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Spectroscopic Methods for Fresh Food Authentication: An Overview

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6.1 Introduction

Consumers recognize the value of the information supplied on food labels, including the description of the ingredients and information about the production processes applied to the final product. The food consumer's choice often reflects lifestyle, religion, awareness of the nutritional properties of food and health concerns. In fact, the identification and authentication of food play an important role in a healthy diet. The verification and reporting of food product components is therefore needed to prevent the practice of adulteration [40].

Consumers have, in particular, become more demanding in their meat and fish consumption, in terms of quality, safety, and the origin of the products they consume. Recent reports into the occurrence of food fraud suggest that an effective identification of the species as part of food authentication is required (Andrée et al. 2010, Ballin 2010, Standal et al. 2010, Lin et al. 2014).

Animal products, particularly meat and fish, can be targets of adulteration, such as the substitution or removal of ingredients, addition of other proteins from various origins, and the addition of food additives and genetically modified organisms (GMO) not described on the label, often contributing to increased financial profits. The authentication and determination of quality meat and fish is of great importance in preventing fraud which negatively impacts the food industry and causes health problems for the consumer (Meza-Márquez et al. 2010, Cawthorn et al. 2013). For example, the substitution of fresh meat and fish for frozen-then-thawed products is a typical commercial fraud which may cause economic loss and food safety and quality issues for consumers. These products are characterized by an increased susceptibility to microbial grow and color changes. Temperature fluctuations can result in the formation of ice crystals (Cozzolino and Murray 2004, Ballin and Lametsch 2008, Fajardo et al. 2010, Standal et al. 2010, Alamprese et al. 2013, Ottavian et al. 2013, Lin et al. 2014) due to the migration of water vapor from the product to the surface, resulting in poor quality food products. This defect is recurrently found in frozen foods which have been inadequately controlled.

Food authentication depends on the establishment of databases that contain information about the origin of food including the biological and geographic origin, species, production methods, and other critical information. However, there is a need for reliable analytical methods that can verify the geographic origin of food apart from their biological origin. Un-targeted spectroscopy approaches combined with chemometric analysis were investigated for their potential to classify the geographical origin of meat and predict its value (Sun et al. 2012b). An overview of analytical methods for determining the geographical origin of food products can be found in Luykx and van Ruth's (2008) paper.

Modern food inspection is under an ever increasing demand for efficiency in the use of resources, either human or material, and for achievement of purpose through optimal inspection planning and the use of new methodologies. Spectroscopy, based on analytical technology tools, in combination with dynamic predictive models may bring these goals closer to reality (Thygesen 2012). Dynamic chemometric methods have been used in food inspection for quality monitoring in food processing industries (Singh and Jayas 2013). Singh and Jayas (2013) present a discussion on three broad categories of optical sensing techniques, namely, spectroscopic, fiber optic, and imaging. In their work, they describe the working principles, instrumentation, advantages, disadvantages, and the limitations of these techniques. For instance, an ultra-low field magnetic resonance imaging (MRI) system using a high-temperature superconducting quantum interference device (HTS-SQUID) for food inspection was reported in Kawagoe et al.(2016).

There are several methods for the detection of low levels of adulteration (Ballin and Lametsch 2008). Replacement products are often similar to the main material from a biochemical point of view and therefore, adulterant

identification can be particularly difficult (Ghovvati et al. 2009). Recently, researchers have applied various analytical techniques in the detection of food industry fraud. The protein-based methods (Al Ebrahim et al. 2013, Mamani-Linares et al. 2012), the deoxyribonucleic acid (DNA) based methods (Ali et al. 2012, Cammà et al. 2012, Mamani-Linares et al. 2012, Sakaridis et al. 2013, Zhang 2013, Karabasanavar et al. 2014, Lin et al. 2014), the real-time polymerase chain reaction (PCR) techniques and analysis of triacylglycerol (Kesmen et al. 2009, Fajardo et al. 2010, Soares et al. 2010, Druml et al. 2015) and methods based on fat (Abbas et al. 2009, Rohman et al. 2011), have become increasingly important. However, these methods are laborious, technically demanding, slow, invasive, expensive, destructive, and require sophisticated laboratory procedures and highly qualified employees. Moreover, they are not suitable for real-time applications (Damez and Clerjon 2008).

The various multidimensional analytical approaches that permit authentication of food can be divided into targeted and un-targeted methods. The classical authenticity assessment of food is usually based on the analysis of specific marker compounds, which are indicative for a certain property of the product (Herrero et al. 2012). Given that most adulterants are unknown, they are difficult to recognize using the targeted screening approaches typically used in food laboratories. The industry needs non-targeted methods to analyze samples for adulterants to provide proof of origin or to prevent deliberate or accidental undeclared admixture of food samples (García-Cañas et al. 2012). Food fingerprinting approaches are based on a high-throughput screening of samples with the purpose of differentiation or classification of samples. The investigation of food fingerprints provides high potential with regard to the characterization and verification of food identity. These approaches are usually based on spectroscopic and spectrometric data, providing the ability for a comprehensive characterization of the investigated matrices. The aim is to differentiate various food fingerprints in terms of, for instance, possible adulterations or their botanical or geographical origin (Esslinger et al. 2014).

There is a growing interest in methods based on spectroscopic techniques because they offer several advantages. Emerging nondestructive mapping technologies for authentication and traceability include nuclear magnetic resonance (NMR) imaging, fluorescence (FS), visible (VIS), near infrared (NIR), mid infrared (MIR), and Raman (RS) spectroscopy, sometimes coupled with Fourier transform technique, and multispectral (MSI) and hyperspectral (HIS) imaging. These are simple, non-destructive, non-invasive, low cost, and allow real-time analysis [32]. All spectroscopic techniques require small samples and no further preparation is necessary. They are powerful tools for conducting adulteration tests (Mamani-Linares et al. 2012). The methods presented in this work might be used as a complement or even constitute an alternative to PCR based DNA (Schmutzler et al. 2015).

The techniques NIR and MIR combined with Fourier transform (the latter so called FTIR—Fourier transform infrared) (Cozzolino and Murray 2004,

Ortiz-Somovilla et al. 2005, Rodriguez-Saona and Allendorf 2011, Mamani-Linares et al. 2012, Alamprese et al. 2013, Morsy and Sun 2013b, Rohman et al. 2011, Meza-Márquez et al. 2010), RS (Abbas et al. 2009, Boyaci et al. 2014, Zając et al. 2014, Zhao et al. 2015) and NMR (Rezzi et al. 2007, Aursand et al. 2009, Standal et al. 2010) combined with multivariate statistical methods were largely applied in the authentication of foodstuffs.

In addition, ultraviolet (UV) based spectroscopic methods were used in meat and fish adulteration studies (Alamprese et al. 2013). In recent years, these techniques have received much attention for safety inspection and the quality of food and meat and related products (Kamruzzaman et al. 2013, Barbin et al. 2013, Kamruzzaman et al. 2016, Kamruzzaman et al. 2012, Ma et al. 2015).

This work is an up-to-date literature revision applied to the detection of fresh meat and fish adulteration using spectroscopic methods which could be important for future research and in the development of equipment and methods for commercial markets, allowing detection of adulteration analysis very quickly.

6.2 Methods of Vibrational Spectroscopy

6.2.1 Visible and Near Infrared Spectroscopy

VIS and NIR spectroscopies offer a number of important advantages when compared to traditional chemical methods. These methods deal with the VIS and the NIR region of the electromagnetic spectrum, from about 750 to 2500 nm, corresponding to a photon of energy from 4000 to 13000 cm^{-1} (Huck 2015). When using the NIR region, the spectra can be recorded in reflection or transmission. The interaction of the radiation with matter provides information about the presence of functional groups (Lohumi et al. 2015, Huck 2015, Porep et al. 2015).

In comparison with other vibrational spectroscopic methods, NIR is considered a time consuming procedure and the detector is often a source of noise. However, the use of an interferometer reduces the time of analysis through the single output signal (spectrum) which has all the infrared frequencies encoded therein. A Fourier transform is also necessary to extract the information from the spectrum. The interferometer coupled to Fourier transform has started to receive great attention for its use in the quantitative analysis of edible fats and oils (Gouvinhas et al. 2015). The attenuated total reflectance (ATR), diffuse reflectance, high-yield transmission and diffuse transmission cells are measuring methods used together with Fourier transform (Lohumi et al. 2015).

