

# Chapter 5

## Application of Spectroscopic and Chromatographic Methods for the Analysis of Non-halal Meats in Food Products



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**Abstract** Meat is one of the major food groups which is frequently adulterated for economic reasons. In line with halal certification, the presence of non-halal meats in food products must be identified. Mislabelling and undeclaring meat types are typically seen in meat-based products such as meatballs, burgers, sausages and Salami. Therefore, the application of analytical methods for the detection, identification, and confirmation of non-halal meats is a must. This chapter highlighted the application of spectroscopic and chromatographic-based methods combined with several chemometrics for the identification and confirmation of non-halal meats in food products. Spectroscopy offered more accelerating methods with higher accuracy rates throughout the screening process for non-halal meats. Further confirmation can be done using chromatography by identifying specific markers present in analyzed non-halal meats.

**Keywords** Halal authentication · Pork · Dog meat · Chemometrics · Molecular spectroscopy

### 5.1 Introduction

Meat is considered one of the most commonly consumed foods worldwide due to the nutritional composition found in meats, especially protein. Meats contain essential healthy nutrients and are an excellent source of protein (Hassoun et al., 2020). From an Islamic perspective, meats could be classified as halal-meats that are allowed to

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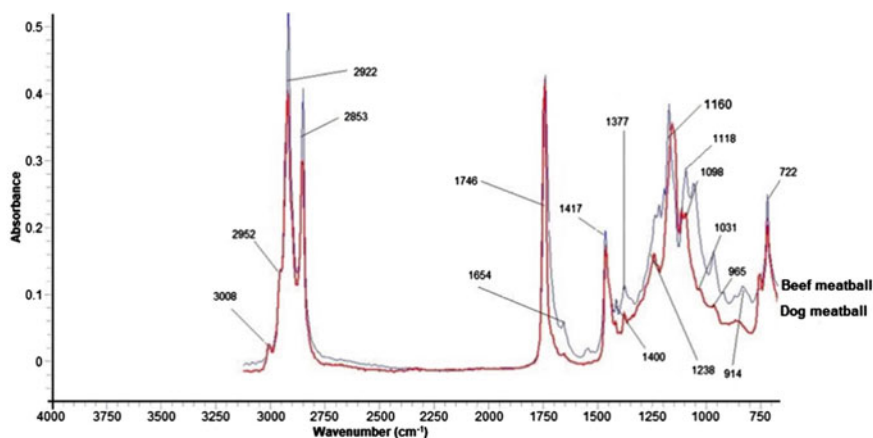
be consumed and non-halal meats that are prohibited to be consumed according to Syariah law (Islamic jurisprudence) (Rohman & Windarsih, 2020).

With the increased awareness among the Muslim population to only consume halal and *tayyib* (*good or pure*) foods, the demand for halal food products also increased, emplacing a huge responsibility on the government, jurisprudence and manufacturers to assure the halalness of food products through halal certifications (Martuscelli et al., 2020). Indonesia has the regulation (Indonesian Act) No. 33 established in 2014 that mandates a halal certification for all halal products which are declared halal. Indeed, meat is one of most essential food components in the food industry and due to the big discrepancy between halal and non-halal meats, some unethical producers intentionally replace halal with non-halal meat (Nakyinsige et al., 2012).

At present, it is estimated that the Muslim population will increase from 1.8 billion in 2014 to 2.2 billion by 2030, with an approximate increased growth of 26.4%. The Muslim population occupies a quarter part of the world; therefore, it is not surprising if the Halal market exhibited a lucrative and significant impact on international business (Adiarni & Fortunella, 2018). However, there are several issues regarding Halal as non-halal meats either because of their source (such as pork) or from the way the animals were slaughtered. Disputes regarding the halal status of animal sources typically arise from thoughts' scholar (*madzhab*) to be followed by Muslim communities (Rohman et al., 2020), while debates on the halal status of various slaughtering methods arise due to the possibility that animals' slaughtering processes did not meet the Halal requirements determined by the Syariah law (Ali et al., 2020).

