Assessment of meat authenticity using portable Fourier transform infrared spectroscopy combined with multivariate classification techniques

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A R T I C L E   I N F O

A B S T R A C T

In the present contribution, the feasibility of portable Fourier transform infrared spectroscopy (FTIR) combined with multivariate classification techniques is assessed for classification of minced beef, lamb, chicken and pork samples. In this regard, both attenuated total reflectance-FTIR (ATR-FTIR) and diffuse reflectance-FTIR (DR-FTIR) methods are evaluated. First, principal component analysis (PCA) was used for exploring FT-IR spectra of four meat species to find similarities and dissimilarities among samples. Additionally, one-class classification (OCC) was utilized as a new approach for halal meat species certification. For OCC, two scenarios were defined: (i) 100% correct classification for pork, and (ii) a most favorable overall classification rate for all species investigated simultaneously. With the OCC approach, both ATR and DR methods were found to produce high false-positive scores in scenario (i), whilst the DR method scored the best in scenario (ii) with an overall score of 89% correct classification. In the next step, partial least squares-discriminant analysis (PLS-DA) and support vector machine (SVM) with radial basis function (RBF) as kernel function were evaluated for meat speciation. On this matter, SVM showed better classification performance in terms of total accuracy for both ATR-FTIR (98%) and DR-FTIR (100%) datasets over PLS-DA (90% and 98%, respectively).

The promising results of both portable ATR-FTIR and DR-FTIR combined with OCC approach and discriminant analysis indicated for the first time their use as successful non-destructive, cost-effective and rapid routine screening methods for on-site analysis of meat speciation and halal meat species certification which could be useful for quality control officers to manage and control meat authenticity at various stages of the supply chain.

1. Introduction

The continuous expansion of the meat supply chain and the participation of different stakeholders in the chain has led to increased concern about meat authenticity for authorities, food producers, and consumers [1]. In the meat supply chain, meat speciation is seen as a significant authenticity issue. For religious (e.g. halal), public health (e.g. red meat) and economic reasons the detection of animal species in the meat supply chain is imperative [2–4]. Nowadays, increasing the demand for halal meat products and implementation of a halal assurance system have become a global concern. From an economic perspective, the Muslim community spend globally 1.37 trillion (US) dollars for food

in 2018 and it is anticipated to reach 2 trillion dollars by 2024 with meat and meat products being an important part of the halal food supply chain [3,5]. Analysis of the DNA and protein for meat speciation are common practice and a notable number of analytical methods have been developed to detect the origin of species [6,7]. These methods include traditional and real-time polymerase chain reaction (PCR) as a DNA-based method and gel electrophoresis, liquid chromatography-mass spectrometry (LC-MS) and immunoassays techniques as a protein-based method [8]. Most of these methods are expensive, laborious and destructive and need complicated laboratory procedures.

Fourier transform infrared (FTIR) spectroscopy is a spectral fingerprinting technique operating in the mid-infrared (MIR) range of the electromagnetic spectrum. When FTIR spectral data is combined with chemometric methods, it can be used as a fast, simple, low-cost, non-destructive and environmentally friendly screening method for meat adulteration and authenticity monitoring [9]. The MIR spectral bandwidth is ranging from 2500 to 25,000 nm and spectra represent the characteristic fundamental stretching (changes in bond length), bending (bond angle), and rotating vibrations of functional groups present in the sample molecules [10,11]. FTIR spectroscopy has different types of sample presentation methods and spectral recording techniques, which are, amongst others, attenuated total reflection FTIR (ATR-FTIR) and diffuse reflection FTIR (DR-FTIR). ATR-FTIR is a direct sample contact technique for measuring the spectra of solids, semisolids, liquids, and thin films. ATR-FTIR has required little or no sample preparation. Previous studies reporting on meat speciation with FTIR mainly deploy the ATR-FTIR spectral analysis on lipid extracts from meat samples [9,12,13]. The classification of different meat species is therefore solely based on triacylglyceride and derived structures and other fat-soluble compounds present in the lipid extracts. Although the fat associated with each meat type may give species discrimination, to reduce the samples preparation procedure, in the current study it was decided to try this spectroscopic method on the lean meat itself without prior extraction.

DR-FTIR is a non-contact technique for measuring the spectra of solids samples. Samples preparation (combined with KBr salt) is necessary before analysis. For the specific case of meat, dehydration of the sample is required, resulting in a sample mass loss of up to 80% (*w/w*). This means that with DR-FTIR, the solids present in the meat sample are present in higher concentrations. For meat speciation, DR-FTIR is a technique that is not often reported upon, due to a relatively laborious sample preparation procedure. Still, in comparison with the effort required for DNA analysis, DR-ATR might serve as an alternative to gather detailed spectral information of meat samples [14,15]. For halal meat certification, the first concern is meat speciation, meaning that a halal meat product is made entirely of halal species such as ovine or bovine and does not contain non-halal species [16]. An FTIR spectra based classification model should therefore ideally have the target class 'pork' or 'non-halal' to efficiently exclude all other halal meats. The second concern is to establish if Islamic ritual slaughtering methods have been applied in a correct way [16]. As it is unknown how this affects the chemical composition of halal meat products versus non-halal meat products, this halal requirement is not considered in this work.

In order to disclose the chemical sample information from the FTIR spectra, usually, multivariate statistics methods are used. In addition to the chemical information, FTIR spectra contain unwanted ambient and instrumental noise and scattering effects, which require a robust data pre-processing and chemometric methodology for the extraction of the relevant chemical sample information from the acquired spectra [17–19]. In previous studies, different multivariate methods such as partial least squares (PLS), support vector machine (SVM), K-nearest neighbor (KNN) and soft independent modelling of class analogy (SIMCA) were used in combination with an appropriate (combination of) data pre-processing method(s) for successful meat speciation. In these studies, there isn't a suitable comparison between OCC approach on the one hand and linear and non-linear discriminant approach on the

other hand, as a tool for halal species certification [2,9,12,13]. As a plausible and more efficient chemometric modelling strategy for finding fraud, one-class classification (OCC) can be considered. OCC only describes a target class, in this case, the non-halal class pork, and returns predictions of samples being out or in the respective target class. Hence, samples that are "in", do contain pork and are therefore not halal and samples that are "out" do not contain pork and may be certified as halal species [3,20,21].

