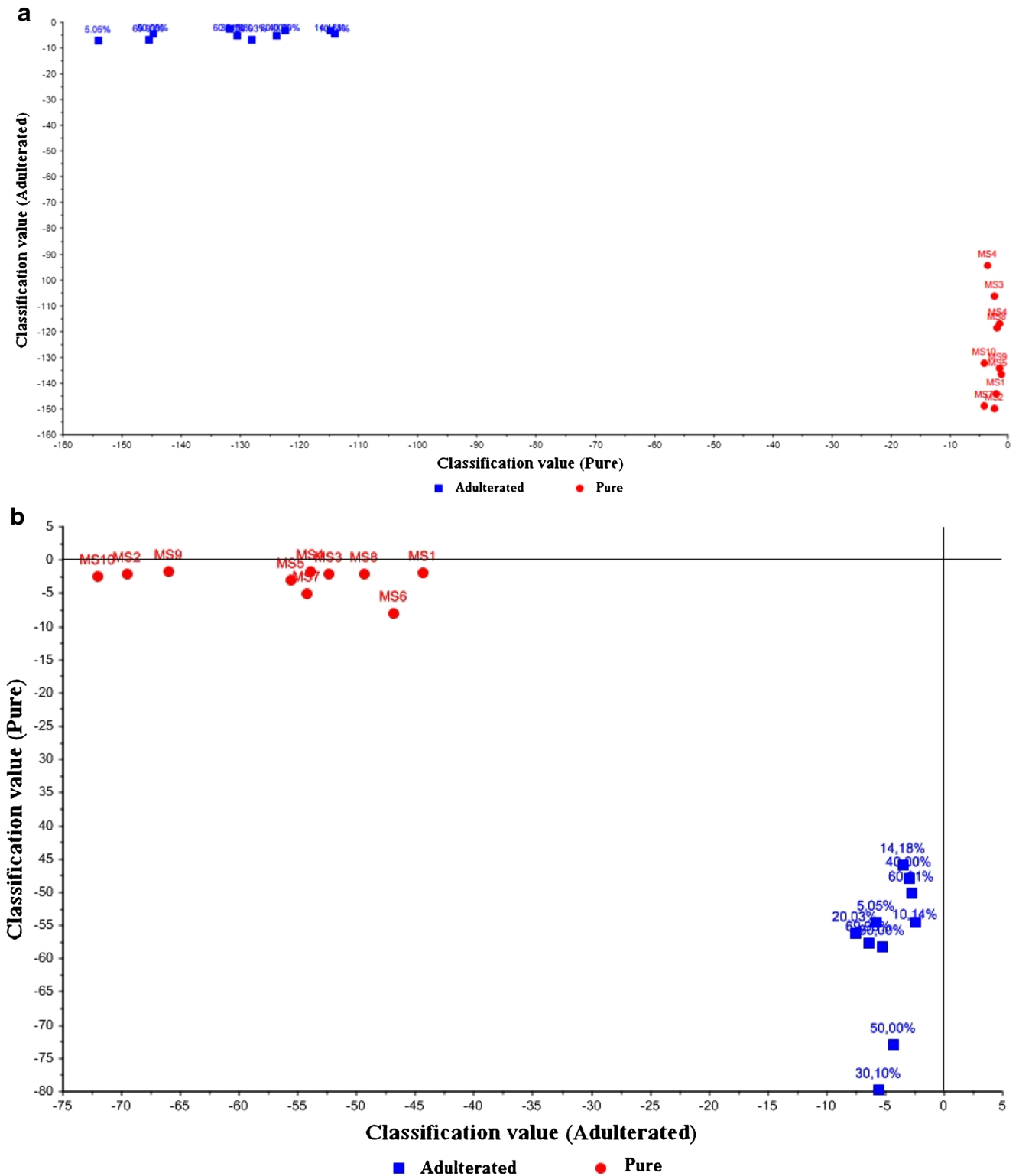


Fig. 4 The LDA class-model of training set; (a) data set 1; (b) data set 2; (c) data set 3; (d) data set 4