## 5.2 Meat's Authentication Using Spectroscopic and Chromatographic-Based Methods

In recent years, consumers have become more concerned about the quality, halalness, and safety of meat-based food products, especially the traceability and authenticity of meat sources (Hassoun et al., 2020). The analytical methods for meat-species identification and detection of adulteration are always needed for quality control and the safety of consumers. The Gold standard method used for halal meat authentication is a type of DNA-based methods, especially analytical methods applying polymerase chain reaction (PCR) with its development and revolution, including real-time PCR using primer species-specific, TaqMan probe and multiplex (Ali et al., 2014; Erwanto et al., 2018a, 2018b; Fajardo et al., 2010; Kumar et al., 2015; Rodríguez-Ramírez et al., 2011). However, PCR techniques involve more complex steps such as starting primer selection, denaturation of DNA, primer annealing and amplification. Therefore, a range of uncomplicated and quick methods were developed and used for analyzing non-halal meats in food products. Some reviews on the application of molecular spectroscopic (Esteki, Shahsavari, et al., 2018; Li et al., 2019; Rodríguez-Saona & Allendorf, 2011) and chromatographic methods (Abbas et al.,



**Fig. 5.1** Typical FTIR spectra of lipid components extracted from meats (beef and dog meat) in meatball samples

2018; Bosque-Sendra et al., 2012; Esteki, Simal-Gandara, et al., 2018) for food authentication and traceability have existed. In this chapter, two groups of methods (spectroscopy and chromatography) were critically assessed for the authentication analysis of non-halal meats in fresh and meat-based food products.

Among the molecular spectroscopic-based methods, Fourier transform infrared (FTIR) spectroscopy is the most applied technique for the analysis of non-halal meats either in fresh meats or meats in food-based products. The analysis of raw meats and meat-based foods is done by extracting the lipid fractions of meats using several extraction methods and then subjecting them to FTIR spectral measurement (Rohman, 2019). Typical FTIR spectra of lipid components extracted from meats are depicted in Fig. 5.1. The intensity is represented by absorbance values (not in transmittance mode) because absorbance can contain quantitative information according to Lambert–Beer law. Each peak and shoulder with specific wavenumbers in FTIR spectra represented functional groups present in lipids which can be correlated with compounds or a group of compounds composing the lipid. The main components of lipids extracted from meats which is represented by functional groups of methyl- ( $\text{CH}_3$ -) present in wavenumbers ( $1/\lambda$ ) of  $2953\text{ cm}^{-1}$  (str asymmetric),  $2875\text{ cm}^{-1}$  (str symmetric),  $1376\text{ cm}^{-1}$  (ben), methylene ( $-\text{CH}_2-$ ) at  $1/\lambda$   $2922\text{ cm}^{-1}$  (str asymmetric),  $2853\text{ cm}^{-1}$  (str symmetric),  $1462\text{ cm}^{-1}$  (ben), carbonyl- ( $\text{C}=\text{O}$ ) at  $1741\text{ cm}^{-1}$  (str)  $1654\text{ cm}^{-1}$  corresponding to cis  $\text{C}=\text{C}$  str,  $1417\text{ cm}^{-1}$  from  $=\text{C}-\text{H}$  cis disubstituted olefins str, and  $\text{C}-\text{O}$  (ether) at  $1/\lambda$   $1117$  and  $1098\text{ cm}^{-1}$ ,  $965\text{ cm}^{-1}$  due to  $-\text{HC}=\text{CH}$ -(trans) in which str stands for stretching, while ben stands for bending vibration (Lerma-García et al., 2010).

The peaks and shoulders in FTIR spectra are rather complex, and they are used as variables for extracting the qualitative and quantitative information regarding the presence and the quantity of certain meats in food products. Therefore, data management using chemometrics was normally used (Nunes, 2014). Table 5.1 compiled the

**Table 5.1** The application of different spectroscopic techniques used for the analysis of pork fresh meat or in meat-based food products