Diffuse reflectance or trans-reflectance spectroscopy has also gained attention in the control of fraud in the food industry. However, adequate overall

reflectance is the most widely adopted method for analyzing the quality and authenticity of the final food product (Porep et al. 2015). Reflectance infrared spectroscopy has allowed discrimination between fresh meat and fish products from frozen-then-thawed and mixtures of species that are not permitted in the final product (Mamani-Linares et al. 2012).

NIR spectroscopy has been applied in industrial online setups using a fiber optic probe and in the laboratory to detect different veal meat adulteration with pork parts (Schmutzler et al. 2015). Samples of veal meat with different percentages of pork parts were analyzed using chemometric methods. Control samples without adulteration (100% veal) and samples with increasing levels of adulteration were prepared in 10% steps from the original until a composition of 50% veal and 50% pork parts was obtained. Figure 6.1 shows the second derivative of spectra from 6200 to 5480 cm^{-1} measured with the laboratory setup as a function of the adulteration of the veal meat with pork. Close connection between the signal intensity and the level of adulteration from genuine (no adulteration) samples up to 50% adulteration were found at 5940 cm^{-1} (1683 nm), 5908 cm^{-1} (1693 nm), 5892 cm^{-1} (1697 nm), 5868 cm^{-1} (1704 nm), 5776 cm^{-1} (1731 nm), 5756 cm^{-1} (1737 nm), 5668 cm^{-1} (1764 nm), 5648 cm^{-1} (1770 nm) and 5492 cm^{-1} (1821 nm). Principal component analysis (PCA) was used to obtain a tridimensional projection of samples and to observe the relation between the genuine product and samples with adulteration. It was possible to notice an absence of associations from 20% to 50% of adulteration, with one and two principal components (PC), for laboratory

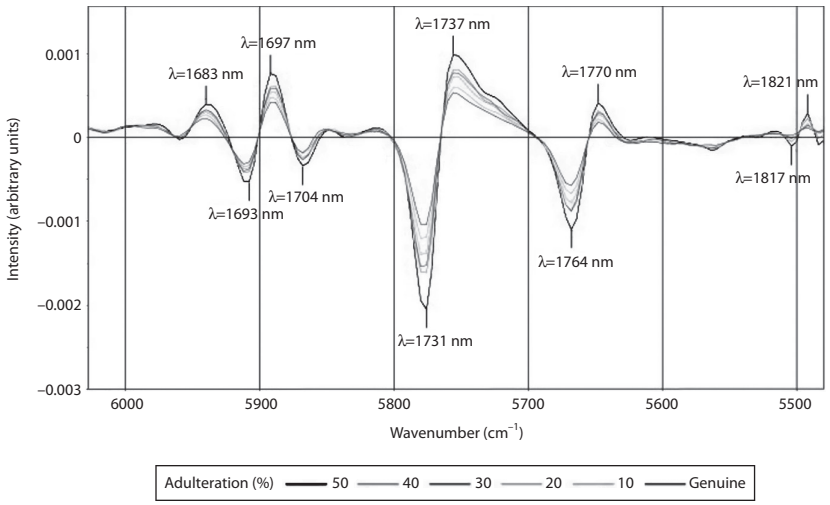

FIGURE 6.1
Second derivative of spectra (from 6200 to 5480 cm^{-1}) measured with the laboratory setup as a function of the adulteration of the veal meat with pork. Adulteration levels from genuine (no adulteration) up to 50%, in 10% steps. (Reprinted with permission from Schmutzler M. et al., *Food Control*, 57, 258–267, 2015.)

and the industrial setups, respectively. However, three PCs were necessary for models applied to the on-site setup. Data from PCA was used as input for classification and validation using support vector machines (SVM). The SVM allowed correct calibration values of discrimination of 94.4% for the laboratory, 91.7% for the industrial and 77.8% for the on-site analyses to be achieved (Schmutzler et al. 2015).

In a study conducted by Mamani-Linares et al. (2012), VIS and NIR reflectance spectroscopy or trans-reflectance methods were used to discriminate meat and meat juices from three livestock species. Meat samples from beef, llamas, and horses were purchased from different butcher shops and supermarkets. 79 samples of *Longissimus lumborum*, 500 g each, were used: 31 of beef, 21 from llama and 27 from horse were thawed at 4°C for 24 h and stored for 4–6 h before measuring the spectra. Another 58 samples of the same muscle (20 of beef, 19 from llama, and 19 from horse) were used to obtain the meat juice. They concluded that the VIS-NIR spectroscopy coupled to PCA, and with partial least squares regression (PLS-R), is a useful tool to discriminate between different species. In addition, it is useful to discriminate the geographical origin and the production system (Mamani-Linares et al. 2012).

The potential of UV-VIS, NIR, and MIR spectroscopies coupled with the chemometric techniques PCA, PLS-R, and linear discriminant analysis (LDA) enabled the detection of minced beef adulteration with turkey meat (Alamprese et al. 2013). Each batch was separately minced and then used to prepare (in duplicate) seven mixtures of bovine meat added with different percentages of turkey meat: 5%–50%, in 5% steps. With NIR, two PCs explained 98% of the total variance and for MIR the first two PCs explained 82.3%. LDA correctly classified 78.6% in the UV-VIS, 88.3% in the NIR and 84.8% in the MIR. PLS-R allowed construction of models with the root mean square error of cross-validation (RMSECV) and the root mean square error of prediction (RMSEP) slightly smaller than for NIR (Alamprese et al. 2013).

NIR has the potential to detect and quantify different adulterants in fresh and frozen-then-thawed minced beef. In addition to the pure beef and pork, fat trimming and offal samples, a series of mixed samples in the range of 10%–90% (w/w) from pork ($n = 144$) and 10%–80% of fat trimming ($n = 112$) was prepared (Morsy and Sun 2013a). The mixtures of samples adulterated with offal were prepared in the range of 2.5%–30%. The PLS-R had determination coefficients (R^2) of 0.96, 0.94, and 0.95 with standard error of prediction (SEP) of 5.4%, 5.1%, and 2.1%. Models based on PLS-R/DA and LDA distinguished between the unadulterated and adulterated classes with a classification of 100% (Morsy and Sun 2013b).

The NIR combined with chemometric analysis was used for data analysis to classify the geographical origins of lamb meat (Sun et al. 2012a). The δ13°C and δ15°N values of defatted lamb meat (Alxa League [37°53′N, 105°23′E, $n = 20$], XilinGol League [42°21′N,115°08′E, $n = 19$] Chongqing City [30°50′N, 108°24′E, $n = 20$] and Heze City [34°48′N, 116°04′E, $n = 20$] and Hulunbuir City [49°06′N, 119°40′E, $n = 20$]) were determined by isotopic ratio mass

spectrometry (IRMS).The analytical precision was lower than 0.2‰ for both. FDA/PLS-R and LDA gave a total correct classification of 88.9% and 75% to the five individual region samples, respectively. The PLS-R/DA and LDA correctly classified 100% of the samples from pastoral and agricultural regions. For PLS-R calibration models, the obtained R^2 value was 0.76 and 0.87 for predicting δ13°C and δ15°N, respectively. The first three PCs explained 98% of the total variance.

The adulteration of beef with pork and chicken was studied by Bilge et al. (2016). The beef samples were adulterated with pork and chicken (concentrations between 10% and 50%). The PLS-R method was used for evaluating laser-induced breakdown spectroscopy (LIBS) spectral data and RMSEC and R^2 values of 2.67 and 0.994 were obtained for beef adulterated with pork. A 83.4% correct discrimination rate between beef, pork, and chicken was achieved by PCA (Bilge et al. 2016).

The samples (43 adulterated and 12 controls), originating from dismantled criminal networks by the Brazilian Police, were analyzed using chemical parameters and ATR in conjunction with FTIR spectroscopy (Nunes et al. 2016). This fraud consisted of injecting aqueous solutions of non-meat ingredients (NaCl, phosphates, carrageenan, maltodextrin, collagen) into bovine meat. The PCA model of ATR-FTIR spectroscopy data was obtained with 4 latent variables (LV), accounting for 95.7% and 26.7% of variance in X and Y blocks, respectively. The PLS-R/DA model correctly detected 91.0% of the adulterated samples (Nunes et al. 2016).