The known analytical techniques used to detect the origin of species include PCR, LC-MS and immunoassays [6–8,22]. These methods are destructive, expensive, laborious, time-consuming, require a specialized laboratory, involve chemical usage and/or need complicated laboratory procedures. Therefore, there is the need to seek other alternatives, and visible and infrared spectroscopies could be very helpful. In this regard, previous studies have mainly used bulky benchtop NIR and FTIR instruments for the application of halal from non-halal meat-species discrimination. Considering the huge meat adulteration from one hand and the need for its rapid detection at various stages of meat supply chain on other hand, there is an urgent need to develop non-destructive, cost-effective and rapid portable and/or handheld spectroscopy techniques for on-site analysis of meat species at various stages of meat supply chain. In this regard, we recently assessed the feasibility of two handheld sensors (400–1000 nm and 900–1700 nm) for meat speciation and halal meat certification [21]. The results showed that Vis-NIR sensor was most successful in the halal certification (OCC approaches) and speciation (discriminant approaches) for both intact and ground meat using SVM. This research, presents the feasibility study on the use of portable ATR-FTIR and DR-FTIR techniques as rapid and cost-effective screening tools combined with multivariate analysis for halal meat species certification (pork vs. other species) as well as for speciation of four different types of meat (lamb, beef, pork and chicken) in food supply chains. We show the application of discriminant chemometric approaches (linear and non-linear methods) and OCC on data obtained using both FTIR spectral recording techniques.

2. Materials and methods

2.1. Sample collection

Forty-eight lamb (*Ovis aries*) muscle samples (fore and hind shank), 53 beef (*Bos taurus*) muscle samples (fore and hind shank, chuck, brisket and round), and 40 chicken (*Gallus gallus domesticus*) muscle samples (breast and drumstick) were obtained at least 24 h and maximum 72 h after slaughtering from local butchers in different cities of Iran. Additionally, 32 pork (*Sus scrofa domesticus*) muscle samples (shoulder and leg) were collected from Azerbaijan country. All meat samples were purchased from December 2018 to August 2020 (for consideration of season variety) in intact form and transported under ice-chilled conditions to the laboratory. Fresh meat samples were stored at 4 °C until the preparation and analysis.

2.2. Sample preparation

Visible skin, fat and connective tissue were excised that could interfere in the analysis, and then about 100–200 g of meat was homogenized by a Moulinex Meat Grinder (1000 W, France) for 30 s. The fresh ground meat samples were used for ATR-FTIR. For compatibility with DR-FTIR, all ground meat samples were dried with a freeze-dryer (Christ Alpha 1–2 LDPlus, France) for 24 h or until dry to remove the excess water. Each dried meat sample was mixed with dry Potassium - bromide (KBr, Merck) salt (1:10 ratio (w/w)) and homogenized with mortar before DR-FTIR analysis.

2.3. Data acquisition

ATR-FTIR spectra of the fresh ground meat samples were acquired on

a Cary 630 FTIR spectrometer (Agilent Technologies, USA). The instrument was equipped with a ZnSe ATR interface. The instrument was equipped with a deuterated triglycine sulfate (DTGS) detector and KBR as the beam splitter. Meat samples were placed in good contact with a horizontal positioned attenuated total reflectance element at room temperature. The surface of the ATR interface was cleaned with ethanol and dried before measuring the next sample. Before each sample scan, a new reference air background spectrum (an average of 32 scans) was acquired. The spectra were recorded in the range between 650 and 4000 cm^{-1} (2500 to 15 384 nm) at a resolution of 16 cm^{-1} with 200 scans. All ATR-FTIR spectra were recorded as transmittance values at each data point but they were converted to absorbance (log1/transmittance). The Cary 630 MicroLab PC software was used for data collection. Finally, the ATR-FTIR data matrix consisted of 160 spectra (160 samples) with 451 points (variables).

For DR-FTIR spectra, each sample was mixed with KBr (1/10 ratio (w/w)) and approximately 500 mg of homogenized samples were transferred to the DR-FTIR sample holder cup and the top of the sample was leveled off for spectral acquisition. The Agilent Cary 630 FTIR spectrometer was operated using the diffuse reflectance sample interface. Each sample was scanned from 650 to 4000 cm^{-1} at a resolution of 8 cm^{-1} with 60 scans. The spectrum of the gold reference mirror was used as background (an average of 20 scans). All measurements were performed in a dry controlled atmosphere at room temperature. All DR-FTIR spectra were recorded as transmittance values at each data point but they were converted to absorbance (log1/transmittance). The Cary 630 MicroLab PC software was used for data collection. Finally, the DR-FTIR data matrix consisted of 173 spectra (173 samples) with 900 points (variables).

2.4. Data analysis

Prior to OCC and discriminant analysis, the FTIR data were visually inspected and further examined using unsupervised principal component analysis (PCA). To evaluate the power of discriminant classification models for each species, classification figures of merit including sensitivity (Sen), specificity (Spe), error rate (ER) and accuracy (Acc) were used. These performance parameters are usually derived from a confusion matrix, to better assess the classification performance.

2.4.1. OCC approach

OCC was applied as previously described by Weesepoel et al [30] and Mueller-Maatsch et al [31] using R 3.6.1 (R Core Team, 2018). In this work, SNV, SNV followed by baseline correction (SNV detrend, DT), 1st or 2nd derivative (Savitzky-Golay) with an 11-point filter length, discrete wavelet transformation (DWT) after interpolating the spectrum into 128 points (applying d2 Daubechies, filter length 2 or a la8 Least Asymmetric, filter length 8 transformations), and spectrum splitting (either full or split into 4 equal quarter spectra modeled separately) was performed. As OCC methods Soft Independent Modelling of Class Analogies (SIMCA), distance to k-Nearest Neighbour (kNN), Principal Components Analysis (PCA) residual, Mahalanobis distance, and One-Class Support Vector Machine (OCSVM) with radial basis kernel were used. The combinations were evaluated using 80 times repeated random cross-validation (70% split) on the target class (pork). The performance of the (combination of) pre-processing and OCC models were evaluated by calculating the 'area under the receiver operating characteristic' (AUROC) of the target class pork against lamb, beef and chicken, respectively. Three models were selected manually as they covered jointly the highest obtained AUROCs for each of the classes. A decision tree was implemented that flagged a sample as "not pork meat" when two or more out of the 3 models classified a sample as 'out-of-class'. The decision tree was optimized for two different scenarios. The first scenario comprises the 100% correct identification of pork, whilst the second scenario was tuned for a balanced false-positive and false-negative rate.