Non-halal meat	Methods	Issues	Chemometrics	Results	References
Pork	Visible and NIR spectroscopy using wavelength 400–2500 nm (vis) and 1/ $\lambda$ . 25,000–4000 $\text{cm}^{-1}$	Analysis of pork in fresh and frozen-thawed minced beef	LDA and PLS-DA for discrimination and PLSR for quantitative analysis of pork	LDA and PLS-DA could discriminate minced beef and that adulterated with pork with 100% accuracy. PLSR could quantify pork with $R^2$ of 0.94–0.96 and SEP of 2.08–5.39% (w/w)	(Morsy & Sun, 2013)
Pork	On-site NIR spectrometer at 1/ $\lambda$ . 6028–5480 $\text{cm}^{-1}$	Analysis of pork adulteration in sausages	PCA and SVM, spectra were preprocessed using 2nd derivative and SNV	PCA and SVM offered the correct classification between authentic and adulterated pork	(Schmutzler et al., 2015)
Pork	NIR spectra at a wavelength of 1000–2500 nm	Quantitative Analysis of pork as an adulterant in beef and chicken meatballs	PLSR for quantification	PLSR could facilitate the prediction of pork in beef meatballs with $R^2$ of 0.88, SECV of 3.45% and bias of 0.14%. For pork in chicken meatballs, $R^2$ of 0.83, SECV of 4.18%, and a bias of 0.22%, respectively	(Vichasilp & Pongchompu, 2014)
Pork	Vis/NIR spectra at 900–1700 nm	Quantitative analysis of pork in beef	PLSR for quantification	PLSR could successfully quantify pork in a binary mixture with beef with high $R^2$ and low RPD	(Rady & Adedeji, 2018)

(continued)

Table 5.1 (continued)

Non-halal meat	Methods	Issues	Chemometrics	Results	References
Pork	Visible-NIR hyperspectral imaging spectroscopy at wavelength 400–1000 nm	Analysis of pork lung adulteration in ground pork	PLSR for prediction of ground pork	PLSR using absorbances at 11 wavelengths based on loading plot (466, 480, 531, 562, 570, 617, 661, 729, 750, 816 and 982 nm) could predict the levels of ground pork with $R^2$ of 0.98, RMSEP of 4.47%, RPD of 7.16, and LOD of 7.58%	(Jiang et al., 2021)
Pork	NIR spectra at 700–850	Analysis of pork as adulterant in beef meatballs	LDA for classification and PLSR for quantification	LDA using the first derivative spectra could accurately classify beef meatballs and those adulterated with pork with an accuracy level of 100%. The prediction results of pork levels using NIR spectroscopy-PLS was in agreement with those using the immune-chromatographic method	(Kuswandi, Cendekiawan, et al., 2015)

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Table 5.1 (continued)

Non-halal meat	Methods	Issues	Chemometrics	Results	References
Pork	FTIR spectroscopy at mid-infrared region at $1/\lambda$ 4000–650 $\text{cm}^{-1}$	Analysis of pork as an adulterant in beef jerky	LDA, SIMCA, SVM	Comparing SIMCA and SVM, LDA offered the best model with an accuracy level of 100%. The LDA model was also in good agreement with the ELISA method	(Kuswandi, Putri, et al., 2015)
Pork	FTIR spectroscopy at $1/\lambda$ 4000–650 $\text{cm}^{-1}$	Rapid discrimination of pork in non-Halal Chinese ham sausages	PLS-DA and LS-SVM. Spectra were pretreated with SNV	PLS-DA and LS-SVM could classify halal and non-halal sausages containing pork. The sensitivity and specificity were 0.913 and 0.929 (PLS-DA) as well as 0.957 and 0.929 (LS-SVM)	(Xu et al., 2012)
Pork	FTIR spectra using $1/\lambda$ 1018–1284 $\text{cm}^{-1}$ and 1200–1000 $\text{cm}^{-1}$	Analysis of pork in meatball broth	PCA (classification) and PLSR for quantification	PLSR using absorbances at 1018–1284 $\text{cm}^{-1}$ could predict the fats extracted from pork meatballs. PCA using absorbances at 1200–1000 $\text{cm}^{-1}$ could classify lipid components extracted from pork and beef meatballs	(Kurniawati et al., 2014)

(continued)

Table 5.1 (continued)