Several strategies have been proposed for determining the substitution of fresh fish with frozen-then-thawed fish (Ottavian et al. 2013). One of the first strategies consists of using the PLS-R/DA method to classify the fresh and frozen-then-thawed status of each sample considering the species altogether. In another approach, a two-level cascade arrangement of PLS-R/DA was developed. In the first level, a PLS-R/DA was used to classify the samples according to their species and in the second level, a different PLS-R/DA discriminated between fresh and frozen-then-thawed samples. In a third strategy, an orthogonal PLS-R/DA was used to remove the information from the spectral data which is not related to the fresh and frozen-then-thawed status of the samples. Depending on the strategy, the overall obtained calibration accuracies ranged between 80% and 91%. The PCA explained 97% of the total variability (Ottavian et al. 2013).

NIR and VIS-NIR spectroscopy has also been used to distinguish fresh from frozen-then-thawed swordfish cutlets (Fasolato et al. 2012). Thirty specimens of swordfish were caught using traditional hooks and fishing. The relevant data was recorded to maintain sample traceability. The samples were vacuum-packed in polyethylene bags and three of them were refrigerated at 2°C. The remaining samples were frozen and stored: 30 samples at −18°C and the remaining 30 samples at −10°C for 30 days. Before analysis, the frozen samples were thawed overnight in the lab at 2°C. The first three PCs of the PCA explained 87.2% of the variability and with milling treatment the

first three PCs explained a higher value (94.8%). The samples were classified using VIS-NIR spectroscopy with a correct classification of 96.7%, whereas this value for NIR was higher than 90.0%.

In another study to distinguish fresh from frozen-then-thawed fish, *Pagrus major* fish were divided into two equal groups and used for further evaluation (Uddin et al. 2005). For fresh or frozen-then-thawed fish, 54 samples were used soon after being killed, whereas the second lot of 54 fish was kept at −40°C. After 30 days, fish were removed and thawed then evaluated as frozen-then-thawed samples. The fresh or frozen-then-thawed status was investigated and discrimination was carried out by soft independent modeling of class analogy (SIMCA), LDA based on PCA. The investigators obtained a classification of 100%. However, the high percentage of water in the fish is a major limitation for the analysis of samples with this application.

Real-time measurement and noise reduction using NIR spectroscopy requires Fourier transform. While this methodology allows detection of small molecules, water interference is a major drawback. However, this method does allow reading through glass or polypropylene containers.

6.2.2 Mid-Infrared Spectroscopy

MIR spectroscopy is fast, non-destructive and does not involve laborious sample preparation. It is an attractive option to identify and quantify adulteration and chemical composition of samples (Rohman and Man 2011). The absorption bands in the MIR region are characteristic of functional groups of molecules (Meza-Márquez et al. 2010, Zhao et al. 2014). The MIR region can be divided in the functional group region, from 4000 to 1500 cm^{-1}, and the fingerprint region, from 1500 to 500 cm^{-1}(Lohumi et al. 2015). MIR spectroscopy associated with FTIR spectroscopy and multivariate analysis require low sample volume and are environmentally friendly. Furthermore, FTIR spectroscopy in combination with PLS-R regression technique and PCA are powerful tools for quantification and classification of adulterants (Rahmania et al. 2015).

Overall, these methods are fast and effective in the detection of contaminants and adulterants (Meza-Márquez et al. 2010, Rodriguez-Saona and Allendorf 2011). Some countries have regulations to determine whether foods are safe, authentic and protect consumers requiring Halal and as such, investigators have conducted studies into the detection of adulterants in this type of food (Kurniawati et al. 2014).

In research investigating the adulteration of Gamo meat (*Dama dama*) with different percentages of goat meat (0%, 25%, 50%, 75% and 100%), samples were stored at 3°C for periods of time between 12–432 h. The methods used were microbiological analysis, measurement of color, lipid oxidation based on the thiobarbituric acid reactive substances method (TBARS), FTIR, sensory analysis, and statistical methods of multivariate analysis. In Figure 6.2, the average FTIR spectrum of different mixture proportions stored at 3°C

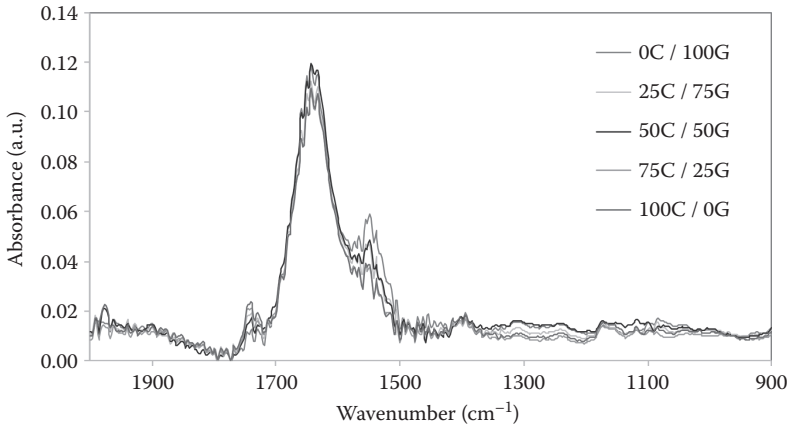

FIGURE 6.2
FTIR spectra of hamburgers containing different percentages of Gamo (G) and Goat (C) from 2000 to 900 cm^{-1}. (Reprinted from Silva, A.C.C. da, Study of adulteration of fresh meat using spectroscopic, microbiological, chemical, physical and sensory methods. B.Sc., School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Portugal, 2014.)

for 0 h between 2000 and 900 cm^{-1} can be observed. A peak at approximately 1639 cm^{-1} due to the presence of water (O-H stretch) with simultaneous contribution of amide I (C=O) is also visible. A second peak at 1550 cm^{-1} can be associated with the amide II (N-H, C-N). The peak at approximately 1460 cm^{-1} can be assigned to fat (ester CO). The absorptions in the region of 950–1200 cm^{-1} may reflect the content of carbohydrates, especially muscle glycogen. The amide content III can be viewed at about 1300 cm^{-1} and amino acid side chains between 1480 and 1800 cm^{-1}. Figure 6.3 shows the graph of observations of a LDA, where the discriminant factors F1 and F2 explain 96.63% of the total variance. With this analysis, the authors obtained a clear distinction between each blending percentage. The accuracy and performance of the model that relates the current and estimated values obtained from FTIR spectra is shown in Figure 6.4 and at $t = 27$ and $t = 0$ h 432 h, respectively. The PLS-R was used in order to validate and calibrate the model used. The PLS-R model was conducted to determine the relationship between the predicted values and the measured values of the mixtures. The R^2 coefficient shows high values and the RMSEC and RMSECV show low values which demonstrates that the PLS-R model has good predictive accuracy and performance.