2.4.2. Discriminant analysis

Discriminant analysis of FTIR data was performed in MATLAB R2013a. For preprocessing of raw data the PLS-Toolbox version, 7.8 (Eigenvector, WA, USA) was used. The duplex method was used for splitting the original spectra into training (70%) and test (30%) sets. Duplex algorithm was utilized to ensure that all species were represented in the test set. It leads to the formation of two balanced sets (with the same diversity) [23]. The training (calibration) set was used for calculating the model. The predictive classification models were validated using cross-validation (Venetian blinds (10 splits and 1 sample per split)) as internal validation. After training, tuning and evaluation of the model, the test set was used for the final performance assessment (external validation). The data analysis pipeline of the presented work is shown in Fig. 1. Data pre-processing of the data was used for removing or reducing redundant information (random noise and/or unwanted systematic variations) without affecting the chemical information present in the FTIR spectra [20,24]. Different data preprocessing techniques were assessed being standard normal variate (SNV), 1st derivative, 2nd derivative (Savitzky–Golay and Gap Segment, 5, 11, 15 and 21-point filter length), mean centering, orthogonal signal correction (OSC), baseline filtering, external parameter orthogonalization (EPO), multiplicative signal correction (MSC), weighted normalization, baseline removal, and median ratio normalization, were assessed. Two discriminant analysis techniques, being PLS-DA and SVM (with a non-linear kernel of radial basis function, RBF) were used in this study. Venetian blinds cross-validation (Number of data split: 10, thickness: 1) was used for cross-validation. In order to use the best combination of pre-processing techniques and the optimum number of latent variables (LVs) for PLS-DA, the lowest values of the root-mean-square error of calibration (RMSEC) and cross-validation (RMSECV) were used as selection criteria. Other attempts such as variable selection using variable importance in projection (VIP) with "greater than one" rule [25] and outlier detection using Q-residuals/Hotelling's T2 [26] were done to increase PLS-DA classification performance of the optimum pre-processing and LV combination. For finding the optimum SVM model, the model's gamma and C parameters combination is optimized by assessing the highest classification rate in a matrix calculation with ranging values gamma (15 values from 10^{-6} to 10, spaced uniformly in log) and cost (11 values from 10^{-3} to 100, spaced uniformly in log). In order to prevent SVM overfitting, values of gamma and C were chosen with a maximum difference of a factor 10 in values [27–29]. To assess the performance of PLS-DA and SVM models, classification figures of merit including sensitivity (Sen), specificity (Spe), error rate (ER) and accuracy (Acc) were used.

3. Results and discussion

3.1. FTIR spectral analysis

ATR-FTIR and DR-FTIR spectra of four meat species (lamb, beef, chicken and pork) are shown in Fig. 2. The mean spectra of the classes show visual differences, especially in 3000–2800 cm^{-1}, 1800–1700 and 1700–1000 cm^{-1} regions that are mainly related to lipid and protein composition. In general, the absorption bands between 3200 and 3500 cm^{-1} correspond to the stretching vibration of O–H and N–H bonds. N–H bonds are associated with protein amino acids and are not visible in the ATR spectra due to the presence of excess water. However, upon removal of water, the DR spectra showed the N–H stretching vibrations [32,33]. The band around 2800 to 3200 cm^{-1} is related to the C–H asymmetric and symmetric stretching and is associated with lipids [32,33]. The region 1800–1600 cm^{-1} allocated to C = C and C = O bonds and these can be related to phospholipids (~1740 cm^{-1}) [32–34]. Furthermore, the bands ~1650 and ~1540 cm^{-1} are associated with amid I and II of protein amino groups. The band ~1650 cm^{-1} is associated with the N–H bond vibration and C=C stretching vibration of alkenes. The bond around 1540 cm^{-1} is linked to the combination of
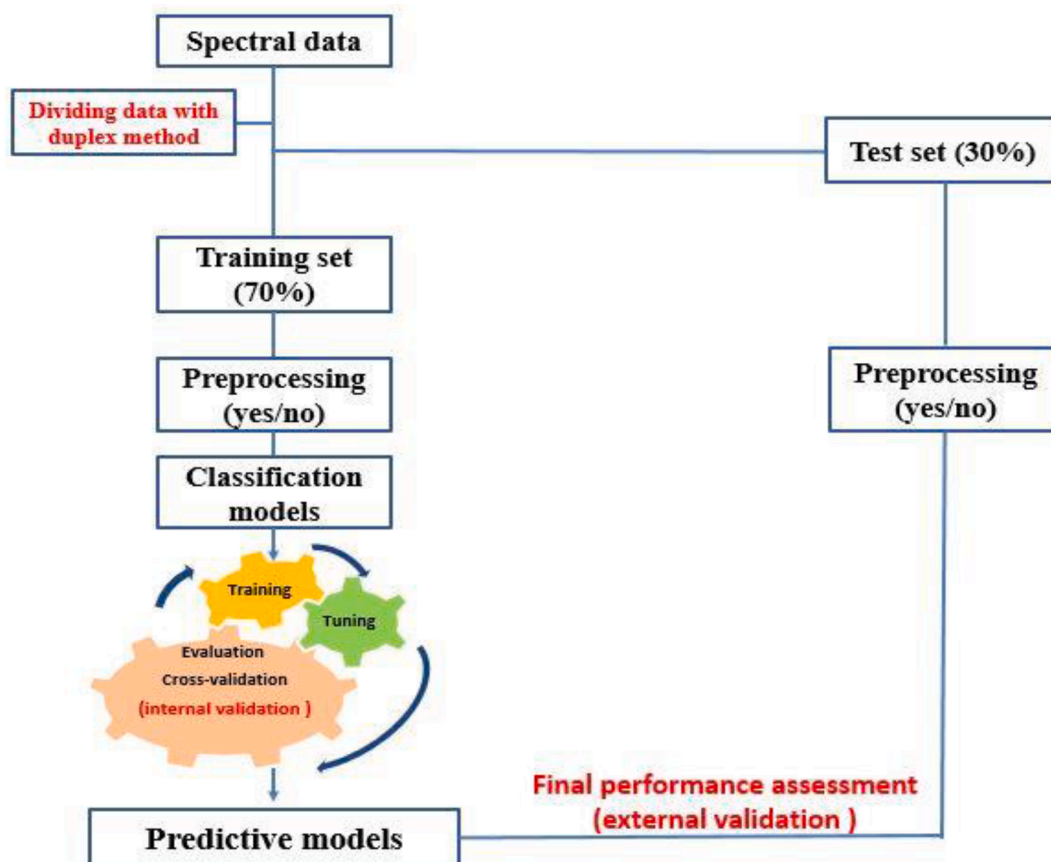
Fig. 1. Data analysis pipeline.