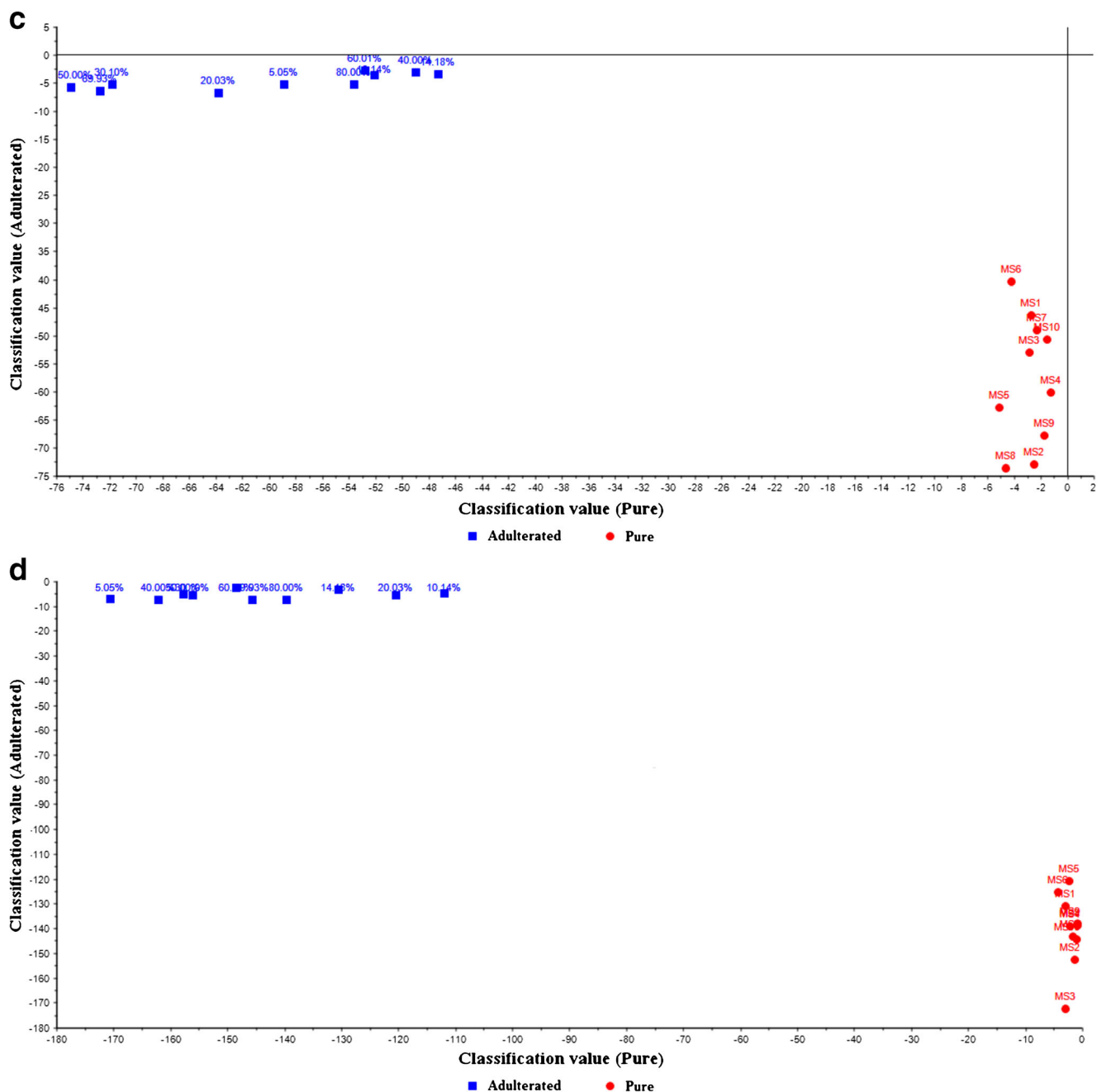


Fig. 4 (continued)

adulterated beef jerky samples, ranging from 5 to 80 % of adulteration as given in Table 1. The representative spectra of jerky powder obtained from the beef and pork adulterated jerky samples as training set are demonstrated in Fig. 1. These spectra look very similar and the difference is only in its intensity. An observation using the naked eyes, the whole range looks very similar because the main component of these meats are similar, i.e. protein. However, due to the fingerprint technique, meaning that there is no two compounds or samples having the same spectra in terms of amount and intensity of peaks, FTIR spectroscopy can be used to

extract a difference among these proteins. However, the identification of functional groups in these proteins through the absorption peaks alone cannot be done with high certainty. Therefore, these spectra were used as prediction of these functional groups. For instance, as it can be observed from the spectra that at the region  $3600\text{--}3200\text{ cm}^{-1}$  could be corresponding to O-H vibration, at the region  $1700\text{--}1600\text{ cm}^{-1}$  could be corresponding to C=O and the amide group for protein (N-H) could be found at the region  $1565\text{--}1520\text{ cm}^{-1}$ . While C-H vibration of the protein could be found at the region  $3000\text{--}2800\text{ cm}^{-1}$  and C=O at the