Non-halal meat	Methods	Issues	Chemometrics	Results	References
Pork	FTIR spectra at 1200–1000 $\text{cm}^{-1}$	Analysis of beef meatballs adulterated with pork	PLSR for quantification	PLSR using absorbance values at 1200–1000 $\text{cm}^{-1}$ could predict pork contents with $R^2$ value of 0.997 and SEC of 0.04%	(Rohman et al., 2017)
Pork	FTIR spectroscopy	Quantitative analysis of pork in the mixture with camel and buffalo meats	PLSR for quantification	The relationship between the levels of pork in camel and buffalo meats and absorbance ratio ( $R_{1,464/1,448 \text{ cm}^{-1}}$ ) offered $R^2 > 0.99$ with acceptable errors	(Lamyaa, 2013)
Pork	FTIR spectroscopy	Identification of pork in the mixture with beef and mutton	PLS-DA and SVM	PLS-DA and SVM using FTIR spectra pretreated with normalization could discriminate beef/ mutton and that adulterated with pork with 100% accuracy	(Yang et al., 2018)
Pork	On-site NIR spectroscopy	Analysis of pork in a binary mixture of pork-beef, pork-chicken and ternary mixture of pork- beef-chicken	PLSR and SVR for quantification	Meats in binary and ternary mixtures could be quantified using PLSR and SVR with acceptable recovery (high $R^2$ ) and precision (low errors)	(Silva et al., 2020)
Wild boar meat (WBM)	FTIR spectroscopy at 1250–1000 $\text{cm}^{-1}$	Analysis of WBM in beef meatballs	PCA for classification and PLSR for quantification	PCA could classify beef meatballs and those adulterated with WBM. The levels of WBM could be predicted with acceptable accuracy and precision	(Guntarti et al., 2015)

(continued)

Table 5.1 (continued)

Non-halal meat	Methods	Issues	Chemometrics	Results	References
WBM	FTIR spectroscopy at 1250–1000 $\text{cm}^{-1}$	Analysis of WBM in beef meatballs	PCA for classification and PLSR for quantification	The equation obtained equaled the predicted value + 0.994 $\times$ actual value + 0.334; with $R^2$ of 0.998, RMSEC of 1.22% and RMSECV of 2.68%. PCA could classify WBM and beef meatballs	(Sari & Guntarti, 2018)

$1/\lambda$  = wavenumbers; LDA = Linear Discriminant Analysis; SIMCA = soft independent modelling of class analogy; SVM = Support Vector Machines; LS-SVM = least squares support vector machine; SEC = standard error of calibration; SEP = standard error of prediction; RPD = relative percentage difference



application of different spectroscopic techniques used for the analysis of pork fresh meat or in meat-based food products.

## 5.3 Analysis of Non-halal Meats in Fresh and Meat-Based Food Products

There are some non-halal meats that are not allowed to be consumed by Muslim communities, namely, pork (pig meat), wild boar meat (WBM), dog meat (DM), monkey meat, cat meat, snake meat, as well as meats from some amphibians and other animals which live in the water and on the ground such as frogs and crocodiles (Ceranac & Bozinovic, 2009).