In separate research, the adulteration of beef meatballs with the meat of rat (*Rattus argentiventer*) was studied by Rahmania et al. (2015). Rat meat was obtained from farmers while beef was purchased from several local markets. During the preparation of calibration samples, rat meat and beef was prepared by mixing rat meat at concentrations of 0%, 10%, 20%, 35%, 50%, 65%, 80%, and 100% in beef. The FTIR spectroscopy in combination with PLS-R

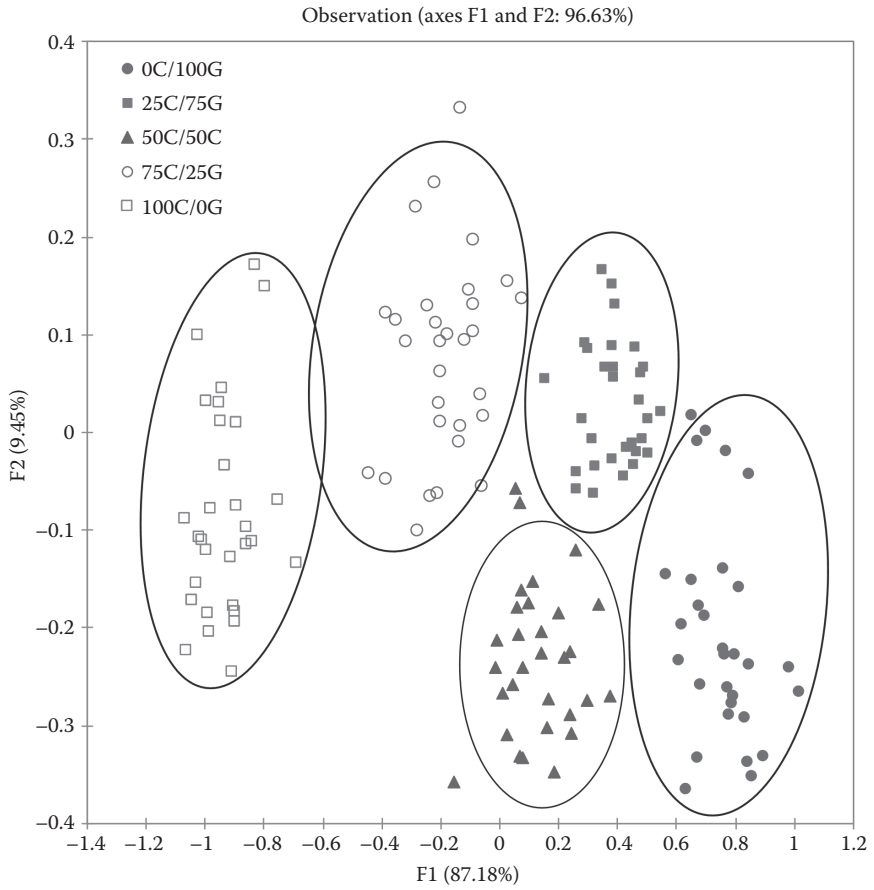

FIGURE 6.3
Projection of the samples according to the storage time (t0 to t14) and the type of meat with two batches where G samples correspond to Gamo, and C samples correspond to goat. (Reprinted from Silva, A.C.C. da, Study of adulteration of fresh meat using spectroscopic, microbiological, chemical, physical and sensory methods. B.Sc., School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Portugal, 2014.)

and PCA multivariate calibrations were used for the differentiation between rat meat and beef meatballs. The frequency region from 750 to 1000 cm^{-1} was selected during PLS-R and a R^2 value of 0.993 and root mean square error of calibration (RMSEC) of 1.79% was obtained. The PCA modeling method correctly classified the meatball sample with 100% rat meat and 100% beef.

In a similar study, the investigators prepared oils of pig (lard), lamb, beef, and chicken. FTIR and GC analyses were performed. For calibration, a training set of 30 samples consisting of lard, body fats of beef, chicken, and mutton with certain concentrations were prepared. Each sample was subjected to FTIR analysis and gas chromatography (GC). PCA showed that PC1

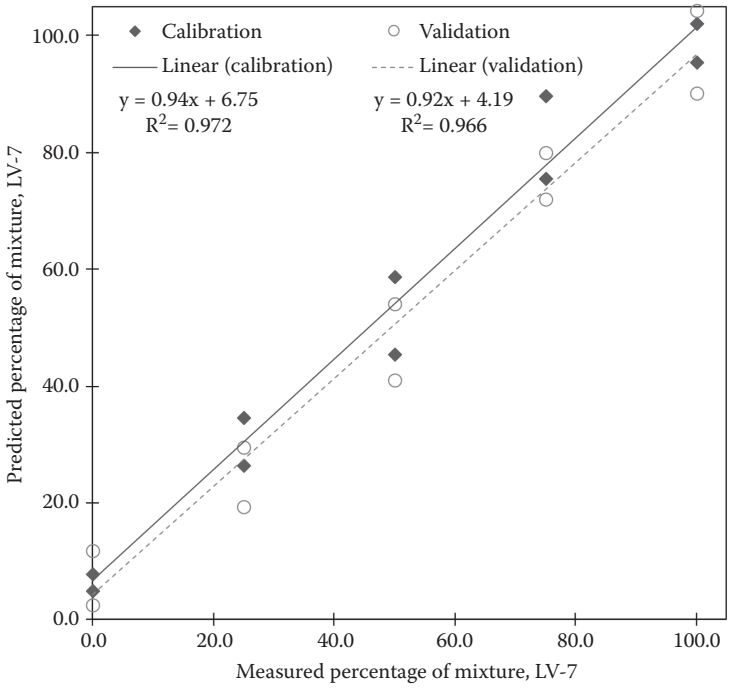

FIGURE 6.4
Illustration of the quality of the PLS-R model conducted to determine the relationship between the predicted values and the measured values of the mixtures. (Reprinted from Silva, A.C.C. da, Study of adulteration of fresh meat using spectroscopic, microbiological, chemical, physical and sensory methods. B.Sc., School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Portugal, 2014.)

accounted for 57% of the variation, while PC2 explained 25% of the variation, and PC3 contributed to 13% of the variation. These three first PCs can describe more than 95% of the overall variation (Rohman et al. 2012).

A separate research study by Rohman et al (2011) investigated the adulteration of beef meatballs with pork. The calibration sets were prepared by spiking pork to beef meatball in the concentrations of 1.0%, 3.0%, 5.0%, 10.0% and 25.0%. Samples containing 100% beef and 100% pork were also made to observe the spectral differentiation. The adulteration was detected using FTIR spectroscopy and PLS-R. This regression method was used to develop a calibration model and a R^2 value of 0.999 was obtained.

In another similar research study, pork fat (lard) and beef fat were obtained through a rendering process of the corresponding animal (Kurniawati et al. 2014). The fatty acid composition of lard and beef fat was carried out using GC with a flame ionization detector (GC-FID). A set of standards consisting of lard in beef fat was prepared by mixing both types of fat in the concentration range of 0%–100%. FTIR spectroscopy in combination with PLS-R

and PCA was used for the detection of the substitution of beef fat with lard. PLS-R was characterized by a high R^2 value (0.998). while PCA was used successfully in the region from 1200 to 1000 cm^{-1}.

The adulteration of high quality beef steak with horse meat, beef fat trimming and soybean protein was studied by Meza-Márquez et al. (2010). The beef steak samples were minced using a food processor. Horse meat samples and beef fat trimming were minced separately in the same way as lean beef. Textured soybean protein was rehydrated according to instructions on the packet label. Samples of each type of adulterated mixture (minced lean beef-horse meat, minced lean beef-textured soy bean, and minced lean beef fat trimmings) ranging from 2% to 90% w/w adulterant concentration were prepared in increments of 2%. A methodology using MIR spectroscopy in tandem with chemometrics was developed to discriminate between pure minced meat and adulterated samples. The results of the developed PLS-R models showed, in the region 1800–900 cm^{-1}, values of R^2 greater than 0.99. The SIMCA model showed 100% correct classification for minced beef and for beef adulterated with horse meat, beef fat trimmings or soy protein.

A common adulteration process is the substitution of fresh food by frozen-then-thawed food. Fresh and frozen-then-thawed samples of offal-adulterated beef burgers were analyzed using ATR-FTIR technique and chemometrics methods (Zhao et al. 2014). The authentic beef burgers were produced in two groups, called lean and fat, which correspond to higher (lean) and lower (fat) quality levels. The beef burgers in each of the two groups were made on separate occasions beginning with the highest lean meat content and moving to the lowest. A total of 82 fresh beef burger samples (36 authentic + 46 adulterated) and 82 frozen-then-thawed beef burger samples (36 authentic + 46 adulterated) were prepared. The first three PCA components accounted for 72.9%, 11.3%, and 8.4% of the variability. From the PLS-R models, 100% were accurately classified in calibration and in validation. The SIMCA efficiency values varied from 0.57 to 0.87 for fresh and from 0.62 to 0.91 for frozen-then-thawed beef burgers.

MIR spectroscopy requires the preparation and dilution of samples. In this methodology the interference of water contained in the food may occur. However, MIR with chemometric methods is a promising technique that allows detection of larger functional groups.