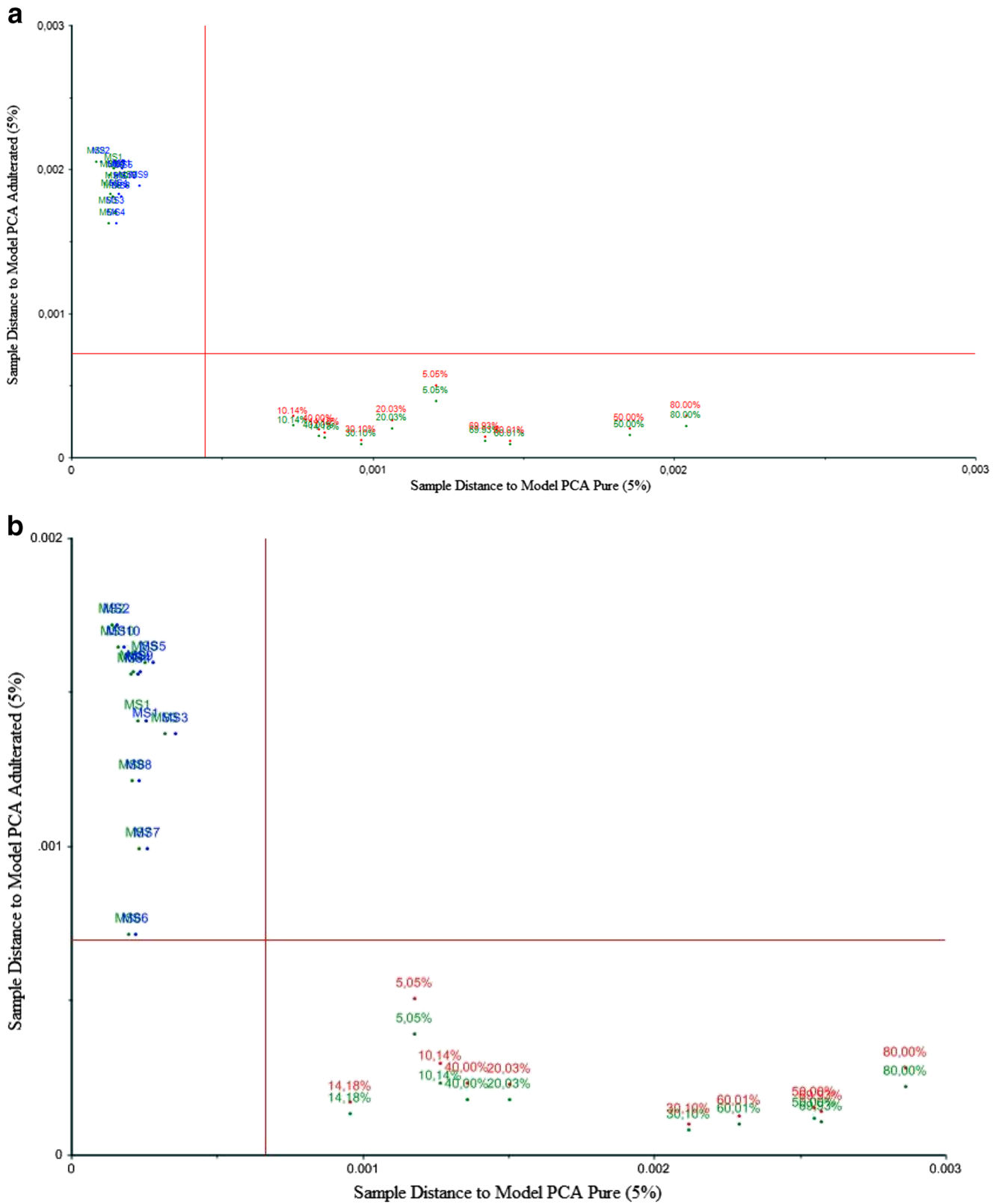