### 5.3.1 Analysis of Pork in Fresh and Meat-Based Food

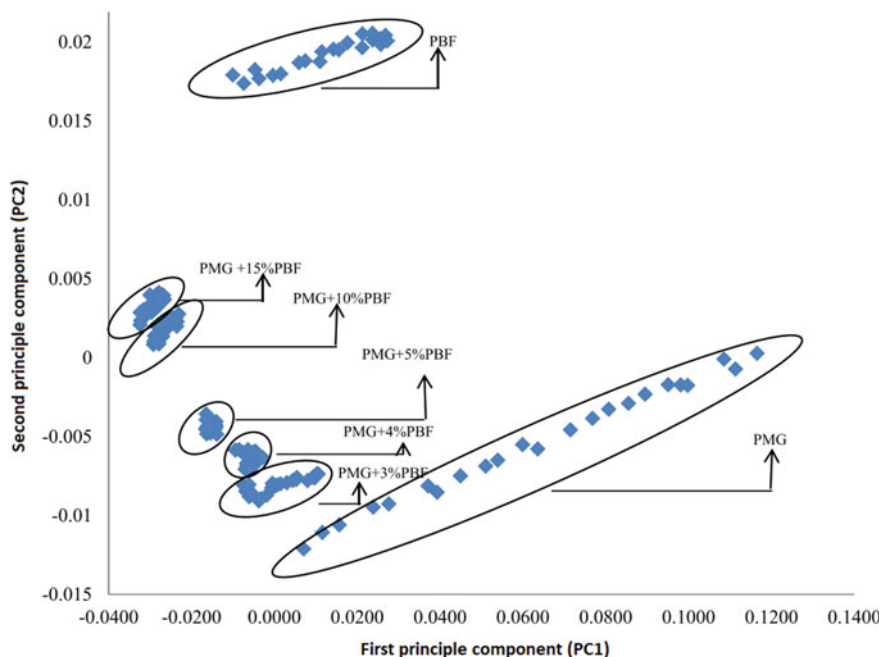
Pork is the most used non-halal meat in food products, and the analytical methods intended for detecting pork in raw components in the mixture with other meats and in meat-based food products such as hamburger, meatballs, and sausages are widely reported. Near-infrared (NIR), mid-infrared (MIR) spectroscopy, gas chromatography and liquid chromatography in combination with some chemometrics techniques have been validated and applied in the analysis of pork for halal authentication. MIR spectra combined with PLS-Kernel have been successfully used for quantifying pork in beef with a limit of detection reaching up to 1.4% wt/wt. The chemometrics of the PLS-Kernel algorithm exhibited good performance results for treating many variables in complex spectral data due to the lower memory storage needed with fewer computation cycles. The absorbance values at wavenumbers of 1900–900  $\text{cm}^{-1}$  were used as variables while preparing the calibration curve for the relationship between actual values and MIR-PLS Kernel predicted values, resulting in the statistical performance of  $R^2$  of 0.9994 and a relative error of prediction (REP) of 3.7%. High  $R^2$  and low REP indicated that MIR spectroscopy combined with PLS-Kernel provided an accurate and precise technique for quantifying pork (non-halal meat) in beef (halal meat) for halal authentication purposes (Abu-Ghoush et al., 2017).

Fourier transform near-infrared spectroscopy (FT-NIR) used in combination with chemometrics of PCA, Partial Least-Squares Discriminant Analysis (PLS-DA) and PLSR has been currently used for the analysis of pork in other meats (lamb, chicken, mutton, beef, camel). PCA was utilized for exhibiting the similarities and differences of pork and other types of meat. PLS-DA was used for the discrimination of pork and other types of meats, while the multivariate calibration of PLSR was applied for the prediction of pork levels in the mixture with other meats. This study involved a large number of samples (>5900 samples from different countries) scanned at

wavenumbers of 10,000–4000  $\text{cm}^{-1}$ . PCA using full NIR spectra that resulted in PC-1 and PC-2 explaining 87 and 8% of the total spectral variability. From PC1 and PC2 curves, it is clear that PCA revealed a complete segregation between pork and other types of meat. All pork samples were grouped in two different categories corresponding to fresh pork and treated pork (dried and smoked pork samples). PLS-DA could distinguish pork from other meats successfully where the lowest level of pork that could be detected is 10%. The prediction of pork levels in the mixture with other meats was optimized using full NIR spectra preprocessed with standard normal variate (SNV) and unit vector normalization transformation. This better model showed a good correlation between actual values and FT-NIR predicted values with  $R^2$  of 0.9774, RMSECV of 1.08% and RMSEP of 1.835%. From these results, it is evident that the combination of NIR spectra and chemometrics of PCA, PLS-DA and PLSR is an effective mechanism for the authentication analysis of distinguishing meats from non-halal meat containing pork (Mabood et al., 2020).

Infrared spectroscopy in mid-region (MIR) combined with chemometrics of PCA, SIMCA and PLS has statistically reported that fats extracted from pork (pig meat) are used as an adulterant in pure ghee (heat clarified milk fat) either qualitatively or quantitatively. Optimizing wavenumbers is capable of providing a good classification using PCA and SIMCA. The combined wavenumbers region of 3030–2785, 1786–1680, and 1490–919  $\text{cm}^{-1}$  which exhibit strong intensities (absorbances) were utilized for these analytical tasks. SIMCA, using this combined region could classify lard, pure ghee and lard mixed with ghee with efficiency of 100%. PCA, using PC1 accounting of 92% and PC2 accounting of 8% variances applying absorbance values at 3030–2785  $\text{cm}^{-1}$  as variables, was capable of clustering pure pork, pure ghee (cow and buffalo), and pure ghee adulterated with pork fat (lard) with different concentrations as shown in Fig. 5.2. The presence of lard of about 5% in pure ghee revealed a clear separation, while lard with a lower rate of 5% (especially 3%) displayed an overlapping separation. The wavenumbers used for PCA were also successfully applied for the prediction of lard in pure ghee with  $R^2$  for the correlation between the actual and FTIR predicted value of  $> 0.99$  and a detection limit of 3% (Upadhyay et al., 2018).