6.2.3 Fluorescence Spectroscopy (FS)

Fluorescence is a physical process associated with the emission of photons upon molecular transition from the electronic excited state to the ground state. The emission of photons occurs at a higher wavelength than the wavelength of the incident excitation source (Bridier et al. 2015). FS involves the application of a light beam in the sample, causing excitation of electrons in molecules of certain compounds and the emission of low energy light. It's a fast, sensitive, and non-destructive technique (Karoui et al. 2006, Damez

and Clerjon 2008). A stable fluorescent label is of crucial importance for the sensitivity of quantitative and qualitative detection as well as for the contrast of fluorescent microscopic imaging. Covalently bound fluorescent labels are a promising tool for obtaining highly stable fluorescent labeled particles for a considerable period of time. However, negligible leakage and low signal intensity have also been reported (Weiss et al. 2006).

This method, combined with multivariate statistical analysis, is an effective tool for the discrimination of different beef muscles in relation to the age of the animal, while chemical and mechanical properties make it possible to evaluate the quality and adulteration of the food (Sahar et al. 2016). There are different applications of FS: heterocyclic particular aromatic amines (HAA), tryptophan fluorescence, and nicotinamide adenine dinucleotide phosphate oxidase (NADPH) (Karoui et al. 2006). The NADPH fluorescence spectrum enables differentiation of fresh from frozen-then-thawed fish and the simplicity of this method also allows the extension of the VIS spectroscopy characterization efficiency of the fish (Karoui et al. 2006).

FS also enables aromatic acids of the amino acids to be observed. When using excitation at 250 nm the emission will be at 305–400 nm. For observation of proteins folding, tryptophan fluorescence can use excitation at 290 nm and emission at 305–400 nm (Albani 2012).

This technique can therefore use the presence of fluorescent molecules such as tyrosine, phenylalanine and tryptophan in proteins to detect the environmental and biological origin of samples (Karoui et al. 2006). Adipose tissue contains fluorescent molecules that are specific for fat. Few studies have been conducted with this method in food. However, NADH/FS and tryptophan fluorescence in combination with chemometric methods enables identification of both fresh and frozen-then-thawed fish fillets (Karoui and Blecker 2011, Karoui et al. 2006). Regarding the PCA of tryptophan fluorescence spectra, the first two PCs explained 55.9%and 36.9% of the total variance. On the other hand, the PCA of NADH fluorescence spectra led to 84.9% and 12.1% variance for the first two PCs. Then, PCA applied to the factorial discriminant analysis (FDA) method obtained a 100% accuracy when using the calibration set (Karoui et al. 2006).

The conventional and the synchronous fluorescence spectroscopic method of excitation-emission in combination with chemometric methods, namely predictive and descriptive methods, determines the changes in foodstuffs during technological process and storage. Front-face FS has the potential to reduce the analysis time and costs compared to the enzymatic and biochemical methods (Karoui and Blecker 2011).

6.2.4 Raman Spectroscopy

Raman spectroscopy can be used to observe vibrational, rotational, and other low-frequency modes in a molecule and/or a system. The vibrational modes provide a major contribution to knowledge of the chemical constitution of a

specific analyte. Raman spectroscopy depends upon the inelastic scattering of monochromatic light, usually from a laser in the ultraviolet, visible or near infrared wavelengths (Li-Chan 1996).

Raman spectroscopy was used to determine authentication and quality of foodstuffs (Lohumi et al. 2015). This technique provides specific information about and allows the determination of lipids, proteins, and carbohydrates, and it can be employed to classify microorganisms (Argyri et al. 2013). It has the capacity to provide information on the chemical structure of molecules without causing changes in the samples (Boyacı et al. 2014). It is a very promising method and has high potential for evaluating the quality of foodstuffs during handling, processing, and storage (Boyacı et al. 2014, Zając et al. 2014).

There are techniques that can be used to improve the Raman signal, in particular scattering Raman spectroscopy (SRS) (Tipping et al. 2016), coherent anti-stokes Raman (CARS) (Roy et al. 2010), resonance Raman spectroscopy (RRS) (Wächtler et al. 2012), shifted excitation Raman difference spectroscopy (SERDS) (Sowoidnich and Kronfeldt 2012), and surface-enhanced Raman scattering (SERS) (Hakonen et al. 2015). Reducing the Raleigh scattering allows high-quality spectra extension to be obtained. SERS is a powerful tool for characterizing a wide range of analytes when combined with biologically relevant nanostructures (Shrestha et al. 2014). The combination of RS with Fourier transform provides high spectral resolution with effective wavelength accuracy and allows the degree of opening of fatty acids in foods to be estimated (Lohumi et al. 2015).

The RS coupled with PCA was developed for the rapid determination of beef adulteration with different concentrations of horse meat. The beef samples were provided from local supermarkets while horse meat samples were bought from local markets. In the scope of study, beef samples containing 0%, 25%, 50%, 75%, and 100% by weight of horse meat were investigated (Boyaci et al. 2014). The PCA exhibited a first PC explaining 96.3% and a second PC explaining 3.2% of the total variance. The developed model system was good enough to differentiate adulterated samples. This method has shown good results because of the short time analysis and simple preparation of the sample (Boyaci et al. 2014). Figure 6.5 illustrates the Raman spectra of meat samples collected between 200 and 2000 cm^{-1}. Raman bands at 555, 678, 815, 1032, 1265, 1392, 1611, and 1706 cm^{-1} were observed in the spectra of both horse and beef samples. The spectral difference between the samples arose from the unique bands of horse fat that were positioned at 919, 974 and 1215 cm^{-1}.

In another study, fresh meat species (cattle, sheep, goat, buffalo, pig, fish, chicken, and turkey) were purchased from the local markets and slaughterhouses and kept in refrigerated conditions (Boyaci et al. 2014). These were utilized in the preparation of salami products and fat was extracted from each meat sample. Raman spectroscopy coupled with PCA differentiated the origin of the meat and meat products. Principal components PC1 and PC2 explained 85.1% and 6.4% of the variance, respectively. After the third

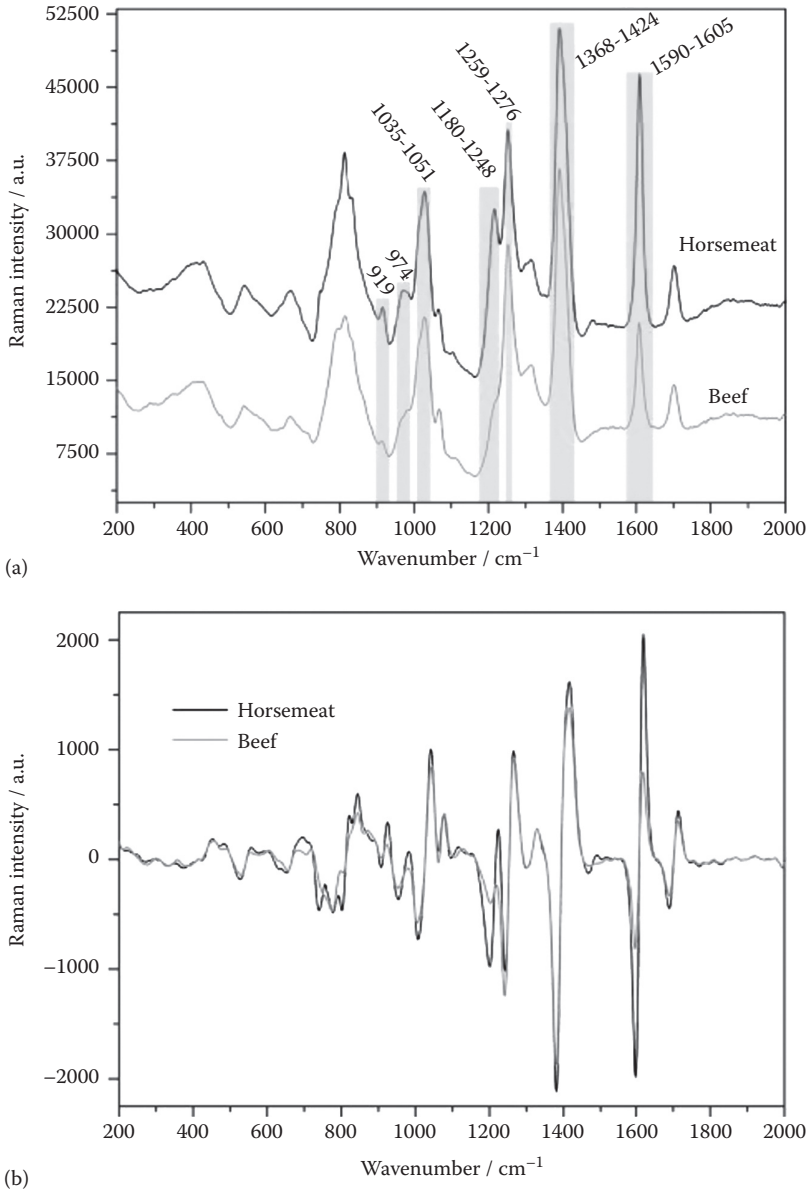

FIGURE 6.5
Original (a) and first derivative (b) Raman spectra of horse meat and beef samples. (Reprinted with permission from Boyaci, I.H. et al., *Food Chemistry*, 148, 37–41, 2014.)

derivative was applied to the spectra, PC1 and PC2 explained the variance of 96.3% and 2.2%, respectively (Boyaci et al. 2014).