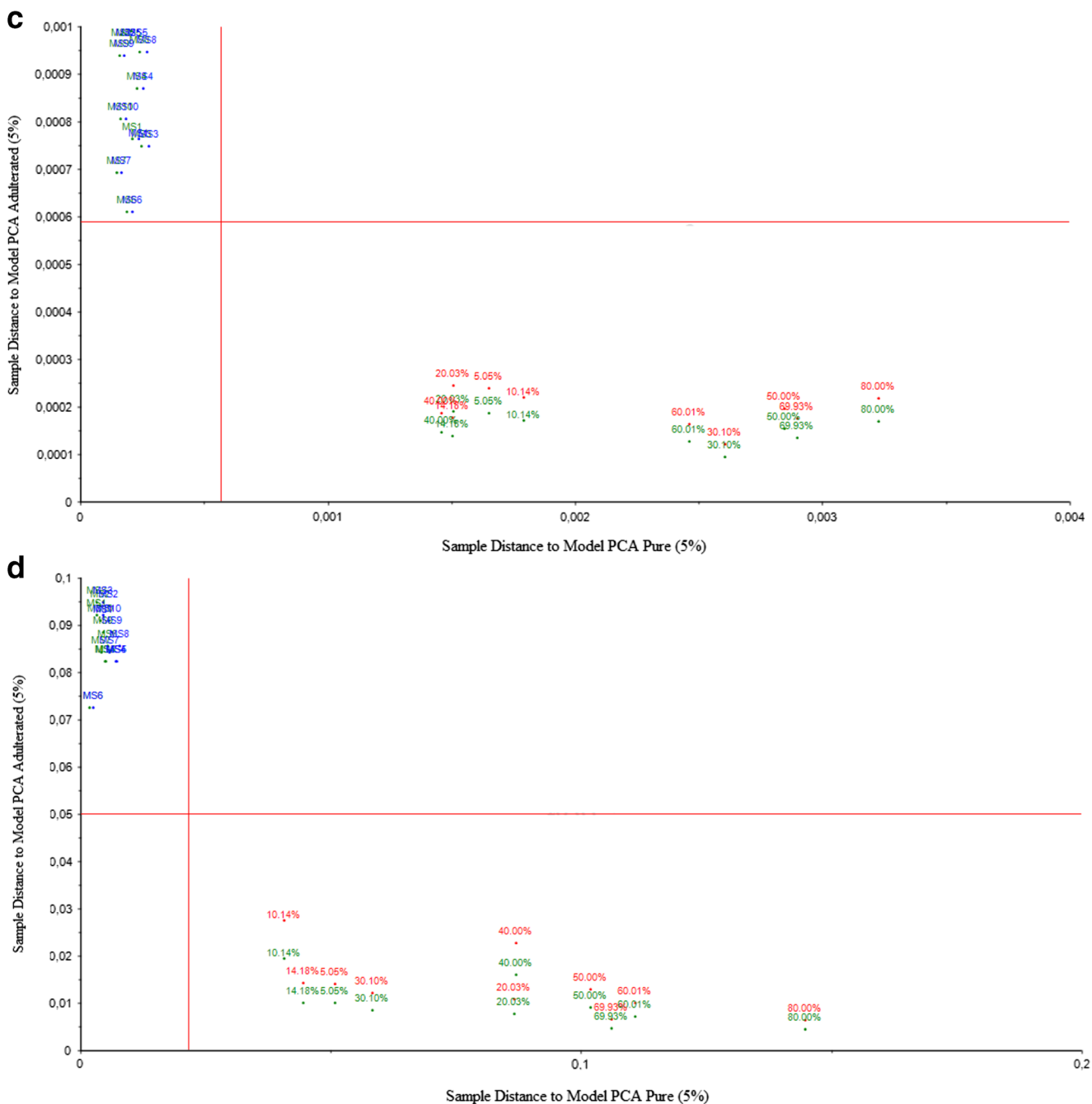