Raman spectroscopy combined with PCA is also a successful approach for a swift identification of pork meat and other meats (sheep, cattle, fish, goat, poultry, and buffalo) and in salami products in a shorter period of time (about 30 s) after the extraction of fats from the corresponding meats. PCA was performed using variables of absorbance values at 200–2000  $\text{cm}^{-1}$ . The first two PCs (PC1 and PC2) having the highest variances with 65.86 and 20.98% were chosen, and the plotting of PC1 and PC2 was capable of identifying pork and other meats in Salami products (Boyaci et al., 2014).



**Fig. 5.2** PCA based on principal component (PC1) and second principal component (PC2) using absorbance values of  $3030\text{--}2785\text{ cm}^{-1}$  for clustering pure pork (PBF), pure mutton ghee (PMG), and PMG adulterated with PBF with different concentrations. Adopted from (Upadhyay et al., 2018)

### 5.3.2 Analysis of Wild Boar Meat as Meat Adulterants

Currently, Wild boar meat, which is similar to pork, has been reported to be used as an adulterant in beef meatballs in Indonesia and Malaysia. Therefore, FTIR spectroscopy is developed for the identification and quantification of WBM in meatballs. The selection of fingerprint regions in FTIR spectra was typically carried out before modeling using chemometrics techniques. Some studies usually involve the addition of WBM into beef meatballs with varying concentrations. PCA using PC1 and PC2 based on absorbance values at combined wavenumbers of  $999\text{--}1481$  and  $1650\text{--}1793\text{ cm}^{-1}$  has successfully distinguished WBM and beef meatballs. In addition, PLSR using the first derivative spectra at the combined wavenumbers of  $999\text{--}1481$  and  $1650\text{--}1793\text{ cm}^{-1}$  could predict the levels of WBM with  $R^2$  value of 0.9991, RMSEC of 1.028%, and RMSECV of 0.30%, respectively. This result indicated that FTIR spectroscopy combined with PCA and PLSR is effective for authenticating halal meatballs from WBM (Ahda et al., 2020).

### 5.3.2.1 Analysis of Frog Meats

Frog meat (FM) is a popular food also known as “*swike*” (in Indonesia and Malaysia) and is considered a non-halal meat according to *madzhab Syafi'i*. FTIR spectroscopy using unsupervised pattern recognition of PCA was applied for fingerprinting profiling of fats extracted from FM and other animal fats, marine oils and vegetable oils. PCA using PC1 and PC2, which are accounting for 88 and 7% variances, could classify fats extracted from FM and others with a distinct separation. The absorbance values at wavenumbers of 2922 (corresponding to  $-\text{CH}_2-$ ), 2853 ( $\text{CH}_2-$ ), 1745 (carbonyl),  $1158\text{ cm}^{-1}$  ( $\text{C}-\text{O}$ ), and  $721\text{ cm}^{-1}$  (*cis*- $\text{CH}=\text{CH}$ ) were considered as the most discriminating variables that are capable of separating fats extracted from FM and others, as explained in the loading plot. This fundamental study has clear implications on the identification of FM fat from its marine and vegetable oils for the potential detection of FM adulteration in various fat-based food products (Ali et al., 2015).