C–H bond stretching vibration and N–H bond bending. Approximately, the range from 1500 to 1000 cm-1 was related to the "fingerprint region", and many different bands appear in this region. There is a clear difference between pork and other species in fingerprinting region. The interpretation of this region for meat matrices cannot be unequivocally done as the many molecules present to contribute to the FTIR spectra by specific types of vibrations. Different vibrations, including C-0, C–C, and C-N single bond stretches and C–H bending vibrations are found in this wavenumber region [32–34]. Fig. 1a and 1b reveal that beef and lamb resemble each other closely. The lipid content and composition (particularly 1700–1800 and 2800–3000 cm^{-1}) are a major source of variation between the different types of species especially pork samples. On the one hand, lipid composition can be linked to the type of animal and the age of the animal, whilst on the other hand, the leanness and the type of cut can also play a role [2]. For the ATR-FTIR, the average pork spectrum is deviating from the other three types of samples. A similar observation can be done in the DR-FTIR spectra. It can be concluded from observation of the spectra that a chemometrics approach would be feasible to use ATR and DR-FTIR for speciation of the four meat sample types.

3.2. Exploratory data analysis using PCA

PCA was used for the exploration and evaluation of differences between four meat species spectra. After visual assessment of different preprocessing methods, the suitable preprocessing methods were baseline correction (automatic Whittaker filter) followed by EPO (2PCs) for ATR-FTIR data and mean centering followed by EPO (3 PCs) for DR-FTIR spectra. Fig. 3a and 3c show the ATR-FTIR and DR-FTIR PCA scores plot for lamb, beef, pork, and chicken spectra. The PC1 scores for ATR were accounted for 52% of the explained variance and less separation

between classes could be related to the high water content of the samples. Relatively samples separation can be observed on PC3 and PC4. Therefore, the remaining variance in spectral data did not result in full clustering into four different groups. For the DR spectra, better clustering was observed in the PCA plot, due to the removal of the excess water. In contrast, in DR-FTIR a better sample separation can be observed on PC1 (40% variance) and there was less scattering of the samples. In summary, PCA analysis can provide an estimation of the sample's distribution based on their ATR-FTIR and DR-FTIR spectra (Fig. 3a and c). However, DR-FTIR can provide better discrimination (clustering) of the samples compared to ATR-FTIR. The better separation between the species in PCA score plots of DR-FTIR spectra compared with ATR-FTIR spectra may be caused by the type of information collection and removal of water content from meat samples [14,15,33]. Furthermore, the loading plots of the PC1, PC2 and PC3 for ATR-FTIR and DR-FTIR are represented in Fig. 3(b and d). Wavenumbers with higher value are important to explain the variance and are potential wavenumbers to differentiate the four species. Analysis of the PCA loading plots reveals that the major features are mostly related to the lipid and protein content of the samples. Namely, moreover the bands at ~1650 and ~1540 cm^{-1}, are related to amide I and II, the bands at 2800–3200 cm^{-1} and most important, the band at ~1740 cm^{-1} are due to lipid content.

3.3. OCC modeling

The application of OCC for pork speciation was a powerful tool to identify any type of sample which do not belong to the pork class. By only defining the 'pork'-class, potential unknown out-of-class samples can be identified and only one sample class needs to be measured. As OCC models are known to be less sensitive or accurate than the usual
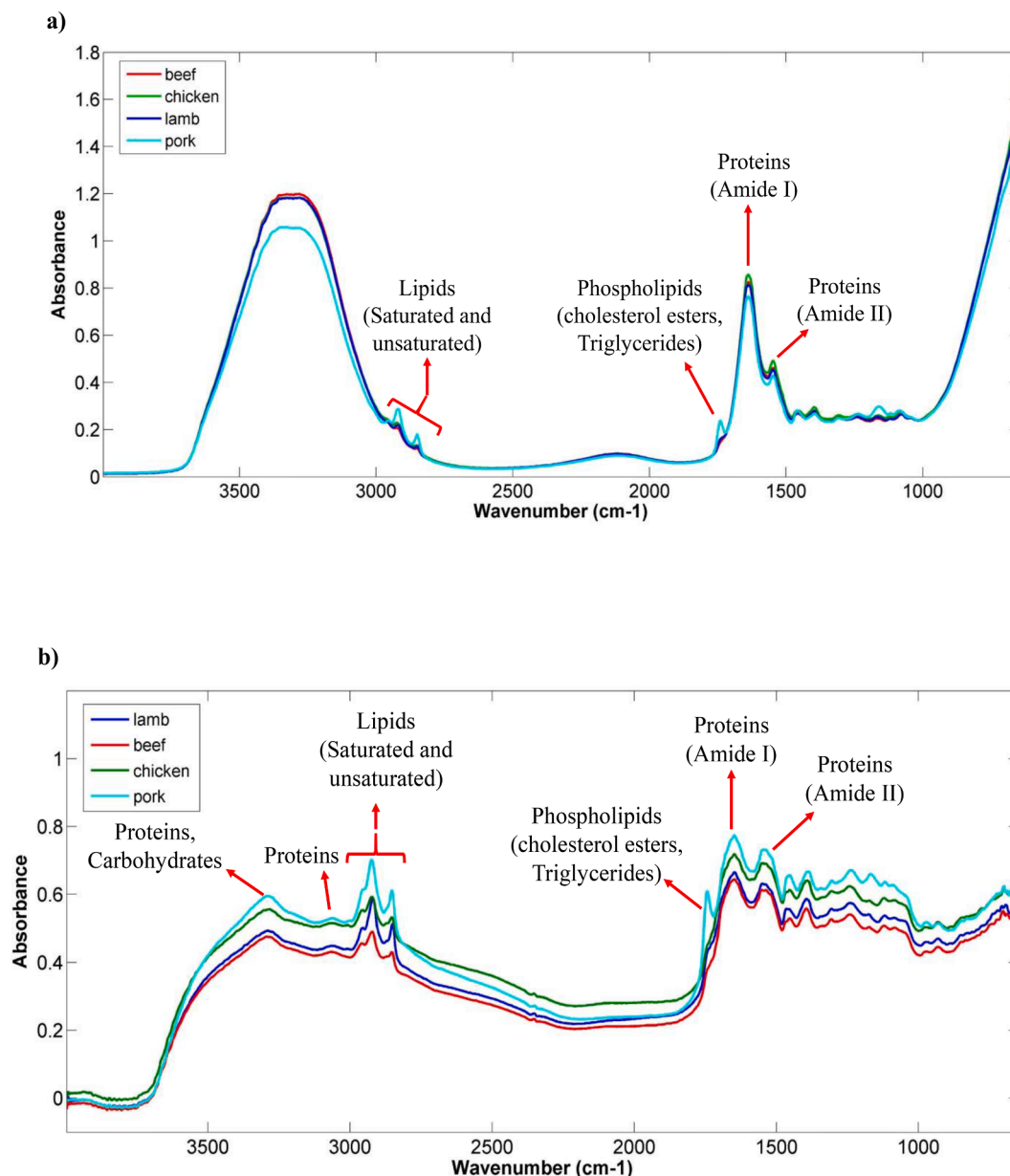
a)