Fig. 5 The SIMCA class-model for training set; **a** data set 1; **b** data set 2; **c** data set 3; **d** data set 4

region 1745–1725  $\text{cm}^{-1}$ . The =C-H of ester group, and alkene of fat could be found at 996  $\text{cm}^{-1}$  (Guillen and

Cabo 1997; Stuart 2004; Vlachos et al. 2006). Hence, these frequencies, which FTIR spectra variations were observed,



**Fig. 5** (continued)

are used as a basis approach for choosing the spectral regions in the classification of pork in beef jerky samples.

### Test set

In order to test, the reliability of the training set used, the class-models were test with the test set. The composition of the test set used as the sample was consist of 5 samples, and the composition is given in Table 2. The representative spectra of the sample obtained from the test set are given in Fig. 2. These spectra also look very similar as well, and the different

only in absorbance intensity according to the degree of pork adulteration in beef jerky. Therefore, these frequencies, which FTIR spectra variations were observed, they were used as a basis approach for the classification between pure and pork adulterated beef jerky samples.

### Training set class-models

Before the chemometric models were constructed, the data were grouped into four (4) data sets, i.e. (a) spectra in the whole region as data set 1; (b) spectra in the fingerprint region

(1500–600 cm<sup>-1</sup>) as data set 2; (c) spectra in the whole region with treatment as data set 3; and (d) spectra in the fingerprint region with treatment as data set 4, as given in Fig. 3. In this regards, the classification of spectra into various data sets as whole region and fingerprint region could increase chemometric analysis results (Gallardo-Velázquez et al. 2009). While in this case, the spectral treatment could reduce false classification due to noise and shifting in the spectral data (Berrueta et al. 2007).

The results of the class-model of LDA as model for classification between pure and pork adulterated beef jerky for data set 1 to 4 of the training set is given in Fig. 4 as graphical classification, while the class-model of SIMCA is given in Fig. 5, respectively. The SVM model only produces the classification predicted table as given in Table 3. Figures 4 and 5 show that the training set of all data sets (data set 1 to 4) given have classification in 100 % accuracy of both LDA and SIMCA class-models, while Table 3 show that the training of both data set 3 and 4 have classification accuracy of 100 %, while data sets 1 and 2 have accuracy of classification of 90 % and 80 % respectively.

**Evaluation of class-models**

In order to test the reliability of the chemometric model constructed, the class-models were evaluated using the test set. Here, the test set can be used as simulated samples to evaluate the accuracy of the constructed class-models (LDA, SIMCA & SVM) for classification of each data in test set, between pure and pork adulterated beef jerkys classification.

The summary of the ability of the models constructed (LDA, SIMCA and SVM) based on the training set and its prediction (%) using test set is given in Table 4. Based on Table 4, it can be concluded that the best model constructed for classification between pure and pork adulterated beef jerkys is LDA model using data set 1 (whole range of spectra without treatment). Since, this LDA model could classified and predicted the sample in 100 % accuracy of classification. This mean that the LDA model could classify all the data in the training set (20 data samples) 100 % correct in their classification and have ability to predict all of the data in the test set (5 data samples) 100 % correct in their classification as well (Stanimirova et al. 2010). In other word, if the class-model could classify and predict classification data samples less than 100 % accuracy, meaning the model could not classify the training set data 100 % correct and could not predict the test set data 100 % correct as well. Thus, as a consequence the class-model constructed consists errors, like in the case of SIMCA and SVM for all data set used. Therefore, the LDA model using data set 1 (whole range of spectra without treatment) was used for further application using real beef jerky samples available in the market.

**Table 3** The SVM class-model of training set

Data Set	No.	Pork concentration (w/w%)	Prediction
1	1	10.14 %	Adulterated
	2	14.18 %	Adulterated
	3	20.03 %	Adulterated
	4	30.10 %	Adulterated
	5	40.00 %	Adulterated
	6	5.05 %	Adulterated
	7	50,00 %	Pure
	8	60,01 %	Adulterated
	9	69,93 %	Adulterated
	10	80,00 %	Pure
	11	0 %	Pure
	12	0 %	Pure
	13	0 %	Pure
	14	0 %	Pure
	15	0 %	Pure
	16	0 %	Pure
	17	0 %	Pure
	18	0 %	Pure
	19	0 %	Pure
	20	0 %	Pure
% Classification =90 %			
2	1	10.14 %	Adulterated
	2	14.18 %	Adulterated
	3	20.03 %	Adulterated
	4	30.10 %	Adulterated
	5	40.00 %	Adulterated
	6	5.05 %	Adulterated
	7	50,00 %	Pure
	8	60,01 %	Adulterated
	9	69,93 %	Adulterated
	10	80,00 %	Pure
	11	0 %	Pure
	12	0 %	Pure
	13	0 %	Pure
	14	0 %	Pure
	15	0 %	Pure
	16	0 %	Adulterated
	17	0 %	Adulterated
	18	0 %	Pure
	19	0 %	Pure
	20	0 %	Pure
% Classification =80 %			
3	1	10.14 %	Adulterated
	2	14.18 %	Adulterated
	3	20.03 %	Adulterated
	4	30.10 %	Adulterated
	5	40.00 %	Adulterated
	6	5.05 %	Adulterated
	7	50,00 %	Adulterated