In a similar study, fresh samples of horse back muscles were purchased from a local butcher (Zając et al. 2014). The meat mixture was prepared from horse meat and beef in a composition of 1:4, 1:2, 3:4, respectively. The content of horse meat in the samples with beef was detected using the Raman bands at 937, 879, 856, 829, and 480 cm^{-1} (Zając et al. 2014).

Al Ebrahim et al. (2013) applied a 671 nm (50 mW) microsystem diode laser to study the applicability of the RS in the distinction of beef and horse. The fresh muscles of beef and horse were purchased from local butcher shops. The muscles were cut into 2 cm thick slices and packed separately. All slices were stored at 5°C for a period of 12 days in a laboratory refrigerator. The PCA method was applied for data evaluation and presented the PC1 and the PC2 explaining 79% and 18% of the total variance, respectively. Raman spectroscopy showed changes in the spectra for proteins, lipids, and water muscle meat.

In another study, SERDS was applied for separation of the meat samples into distinct groups (Sowoidnich and Kronfeldt 2012). For each animal species, beef (rump steak), pork (loin chops), chicken (breast), and turkey (breast), 12 randomly chosen slices of fresh meat were bought in a local supermarket and measured at the day of purchase for separation of the meat species into four distinct groups with the PCA. The SERDS method showed enormous potential and demonstrated a quick breakdown for classification of different species of meat.

In a study conducted by Ellis et al. (2005), RS was applied to the identification of meat and poultry based products and showed potential for the rapid assessment of adulteration of food. Samples of pre-packed meat (lamb, beef, pork) and poultry (chicken [skinless breast fillets] and turkey [skinless breast fillets]) were acquired, and for the subsequent experiments, chicken (skinless breast fillets and legs with skin) and turkey (skinless breast fillets and legs with skin) were purchased from retail outlets and stored at 4°C. Raman spectra were collected using an infrared diode laser at 785 nm, using a Renishaw 2000 Raman probe system together with the Renishaw WiRE Grams software package and a CCD detector. Spectra were collected for 10 s and 1 accumulation over the wave number range 100 cm^{-1} to 3000 cm^{-1}. PCA and genetic algorithms multiple linear regression (GA-MLR) and discriminant multiple linear regression (D-MLR) were used.

The Fourier transform Raman spectroscopy (FT-RS) was chosen for the discrimination of animal fat (Abbas et al. 2009). To assess the technique, four mixtures were analyzed: mixture 1 contained 50% bovine, 50% ovine–porcine; mixture 2 contained 80% bovine, 20% porcine; mixture 3 contained 50% bovine, 50% ovine–porcine–avian–former foodstuffs; and mixture 4 contained 55% bovine, 15% ovine, 30% porcine, and traces of avian fat. PCA was applied and the first PC represented 67% of the variance while the second one explained 24% of the variance. PLS-R/DA model allowed

discrimination between poultry samples and other components (porcine, bovine, ovine fats, and fish oils) obtained a sensitivity and specificity of 0.917 and 1.000, respectively

In a study by Beattie et al. (2007), RS was used to classify adipose tissue from four different species (chicken, beef, lamb, and pork). The samples used in this investigation were from beef, lamb, pork, and from the breast of chicken. In order to obtain a wide range of variation within each species, the samples were obtained from a number of commercial outlets and encompassed a wide variety of breeds and feeding regimes. Complementary fatty acid composition was determined by GC. PCA data reduction on the adipose Raman spectral data set was followed by LDA and allowed 97.6% correct classification of the samples, while using the PLS-R/DA method further improved the correct classification rate to 99.6%

Beef offal adulteration of beef burgers was studied using dispersive Raman spectroscopy and multivariate data analysis to explore the potential of these analytical tools for detection of adulterations in comminuted meat products with complex formulations (Zhao et al. 2015). Fresh beef (brisket), beef offal (kidney, liver, lungs, and heart) and beef fat were purchased from local stores and stored overnight at 4°C. Authentic beef burgers comprised two groups, higher quality burgers contained only lean beef and beef fat; lean meat content varied between 80% and 100% of the burger in 2.5% increments, with fat accounting for the remainder and lower quality burgers contained rusk (5%) and water (20%) in addition to lean beef (45%–65% in 2.5% increments) and beef fat (30%–10% in 2.5% increments). Adulterated beef burgers were formulated with lean beef, beef fat, water, rusk, and offal (liver, lung, kidney, and heart). Multivariate data analysis methods of the DRS spectra comprised PLS-R/DA, SIMCA, and PCA. In relation to the PCA, the first three PCs described 61%, 34%, and 3% of variance, respectively, in the frozen-then-thawed beef burger spectral data set. PLS-DA models correctly classified 89%–100% of authentic and 90%–100% of adulterated samples. The SIMCA has a specificity of 0.64–0.89 and a sensitivity of 0.95–1. In comparison with other studies by these authors (Zhao et al. 2014), using the model of PLS-R/DA, adulterated samples obtained a 74%–91% value for NIR, 73%–100% for Fourier transform-NIR, and 81%–100% for RS. The SIMCA efficiency was 0.62–0.91 for NIR, 0.81–0.94 for Fourier transform-NIR and 0.88–0.97 for DRS.

Raman spectroscopy provides a high rating of detection of adulteration compared to other spectroscopic methods. Water interference doesn't occur when using the RS technique, providing specific information on the matrices of the samples. Samples can be read through glass or polymer packaging. However, the heating from the laser radiation can destroy the samples or hide the Raman spectrum. This process requires only a small sample. It has the ability to supply information about the chemical structure of molecules without causing any alterations. This technique is a new approach to the determination of meat adulteration and showed reasonable results for the determination of fraud meat mixtures.

6.2.5 Nuclear Magnetic Resonance Spectroscopy

NMR is based on the emission and absorption of energy in the radio frequency range of the electromagnetic spectrum. The most commonly measured nuclei are ^{1}H and ^{13}C, the first for proteins because they are rich in hydrogen and the second for larger proteins and lipids (triglycerides) (Aursand et al. 2009, Jakes et al. 2015). The shielding effect of electrons, which decreases resonance frequencies of nuclei, varies with the chemical environment and is, therefore, characteristic of specific structural fragments of organic compounds (e.g., methyl, methylene, or methine^{1}H nuclei) and their substituents (e.g., OH, NH_2, NH, COOH, CONH) (Mlynárik 2016).

This technique presents advantages compared with other spectroscopic methods for foods with a high water percentage because the protons are easily detected. However, it is expensive and time consuming (Aursand et al. 2009, Jakes et al. 2015, Santos et al. 2014). Low-resolution NMR or time-domain ^{1}H nuclear magnetic resonance (TD-NMR) is an excellent alternative to traditional methods because it is rapid, simple, and has the potential for online and in situ measurements and using permanent magnet technologies, significantly reduces the overall system and running costs (Santos et al. 2014). These benefits make NMR particularly interesting for analyzing the safety of food and consequently, there has been an increase in its use in the food industry (Damez and Clerjon 2013). For example, NMR has shown high sensitivity in differentiating between the structure of muscles in wildlife and farmed animals (Standal et al. 2010). The ^{1}H NMR is also an effective technology for analysis and quantification of triglyceride samples and the use of high resolution (HR) ^{13}C NMR in the analysis of lipids is increasing with lipid analysis being a potential tool for authentication of fish and marine oils (Standal et al. 2010). However, there are few studies with NMR for authentication of meat or fish products.