b)

Fig. 2. Mean of (a) ATR-FTIR spectra and (b) DR-FTIR spectra of lamb, beef, chicken, and pork samples.

supervised classification methods, it was chosen to use the results of the three OCC models with the most favorite AUROC scores in the four different settings (i.e. pork vs lamb, pork vs beef, pork vs chicken and pork vs all) as displayed in Table 1. The three individual models were then optimized for two different scenarios by using different class limits and thresholds (Table 2). In scenario 1, 100% of the pork samples were correctly identified as pork. For Halal meat certification, a 100% correct identification of fraudulent samples is important, as false negatives are unacceptable in this setting. As a draw-back of this scenario, many false positives will be found, leading, therefore, to an increase in laboratory reference method work. This was especially the case for lamb and beef in the ATR setting, whilst a very low correct rate of 23 % was observed for chicken meat in the DR configuration. These low scores are a direct artifact of the forced zero false-negative rates set for the pork. Due to these extreme settings, scores from negative classes can be reduced dramatically with no obvious physical or chemical reason. Therefore in scenario 2, the number of false positives and false negatives was balanced to counter the high amount of false positives from scenario 1. In scenario 2, approximately 75–77% of the pork samples were

identified correctly. Especially for the DR setting in scenario 2, the scores of the negative samples were all above 90 %, whilst for the ATR setting negative sample rates were slightly lower. It can therefore be concluded that the added value of the DR set-up with its more elaborated sample preparation, gives a slight advantage over the ATR methods. Both sample presentation methods may be used for the preliminary screening of meat samples using the OCC approach and the scenario which fits best to the envisioned application. In this work, we have only demonstrated 2 OCC scenarios, though one can imagine that several different scenarios can be possible. Also, OCC can be combined with supervised discriminant models (Section Discriminant modeling). When a sample in the OCC approach is detected as a halal meat species (out-of-class), the discriminant approach may be used for the discrimination of chicken, beef, lamb and pork samples.

3.4. Discriminant modeling

The ATR and DR FTIR spectral data were subjected to supervised discriminant analysis. Both PLS-DA and SVM methods were explored to
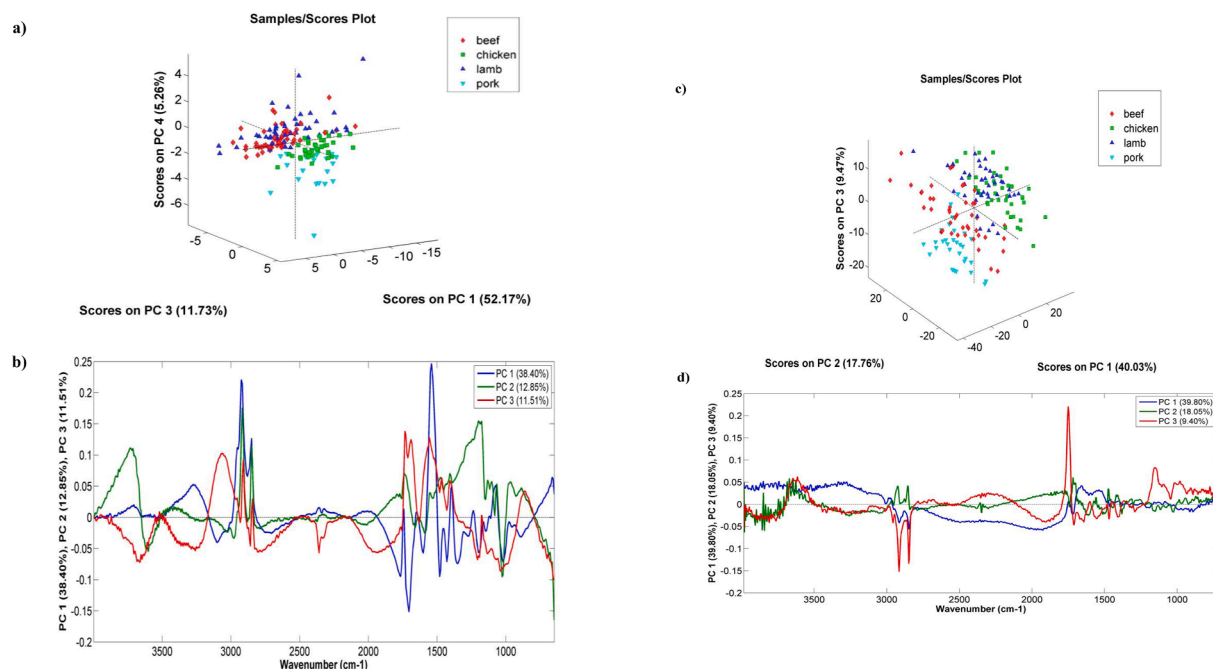
Fig. 3. The PCA score and loading plots, (a) PCA score plot of ATR-FTIR (3000–2830 and 1760–1000 cm^{-1}) preprocessed with baseline correction (automatic Whittaker filter) + EPO (2PCs), (b) PCA loading plot of ATR-FTIR (full spectra) preprocessed with baseline correction (automatic Whittaker filter) + EPO (2PCs), (c) PCA score plot of DR-FTIR preprocessed with mean center + EPO (3 PCs), (d) PCA loading plot of DR-FTIR (full spectra) preprocessed with mean center + EPO (3 PCs).