### 5.3.2.2 Analysis of Dog Meat

Dog meat (DM) is occasionally added into beef meatballs intentionally or unintentionally. FTIR spectroscopy in combination with PLSR is used for the quantification of DM in beef meatballs. During the preparation of the prediction and validation samples, DM was added into beef meatballs in the range of 0–100% wt/wt. All samples were subjected to fat extraction using the Folch method applying chloroform–methanol (2:1 v/v) as extracting solvents, and the lipid extracts obtained were scanned at  $4000\text{--}650\text{ cm}^{-1}$ . PLSR applying absorbance values at combined wavenumbers region of  $1782\text{--}1623$  and  $1485\text{--}659\text{ cm}^{-1}$  using normal spectra with a high accuracy as indicated by high  $R^2$  either in the calibration model (0.993) or in the validation model (0.995). The errors were also low in both calibration (RMSEC of 1.63%) and in cross validation using the leave-one-out technique with a RMSECV of 2.68%. FTIR spectroscopy combined with PLSR provided an accurate and precise technique for the quantitative analysis of DM in beef meatballs (Rahayu, Rohman, et al., 2018).

The classification and quantification of DM in beef sausages have been carried out. Based on the optimization in terms of FTIR spectra model and wavenumbers region, the authors discovered that the normal spectra at wavenumbers of  $1124\text{--}688\text{ cm}^{-1}$  was suitable for the quantification of DM, which produced the equation of predicted value =  $0.9999 \times \text{actual value} + 0.0004$ , with statistical parameters of  $R^2$  of 0.9999, RMSEC of 0.30%, RMSEP of 0.05% and RMSECV of 0.05%. Based on PCA results, samples 1 and 5 (sausages obtained from a commercial market) had the closest point to that of DM (Guntarti & Ayu Purbowati, 2019).

Rahayu et al. have compared two extraction techniques, namely Bligh-Dyer and Folch methods for fat extraction from dog meat in meatballs formulation. The lipids obtained were scanned in FTIR spectrophotometer at the mid-IR region ( $4000\text{--}650\text{ cm}^{-1}$ ). FTIR spectral bands correlated with fats of beef, dog and the mixture of

DM and beef were analyzed. The small variations among spectra were utilized as basic tools to differentiate between DM and beef. PCA using variables of absorbance values at  $1700\text{--}700\text{ cm}^{-1}$  was capable of identifying DM in meatballs (Rahayu, Martono, et al., 2018).

### 5.3.2.3 Analysis of Rat Meat

Our group has reported the application of FTIR spectroscopy combined with chemometrics of PCA and PLSR for qualitative (identification) and quantitative analysis of rat meat (RM) in beef meatballs. Lipids in meatballs were extracted using Soxhlet, applying hexane as an extracting solvent according to the AOAC method. Lipids components scanned at mid-IR region ( $4000\text{--}650\text{ cm}^{-1}$ ) were classified using PCA for grouping the meatballs composed of beef and rat meat. In this study, some wavenumber regions were optimized for getting to acquire the best classification and prediction models. Finally, absorbances at wavenumbers of  $750\text{--}1000\text{ cm}^{-1}$  were preferred for PCA PLSR. PCA using PC1 and PC2 could classify beef and rat meatballs. In addition, for the quantitative analysis, the  $R^2$  value showing the correlation between the actual values and FTIR predicted values was 0.993 with the obtained equation for such relationship of predicted value =  $0.9417 \times \text{actual value} + 2.8410$  with RMSEC of 1.79% (Rahmania et al., 2015).

Assisted with chemometrics of PCA and multivariate calibrations, FTIR spectroscopy is reported to have been used for the identification and quantification of rat meat (RM) in food products such as sausages. Some lipid extraction techniques were carried out during this task, including Bligh and Dyer, Folch, and Soxhlet. The lipid extracted was subjected to FTIR measurement at the wavenumbers region of  $4000\text{--}650\text{ cm}^{-1}$ . PCA was successfully applied for the classification of fats extracted from RM sausages and fats extracted with different extraction methods from beef sausages using variables of FTIR spectral absorbances at  $1/\lambda$   $1800\text{--}750\text{ cm}^{-1}$  with PC1 and PC2 contribution and Soxhlet was 97.57% and 1.28% (Bligh and Dyer), 85.50 and 10.64% (Folch) and 97.86 and 2.02% (Soxhlet). In addition, quantitative analysis of RM in sausages extracted using three extraction techniques revealed good accuracy results as indicated by a high coefficient of determination ( $R^2$ ) between actual values of RM prepared in sausages and FTIR predicted values, namely using the Soxhlet method. The results were 0.945 (Bligh and Dyer), 0.991 (Folch), and 0.992 (Soxhlet). The precision method was also acceptable as indicated by the low values of error with RMSEC of 2.73, 1.73 and 1.69%, respectively, using Bligh and Dyer, Folch, and Soxhlet. In validation models using sausage samples extracted by Folch and Soxhlet, the  $R^2$  values and RMSEP values obtained were 0.458 and 18.90% (Folch), and 0.983 and 4.21 (Soxhlet). Guntarty et al. also used a similar method (FTIR spectra combined with PCA and PLSR) for the identification and quantification of RM in meatball samples using absorbance values at wavenumbers of  $1800\text{--}750\text{ cm}^{-1}$ . The extraction method used was Soxhlet using hexane as an extracting solvent (Guntarti & Prativi, 2017). Meatballs from RM and beef could also be differentiated using FTIR spectroscopy combined with two unsupervised pattern recognitions of PCA