In a study by Santos et al. (2014), TD-NMR spectroscopy, when combined with univariate and multivariate analysis, provided a valuable tool for tracing the sex and bull race of beef samples. It has been demonstrated that NMR is a fast and accurate method for measuring conjugated linoleic acid (CLA) content in beef samples (Manzano Maria et al. 2010). The beef samples were collected from calves (43 heifers and 56 steer) from different bull race (Angus, Bonsmara, and Canchim) and cows (Simmental-Nellore and Angus-Nellore for cows). The calves were designated according to the bull race and sex, resulting in 14 Angus heifers, 21 Angus steer, 17 Bonsmara heifers, 19 Bonsmara steer, 12 Canchim heifers, and 16 Canchim steer. Carr-Purcell-Meiboom-Gill (CPMG) and Continuous Wave Free Precision (CWFP) pulse sequences were used to obtain time-domain ^{1}H NMR. The PLS-R/DA showed a correct classification higher than 79% either for CPMG or CWFP decays (validation set). The k-nearest neighbor (KNN) showed a correct classification of 75% and 76%, while SIMCA showed a correct classification 66% and 78%, respectively, for the CWFP and CPMG dataset. The SIMCA method obtained a best predictability for the CWFP dataset with correct classification between 85% and 89% for beef samples. ^{1}H NMR coupled CPMG

CWFP and with univariate and multivariate methods obtained a correct classification of more than 80%. The ^{1}H TD-NMR method allowed for authentication and traceability when applied to meat samples.

The 60 MHz ^{1}H NMR method was used to differentiate samples of fresh beef and horse. Peak integration was sufficient to differentiate samples of fresh beef (76 extractions) and horse (62 extractions) using Naïve Bayes classification. Fresh meat samples were purchased from a variety of outlets. It was possible to obtain 100% correct classification of the different samples of beef and horse, exploiting the differences in triglyceride compositions. In relation to the PCA, principal components 1 and 2 described 83% and 12% for lab 1 and 81% and 13% for lab 2, respectively, of variance in fresh beef and horse spectral data set (Jakes et al. 2015). The first two PC scores are plotted against one another in Figure 6.6(a) and (b), with symbols coded according to species. In both cases, the first dimension contains most of the relevant information relating to the difference between the two species. Furthermore, regions of the loading corresponding to the olefinic and *bis*-allylic peaks are positively associated with horse samples in Figure 6.6 (c) and (d).

The differences in quality and price between different species of fish are reasons for falsification and therefore, it is necessary that methods are able to verify the traceability of the correct information to protect consumer rights (Aursand et al. 2009).

The following species and stocks of lean fish were caught outside the coast of Vikna, Nord-Trondelag: Norway north-east arctic cod, Norwegian coastal cod (*G. morhua L.*), haddock (*M. aeglefinus*), saithe (*P. virens*), and pollack (*P. pollachius*). Approximately 90 mg of the oil sample was transferred to 5 mm NMR tubes and diluted with 0.6 mL deuterated chloroform ($CDCl_3$, 99.8% purity, Isotec Inc., Matheson). Lipid was extracted from white fish muscle under the back dorsal fin according the Bligh and Dyer method (Bligh and Dyer 1959). Before analyzing the lipid extract by NMR, parts of the chloroform phase were removed by evaporation. ^{13}C NMR spectroscopy coupled with chemometric methods PCA, LDA and Bayesian belief networks (BBN) authenticated five different gadoid fish species. With PCA, groupings were obtained and the first two principal components accounted for 36% and 9% of the variance. PCA was used as input variables in the LDA. LDA with the three PCs obtained 21/27 correct classifications (78% correctly classified) and the Bayesian belief networks (BBN) showed 100% correct classifications (Standal et al. 2010).

^{13}C NMR spectroscopy in tandem with chemometrics methods classified the Atlantic salmon in relation to its geographical origin and to its identity as wild or farmed. The probabilistic neural networks (PNN) and support vector machines (SVM) showed an excellent breakdown of 98.5% and 100%, to wild and farmed salmon. The geographical origin obtained correct classification of 82.2% to 99.3% for PNN and SVM, respectively (Aursand et al. 2009).

A similar study with NMR and chemometrics methods tried to classify *Gilthead pargo* in accordance with wild or farmed and geographical origin (Rezzi et al. 2007). The LDA and PCA made a clear distinction between wild

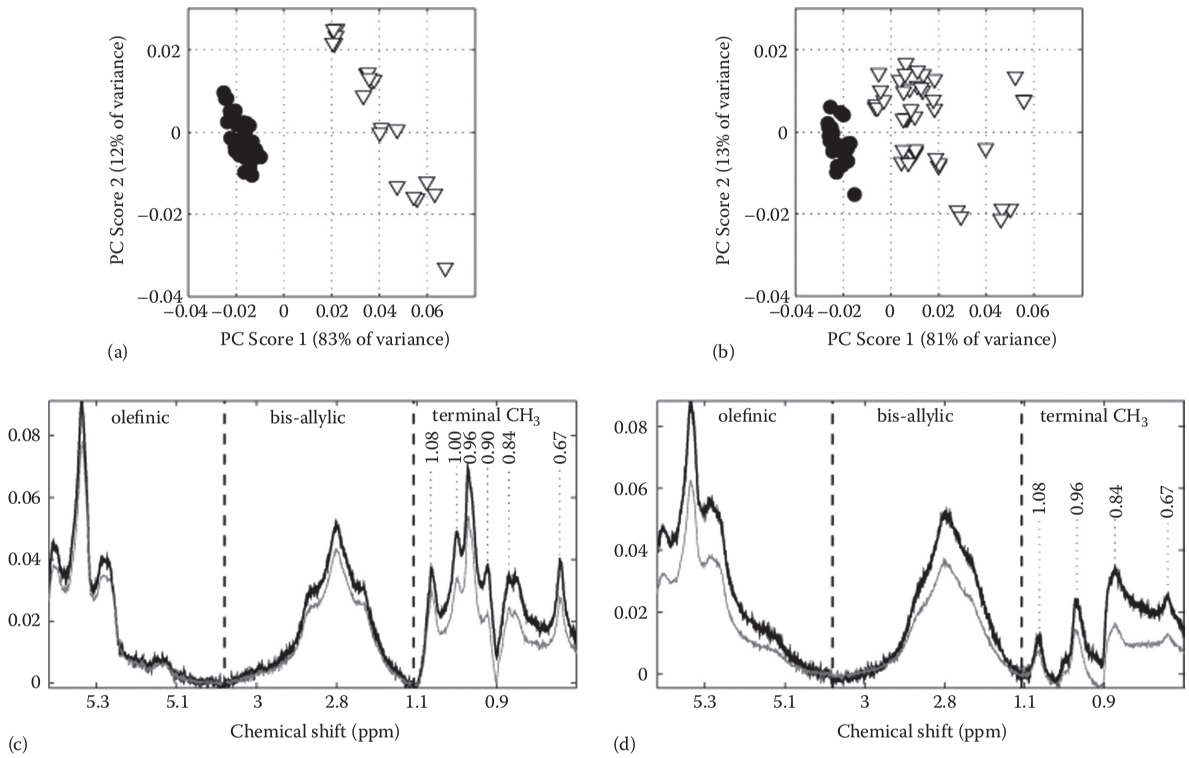

FIGURE 6.6
First versus second principal component scores plots for (a) Lab 1 Training Set data, and (b) Lab 2 Training Set data (black disks = beef, open triangles = horse). (c) and (d) Corresponding loadings plots (black trace), together with the covariance of each dataset with the group membership (grey trace) and peaks picked from the loadings in the CH_3 region. (Reprinted with permission from Jakes, W. et al., *Food Chemistry*, 175, 1–9, 2015.)

and farmed samples. This method showed a rating of 100% of the samples in accordance with wild or farmed and 85%–97% for the geographical origin.