Table 1

Top 3 of pre-processing and OCC algorithm combination and AUROCs on the manually picked OCC models for ATR-FTIR and DR-FTIR respectively.

| | Model | Pre-processing | | | | Algorithm | AUROC | | | |
		SNV	Derivative	Subset	DWT		Pork vs lamb	Pork vs beef	Pork vs chicken	Pork vs all
ATR-FTIR	1	–	1st	4th	–	kNN *(2neighbors)*	0.81	0.86	0.92	0.86
	2	–	2nd	2nd	–	kNN *(2neighbors)*	0.82	0.84	0.89	0.85
	3	–	–	2nd	–	Mahalanobis	0.75	0.68	0.85	0.75
DR-FTIR	1	–	1st	3rd	–	kNN *(2neighbors)*	0.92	0.94	0.95	0.94
	2	–	1st	(full)	–	kNN *(2neighbors)*	0.88	0.9	0.88	0.89
	3	–	1st	3rd	–	Mahalanobis	0.92	0.84	0.85	0.87

Table 2

Correct classification rate of samples in two scenarios.

| Scenario | ATR-FTIR | | DR-FTIR | |
	1	2	1	2
Pork	100%	77%	100%	75%
Lamb	52%	85%	69%	90%
Beef	58%	87%	57%	94%
Chicken	70%	93%	23%	98%

gain classification models for allocating categories to meat samples. In this regard, different preprocessing strategies were assessed and the most appropriate preprocessing strategy was chosen according to the highest Sen, Spe and lowest Err. The best PLS-DA models for ATR-FTIR and DR-FTIR were achieved with EPO (2 PCs) + OSC and extended multiplicative signal correction (EMSC) + OSC, respectively (Fig. S1). The DR method results in spectra where the pork and lamb can be easily distinguished from the beef and chicken. In the test set, the greatest total accuracy values of PLS-DA for ATR-FTIR and DR-FTIR were 90% and 98%, respectively (Tables 3 and 4). Furthermore, outlier detection using

Q-residuals/Hotelling's T2 and variable selection using variable importance in projection (VIP) was performed to improve PLS-DA classification but did not result in any significant improvement in both methods (Figure S2 and Table S1). After that, for assessing non-linear methods, SVM (RBF kernel) model was used. The best SVM models for ATR-FTIR and DR-FTIR were obtained with EPO (4 PCs) and Gap Segment 1st derivative (gap: 5, segment: 5), respectively. The maximum total accuracy values of SVM for ATR-FTIR and DR-FTIR were 98% and 100%, respectively (Tables 3 and 4). According to the above results, DR-FTIR and SVM model (with non-linear kernel function) presented a relatively better performance rather than ATR-FTIR and PLS-DA. These findings may be associated with types of information collected from samples that in ATR-FTIR is mainly from a solid surface, while DR-FTIR presents information from the entire solid matrix and its information is a combination of internal and external reflection. DR-FTIR presented spectra with higher intensity of absorption in comparison to ATR-FTIR and removal of the water content of meat samples could improve spectral interpretation. However, DR-FTIR requires more sample preparation than ATR-FTIR, and samples must be dried and combined with KBr salt prior to analysis [14,15,33]. Furthermore, the results of this

Table 3

Classification performance (in %) of PLS-DA and SVM (kernel function: RBF) models[1] for classification of lamb, beef, chicken and pork with ATR-FTIR.

		PLS-DA[2]			SVM[3]		
		Train	CV	Test	Train	CV	Test
Lamb	Sensitivity	97.0	81.0	83.0	97.0	70.0	87.0
	Specificity	96.0	86.0	80.0	100.0	90.0	97.0
	Accuracy	96.5	83.5	81.5	98.5	79.4	91.8
	Error	3.5	16.5	18.5	1.5	20.6	8.2
Beef	Sensitivity	84.0	76.0	91.0	100.0	79.0	93.0
	Specificity	79.0	75.0	84.0	99.0	85.0	91.0
	Accuracy	81.5	75.5	87.4	99.5	82.0	92.0
	Error	18.2	24.5	12.6	0.5	18.0	8.0
Chicken	Sensitivity	100.0	97.0	96.0	100.0	97.0	88.0
	Specificity	100.0	95.0	98.0	100.0	99.0	100.0
	Accuracy	100.0	96.0	97.0	100.0	98.0	93.8
	Error	0.0	4.0	3.0	0.0	2.0	6.2
Pork	Sensitivity	92.0	85.0	100.0	100.0	85.0	93.0
	Specificity	97.0	93.0	91.0	100.0	100.0	98.0
	Accuracy	94.5	89.0	95.4	100.0	92.0	95.5
	Error	5.5	11.0	4.6	0.0	8.0	4.5

[1] Cross validation: Venetian blinds (Number of data split: 10, thickness: 1).
[2] PLS-DA (LVs: 6): pre-processed with EPO (2 PCs) + OSC.
[3] SVM: pre-processed with EPO (4 PCs).

Table 4

Classification performance (in %) of PLS-DA and SVM (kernel function: RBF) models[1] for classification of lamb, beef, chicken and pork with DR-FTIR.