and cluster analysis employing variables of absorbance values at  $4000\text{--}400\text{ cm}^{-1}$  (Rosyidi & Khamidinal, 2019).

The analysis of fatty acid composition using gas chromatography with a flame ionization detector exhibited significant differences between the contents of fatty acids in fats extracted from beef and rat meat sausages. The contents of fatty acids of C12:0, C16:0, C16:1 cis 9, and C18:0 in rat lipid were higher than those in beef lipid, and the contrary was observed for fatty acids of C14:1 cis 9, C15:0, C17:0, C17:1 cis 10, unsaturated C18, and C21:0. The difference in fatty acid compositions can be used as complementary data for determining the presence of RM in food products (Pebriana et al., 2017). Fatty acid compositions determination by gas chromatography with a mass spectrometric detector combined with PCA's chemometrics were successfully applied for the differentiation of fats extracted from RM and other fats from pigs, cows, chickens, wild boars, dogs, and goats. The dominant fatty acids extracted from Wistar RM were oleate (40.48%), followed by linoleate (30.14%), palmitate (19.08%), stearate (2.55%), palmitoleate (0.73%) and myristate (0.1509%) (Guntarti et al., 2020). A similar procedure was also applied for the analysis of fats extracted from RM strain Sprague Dawley (Guntarti et al., 2021) and black rats (Utami et al., 2018). The combination of FTIR spectroscopy and fatty acid composition could be an effective method for detecting the adulteration of rat meat into beef meatballs (Damayanti et al., 2020).

#### 5.3.2.4 Analysis of Donkey Meat

In China, the adulteration of donkey meat products with a similar species, namely horse meat is becoming a widespread concern. Therefore, the availability of analytical methods for detecting donkey meat is a must (Wang et al., 2020). The analysis of donkey meat as an adulterant in beef meatballs was carried out using FTIR spectroscopy combined with chemometrics of hierarchical cluster analysis (HCA). Before being used for clustering, FTIR spectra were subjected to some spectral treatments. Finally, FTIR spectra were preprocessed using first derivatization, first derivatization + vector normalization and vector normalization at wavenumbers region of  $1480\text{--}1425\text{ cm}^{-1}$  which were capable of distinguishing donkey-adulterated beef meatballs, donkey meatballs and beef meatballs with 100% sensitivity and specificity (Candoğan et al., 2020).

The combination of near-infrared (NIR) spectroscopy and chemometrics has been used for differentiating donkey meat (167 samples) from different parts of the donkey's body, beef, pork and mutton with reflectance models at wavenumbers of  $12,500\text{--}4000\text{ cm}^{-1}$ . The chemometrics of soft independent modeling of class analogy (SIMCA) and least squares-support vector machine (LS-SVM) were applied. Some NIR spectral treatments, including Savitzky-Golay smoothing and derivative spectra (first and second-derivative spectra), multiplicative scatter correction and standard normal variate, were compared and used. LS-SVM using the first 6PCs and SIMCA with the first 8PCs could accurately classify donkey meat and other meats with an accuracy of 100% in the calibration set and 98.96% in prediction sets (LS-SVM) and

100% in calibration and 97.53% in prediction sets (SIMCA). These results indicated that NIR spectra combined with chemometrics methods offered fast and reliable tools for distinguishing DM from other meats (Xiao-ying et al., 2014).

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