**Table 3** (continued)

Data Set	No.	Pork concentration (w/w%)	Prediction
	8	60,01 %	Adulterated
	9	69,93 %	Adulterated
	10	80,00 %	Adulterated
	11	0 %	Pure
	12	0 %	Pure
	13	0 %	Pure
	14	0 %	Pure
	15	0 %	Pure
	16	0 %	Pure
	17	0 %	Pure
	18	0 %	Pure
	19	0 %	Pure
	20	0 %	Pure
	% Classification =100 %		
4	1	10.14 %	Adulterated
	2	14.18 %	Adulterated
	3	20.03 %	Adulterated
	4	30.10 %	Adulterated
	5	40.00 %	Adulterated
	6	5.05 %	Adulterated
	7	50,00 %	Adulterated
	8	60,01 %	Adulterated
	9	69,93 %	Adulterated
	10	80,00 %	Adulterated
	11	0 %	Pure
	12	0 %	Pure
	13	0 %	Pure
	14	0 %	Pure
	15	0 %	Pure
	16	0 %	Pure
	17	0 %	Pure
	18	0 %	Pure
	19	0 %	Pure
	20	0 %	Pure
	% Classification =100 %		

**Table 5** Results summary of the FTIR-LDA classification model Vs. ELISA method

Sample	Analysis classification of beef jerky			
	FTIR-LDA classification model		ELISA	
	Pure	Pork adulterated	Pure	Pork adulterated
1	√		√	
2	√		√	
3	√		√	
4	√		√	
5	√		√	
6	√		√	
7	√		√	
8	√		√	
9	√		√	
10	√		√	

### Application for the real samples

The LDA class-model selected using data set 1 was applied toward the real samples, which consist 10 samples with official halal logo (sample no. 1–2), 2 samples with unofficial halal logo (sample no. 3–4) and 6 samples without halal logo (sample no.5–10). All of the beef jerky sample used were purchased from supermarket in Jember. The real sample of beef jerky have also been tested using ELISA method as comparison, and the results have been summarised in Table 5. Table 5 shows that the LDA class-model developed was in good agreement with the ELISA method. Based on this result, it can be stated that all beef jerky samples tested using the LDA class-model based on data set 1 (whole range of spectra without treatment) can be classified as pure beef jerky. It can be concluded that all beef jerky tested were classified as halal and can be consumed according to Islamic law. Therefore, the proposed method based on IR spectra and LDA class-model as a rapid analytical method for the analysis of pork adulteration in beef jerky as halal authentication has been achieved.

**Table 4** The percentage of classification and prediction of each classification model using LDA, SIMCA and SVM

Data set	LDA		SIMCA		SVM	
	% Classification	% Prediction	% Classification	% Prediction	% Classification	% Prediction
1	100 %	100 %	100 %	60 %	90 %	80 %
2	100 %	80 %	100 %	60 %	80 %	80 %
3	100 %	80 %	100 %	20 %	100 %	80 %
4	100 %	80 %	100 %	0 %	100 %	80 %

% classification = classification ability of the model towards *training set*

% prediction = classification prediction ability of the model towards *test set*

## Conclusion

The adulteration of beef jerky with pork can be analysed by means of IR spectra coupled with the class-model techniques (i.e. LDA, SIMCA, and SVM). The LDA model selected as the best class-model, can be used to classify between pure and pork adulterated beef jerky using whole region spectra (4000–700  $\text{cm}^{-1}$ ) without treatment. The LDA class-model has also been successfully tested to the real beef jerky samples marketed in Jember, and the results showed in good agreement with the ELISA method. Thus, the potential of FTIR spectroscopy and chemometrics as a rapid analytical tool for the analysis of pork adulteration in beef jerky 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.

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## Compliance with ethics requirements

**Conflicts of interest statement** All authors declares that they have no conflict of interest.

**Author contributions statement** First author contributed for whole project starting from experimental work to manuscript preparation.

Second author contributed to experimental work.

Third author contributed to the analysis of data.

Fourth author contributed to evaluation of data analysis and the manuscript.

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