		PLS-DA[2]			SVM[3]		
		Train	CV	Test	Train	CV	Test
Lamb	Sensitivity	100.0	94.0	100.0	100.0	100.0	100.0
	Specificity	94.0	92.0	95.0	100.0	99.0	100.0
	Accuracy	97.0	93.0	97.5	100.0	99.5	100.0
	Error	3.0	7.0	2.5	0.0	0.5	0.0
Beef	Sensitivity	94.0	94.0	100.0	100.0	100.0	100.0
	Specificity	93.0	95.0	88.0	100.0	98.0	100.0
	Accuracy	93.5	94.5	94.0	100.0	99.0	100.0
	Error	6.5	5.5	6.0	0.0	1.0	0.0
Chicken	Sensitivity	100.0	96.0	100.0	100.0	96.4	100.0
	Specificity	94.0	94.0	100.0	100.0	100.0	100.0
	Accuracy	97.0	95.0	100.0	100.0	98.0	100.0
	Error	3.0	5.0	0.0	0.0	2.0	0.0
Pork	Sensitivity	100.0	95.0	100.0	100.0	90.0	100.0
	Specificity	100.0	100.0	100.0	100.0	100.0	100.0
	Accuracy	100.0	97.5	100.0	100.0	95.0	100.0
	Error	0.0	2.5	0.0	0.0	5.0	0.0

[1] Cross validation: Venetian blinds (Number of data split: 10, thickness: 1).
[2] PLS-DA (LVs: 4): preprocessed with EMSC + OSC.
[3] SVM: pre-processed with Gap Segment 1st derivative (gap: 5, segment: 5).

study revealed that SVM (RBF kernel as a non-linear kernel) has a relatively better performance compared with PLS-DA (as a linear model) for meat speciation in both techniques (Tables 3 and 4). The better self-learning and self-adjustment capacity of the SVM technique might be attributed to the optimal performance exhibited by the SVM model. In some studies, it has been shown that SVM models had better performance for authenticity and quality control [35,36]. In previous studies, it has been reported that DR-FTIR (in which the samples are dried) presented a better performance than ATR-FTIR in some authenticity studies [37,38].

4. Conclusion

Regarding increasing meat fraud during these years and demand for screening methods for meat authenticity, in this study, the application of portable ATR-FTIR and DR-FTIR spectroscopy combined with different chemometric methods was assessed for discrimination of lamb, beef, chicken and pork meat. In the present contribution, DR-FTIR and SVM discriminant modeling gave the highest classification rates. The multivariate statistics models investigated in this study, with the performance of 90%-100% accuracy reveal that ATR-FTIR and DR-FTIR combined with appropriate OCC and discriminant approaches have the potential to be used as a screening method and part of a two-tiered system for meat authenticity and halal species certification. Crucial for the performance of this screening method is the choice of sample presentation (ATR and DR) and multivariate data analysis approach. DR-FTIR combined with SVM showed better performance rather than ATR-FTIR followed by PLS-DA. A potential disadvantage is the more elaborated sample preparation required for DR-FTIR. The OCC screening approach with both ATR-FTIR and DR-FTIR can decrease the number of samples to be evaluated and put forward for confirmatory PCR methods, although the OCC models explored in this study could not match the performance of the discriminant models. Further validation of the spectral databases built in this study can be done by adding more sample variability, and by incorporating samples from different geographical areas and seasons.

CRediT authorship contribution statement

Abolfazl Dashti: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Visualization. **Yannick Weesepoel:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Supervision, Project administration. **Judith Müller-Maatsch:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – original draft, Supervision, Project administration. **Hadi Parastar:** Conceptualization, Validation, Formal analysis, Investigation, Supervision, Writing – original draft. **Farzad Kobarfard:** Conceptualization, Investigation, Resources, Supervision. **Bahram Daraei:** Conceptualization. **Hassan Yazdanpanah:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2022.107735.

References:

[1] R. Valand, S. Tanna, G. Lawson, L. Bengtström, A review of Fourier Transform Infrared (FTIR) spectroscopy used in food adulteration and authenticity investigations, Food Addit. Contam. Part A. 37 (1) (2020) 19–38.

[2] G. Downey, J. McElhinney, T. Fearn, Species identification in selected raw homogenized meats by reflectance spectroscopy in the mid-infrared, near-infrared, and visible ranges, Appl. Spectrosc. 54 (6) (2000) 894–899.

[3] J.T.L. Müller-Maatsch, Y.J.A. Weesepoel, E.A.M. Roetgerink, A.M. Wijtten, M. Alewijn, Are low-cost, hand-held NIR sensors suitable to detect adulterations of halal meat?, in, OCM 2021-Optical Charact. Mater. Conf. Proc., KIT Publishers (2021) 1–10.

[4] M.Á. Sentandreu, E. Sentandreu, Authenticity of meat products: Tools against fraud, Food Res. Int. 60 (2014) 19–29.

[5] T. Reuters, D. Standard. State of the global Islamic economy report 2019/20. 2019. Dinar Stand.

[6] Y.H. Hui, Handbook of food science, technology, and engineering, CRC Press, 2006.

[7] N.Z. Ballin, F.K. Vogensen, A.H. Karlsson, Species determination–Can we detect and quantify meat adulteration? Meat Sci. 83 (2) (2009) 165–174.

[8] B. Kuswandi, F.K. Putri, A.A. Gani, M. Ahmad, Application of class-modelling techniques to infrared spectra for analysis of pork adulteration in beef jerkys, J. Food Sci. Technol. 52 (12) (2015) 7655–7668.

[9] H. Rahmania, Sudjadi, A. Rohman, The employment of FTIR spectroscopy in combination with chemometrics for analysis of rat meat in meatball formulation, Meat Sci. 100 (2015) 301–305.

[10] Y.B. Che Man, Z.A. Syahariza, A. Rohman, Fourier transform infrared (FTIR) spectroscopy: development, techniques, and application in the analyses of fats and oils, Fourier Transform Infrared Spectrosc. Nov. Sci. Publ. New York. (2010) 1–36.

[11] A.K. Shukla (Ed.), Spectroscopic Techniques & Artificial Intelligence for Food and Beverage Analysis, Springer Singapore, Singapore, 2020.

[12] I. Saputra, I. Jaswir, R. Akmeliawati, Identification of pig adulterant in mixture of fat samples and selected foods based on FTIR-PCA wavelength biomarker profile, Int. J. Adv. Sci. Eng. Inf. Technol. 8 (2018) 2341–2348.

[13] W.S. Rahayu, A. Rohman, S. Martono, S. Sudjadi, Application of FTIR spectroscopy and chemometrics for halal authentication of beef meatball adulterated with dog meat, Indones. J. Chem. 18 (2018) 376–381.

[14] N. Reis, A.S. Franca, L.S. Oliveira, Discrimination between roasted coffee, roasted corn and coffee husks by Diffuse Reflectance Infrared Fourier Transform Spectroscopy, LWT-Food, Sci. Technol. 50 (2) (2013) 715–722.

[15] N. Reis, B.G. Botelho, A.S. Franca, L.S. Oliveira, Simultaneous detection of multiple adulterants in ground roasted coffee by ATR-FTIR spectroscopy and data fusion, Food Anal. Methods. 10 (8) (2017) 2700–2709.

[16] OIC/SMIIC. General Requirements for Halal Food. 2019. https://www.smiic.org/en/project/24.

[17] H. Parastar, G. van Kollenburg, Y. Weesepoel, A. van den Doel, L. Buydens, J. Jansen, Integration of handheld NIR and machine learning to "Measure & Monitor" chicken meat authenticity, Food Control. 112 (2020), 107149.

[18] Q.i. Li, J. Chen, Z. Huyan, Y. Kou, L. Xu, X. Yu, J.-M. Gao, Application of Fourier transform infrared spectroscopy for the quality and safety analysis of fats and oils: a review, Crit. Rev. Food Sci. Nutr. 59 (22) (2019) 3597–3611.

[19] K. Kucharska-Ambrożej, J. Karpinska, The application of spectroscopic techniques in combination with chemometrics for detection adulteration of some herbs and spices, Microchem. J. 153 (2020), 104278.

[20] P. Oliveri, G. Downey, Discriminant and class-modelling chemometric techniques for food PDO verification, in, Compr. Anal. Chem., Elsevier (2013) 317–338.

[21] A. Dashti, J. Müller-Maatsch, Y. Weesepoel, H. Parastar, F. Kobarfard, B. Daraei, M.H.S. AliAbadi, H. Yazdanpanah, The Feasibility of Two Handheld Spectrometers for Meat Speciation Combined with Chemometric Methods and Its Application for Halal Certification, Foods. 11 (1) (2022) 71.

[22] N.A. Tasrip, N.F. Khairil Mokhtar, U.K. Hanapi, Y.N. Abdul Manaf, M.E. Ali, Y.K. Cheah, S. Mustafa, M. Desa, Loop mediated isothermal amplification; a review on its application and strategy in animal species authentication of meat based food products, Int. Food Res. J. 26 (2019).

[23] T. Puzyn, A. Mostrag-Szlichtyng, A. Gajewicz, M. Skrzyński, A.P. Worth, Investigating the influence of data splitting on the predictive ability of QSAR/QSPR models, Struct. Chem. 22 (4) (2011) 795–804.

[24] R. Gautam, S. Vanga, F. Ariese, S. Umapathy, Review of multidimensional data processing approaches for Raman and infrared spectroscopy, EPJ Tech. Instrum. 2 (2015) 1–38.

[25] C.M. Andersen, R. Bro, Variable selection in regression—a tutorial, J. Chemom. 24 (11-12) (2010) 728–737.

[26] D. Ballabio, V. Consonni, Classification tools in chemistry. Part 1: linear models. PLS-DA, Anal. Methods. 5 (2013) 3790–3798.

[27] C.-W. Hsu, C.-C. Chang, C.-J. Lin. A practical guide to support vector classification. (2003).

[28] O. Chapelle, V. Vapnik, O. Bousquet, S. Mukherjee, Choosing multiple parameters for support vector machines, Mach. Learn. 46 (2002) 131–159.

[29] O. Devos, C. Ruckebusch, A. Durand, L. Duponchel, J.-P. Huvenne, Support vector machines (SVM) in near infrared (NIR) spectroscopy: Focus on parameters optimization and model interpretation, Chemom. Intell. Lab. Syst. 96 (1) (2009) 27–33.

[30] Y. Weesepoel, M. Alewijn, M. Wijtten, J. Müller-Maatsch, Detecting food fraud in extra virgin olive oil using a prototype portable hyphenated photonics sensor, J. AOAC Int. 104 (2021) 7–15.

[31] J. Müller-Maatsch, M. Alewijn, M. Wijtten, Y. Weesepoel, Detecting fraudulent additions in skimmed milk powder using a portable, hyphenated, optical multi-sensor approach in combination with one-class classification, Food Control. 121 (2021), 107744.

[32] B. Smith, Infrared spectral interpretation: a systematic approach, CRC Press, 2018.

[33] M. Lozano, P. Rodríguez-Ulibarri, J.C. Echeverría, M. Beruete, M. Sorolla, M.J. Beriain, Mid-infrared spectroscopy (MIR) for simultaneous determination of fat and protein content in meat of several animal species, Food Anal. Methods. 10 (10) (2017) 3462–3470.

[34] C.W. Huck, Advances of infrared spectroscopy in natural product research, Phytochem. Lett. 11 (2015) 384–393.

[35] H. Lin, Q. Chen, J. Zhao, P. Zhou, Determination of free amino acid content in Radix Pseudostellariae using near infrared (NIR) spectroscopy and different multivariate calibrations, J. Pharm. Biomed. Anal. 50 (5) (2009) 803–808.

[36] E. Teye, C. Elliott, L.K. Sam-Amoah, C. Mingle, Rapid and nondestructive fraud detection of palm oil adulteration with Sudan dyes using portable NIR spectroscopic techniques, Food Addit. Contam. Part A. 36 (11) (2019) 1589–1596.

[37] A.P. Craig, A.S. Franca, L.S. Oliveira, Evaluation of the potential of FTIR and chemometrics for separation between defective and non-defective coffees, Food Chem. 132 (3) (2012) 1368–1374.

[38] Z. Keshavarzi, S. Barzegari Banadkoki, M. Faizi, Y. Zolghadri, F.H. Shirazi, Comparison of transmission FTIR and ATR spectra for discrimination between beef and chicken meat and quantification of chicken in beef meat mixture using ATR-FTIR combined with chemometrics, J. Food Sci. Technol. 57 (4) (2020) 1430–1438.