Fourier transform spectroscopy and micro ^{1}H NMR LF are used in studies of changes in the structure of proteins and secondary water distribution (Rezzi et al. 2007, Damez and Clerjon 2013). The NMR technique permits an easy reading of the characteristics of foods with large amounts of water but is highly sensitive, expensive and time consuming. It is a method that can cause spectra with many peaks. However, TD-NMR is fast, simple and has the potential for online measurement.

6.2.6 Multispectral and Hyperspectral Imaging

Currently, many researchers are using hyperspectral imaging (HIS) methods because they are powerful techniques which can provide spectral data of an object with certain chemical characteristics in a spatially resolved manner (Pu et al. 2015). HIS methods, with the aid of image processing techniques that allow visualization, allow detection of attributes by spectral analysis of the samples. This method is a non-conventional analytical technique, nondestructive, using few reactants and it is fast.

This method allows simultaneous analysis of several samples and was introduced to integrate images and spectroscopy in a system for providing spectral and spatial information of an object (Ma et al. 2015, Lohumi et al. 2015). The images originate three-dimensional data sets that can be analyzed to characterize the object in greater detail than the imaging or spectroscopy techniques (Kamruzzaman et al. 2016). HIS is composed of hundreds of discrete spectral bands for each spatial position for the object (Kamruzzaman et al. 2012). The spectroscopy is used to detect or quantify the biological, chemical and physical properties of samples based on their spectral signature images and transform chemical information steps to viewing space (Kamruzzaman et al. 2013). HIS can be used to ascertain the amount of certain attributes and where they are located in the sample. Spectra can be used to characterize, identify and discriminate classes and types of materials in the image. The most commonly used spectral bands are in NIR, VIS-NIR and VIS (Lohumi et al. 2015). NIR HIS is involved in acquiring a spectrum for each image pixel in micro- and macroscopic scale (Kamruzzaman et al. 2016).

In a study by Kamruzzaman et al. (2013), the identification of the adulteration was conducted using pure minced lamb meat and lamb meat mixed with potential adulterants including pork, heart, kidney, and lungs in 20% proportions. The lamb samples were adulterated by mixing pork in the range of 2%–40%. Both minced lamb (28% fat) and pork (15% fat) were acquired from a local supermarket and transported to the laboratory. NIR HIS detected the level of adulteration of minced lamb using a PLS-R method. PCA was used to interpret and visualize the spectral data to highlight their properties. The first PC represents 87.5% of the variance while the second one explains 8% of the variance. (The two first PCs explained 95.7% variation).With PLS-R

prediction results, it was possible to detect adulteration in minced lamb with high performance in both calibration and cross-validation conditions using five latent variables (LV). The coefficient of determination in calibration (R^2_c) of 0.99, RMSEC of 1.08%, coefficient of determination in cross-validation (R^2_{cv}) of 0.99, and RMSECV of 1.37% were obtained for PLS-R. The calibration model was also evaluated based on the ratio of percentage deviation (RPD) and this value for adulterate detection was 8.51. The multiple linear regression (MLR) model was then built using the reduced spectral data and the results of MLR for predicting adulteration are R^2_c of 0.99, RMSEC of 1.25%, R^2_{cv} of 0.98, RMSECV of 1.45%, and RPD of 8.04. The prediction ability of PLS-R with selected wavelengths was equivalent to the PLS-R with full spectra, with R^2_{cv} (0.99 vs. 0.99), RMSECV (1.42% vs. 1.37%), and RPD (8.51 vs. 8.21).

In a different study, meat samples originating from *Longissimus dorsi* muscles of pork, beef, and lamb were analyzed by Kamruzzaman et al. (2012). The muscles were dissected and then sliced by a mechanical slicer. The slices were labeled and vacuum-packed and transported under refrigerated conditions to the laboratory. HIS with PCA and PLS-R/DA was used for identification and authentication of different red meat species. The first three PCs resulting from PCA explained 99.7% of the variation among samples. The PC1 and PC2 were particularly representative and accounted for 98.9% of the total variance (PC1 – 88.9% and PC2 – 10.1%). The PLS-R/DA showed a classification accuracy of 93.3%, 98.7%, and 97.3% for pork, beef, and lamb, respectively.

NIR HIS coupled with PLS-R/DA was used to distinguish between fresh and frozen-then-thawed samples (Barbin et al. 2013). Fresh samples of pork from the loin muscle were obtained from a commercial food retailer and transported to the laboratory for storage at 4°C. After 24 h, each fresh sample was removed from cold storage and scanned in the NIR hyperspectral system. Pork samples were then vacuum-packed and frozen at –18°C. PLS-R/DA with full cross-validation had coefficients of prediction of 0.97 and 0.89 for R^2_C and R^2_{CV}, respectively, with standard error of calibration (SEC) of 0.23 and standard error of cross-validation (SECV) of 0.46. To verify the potential information carried by the selected wavelengths, frozen-then-thawed samples were correctly identified (sensitivity = 1.00), and no fresh sample was misclassified as frozen-then-thawed (specificity = 1.00). The overall correct classification for this method was 100% to discriminate fresh from frozen-then-thawed samples. The classification of pork samples according to freezing treatment are: fresh samples (85.4%), frozen once (77.9%), frozen twice (60%), frozen three times (70%) and frozen four times (90%). The fresh pork meat and frozen-then-thawed meat was detected with PLS-R/DA and obtained 97.9% accuracy, and with colorimeter method achieved 75% accuracy. The discriminant model PLS-R/DA obtained a variance to LV1 of 58% and LV2 of 39% to identify the fresh and frozen-then-thawed samples. This method can be applied for the benefit of the retail sector and the consumer. Figure 6.7 shows the main configuration of the push room NIR hyperspectral imaging system, reprinted from Barbin et al. (2013).

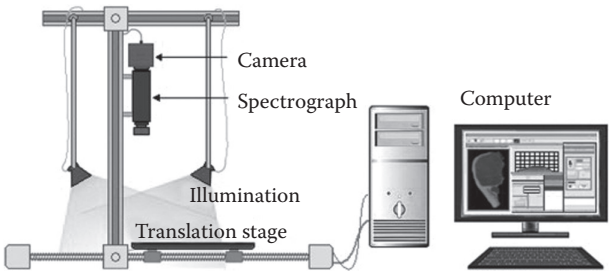

FIGURE 6.7
Hyperspectral imaging system setup. (Reprinted with permission from Barbin, D.F. et al., *Innovative Food Science and Emerging Technologies*, 18, 226–236, 2013.)

In other research, the potential of VIS and NIR HIS with PNN was used for classification of fresh and frozen-then-thawed pork muscles (Pu et al. 2015). Animals with similar conditions (age, weight, feeding environment from the same farm) were obtained for the experiment. The pork samples were divided into three groups: the first group without any freezing treatment was designated as fresh pork meat, the second group was frozen-then-thawed-once, and the third group was frozen-then-thawed-twice. The PC images from HIS were obtained using histogram statistics (HS), gray level co-occurrence matrix (GLCM) and gray level-gradient co-occurrence matrix (GLGCM). For fresh, frozen-then-thawed once and frozen-then-thawed twice meats, PNN showed a correct classification rate of 100% and 97.73% for calibration and validation sets, respectively. The successive projections algorithm (SPA) showed a correct classification rate of approximately 100% for calibration and validation sets. The correct classification rate was reduced to 86.36% and 86.36% for calibration and validation sets, when six optimum wavelengths were used alone. The average classification accuracy of PNN using optimum wavelengths (OW)-GLGCM was the highest (92.0%), followed by OW-GLCM (91.3%), OW-HS (91.3%) and OW (86.4%).

The multispectral imaging (MSI) coupled with PLS-R/DA and LDA was used for the detection of minced beef fraudulently substituted with pork (Ropodi et al. 2015). Different levels of adulteration of minced beef and pork were prepared; fillets were cut into smaller pieces and grinded separately one at a time, using a domestic meat-mincing machine. To achieve different levels of adulteration, ranging from 10% to 90% with a 10% increment, the appropriate amount of each type of meat was used and mixed in conditions that simulate industrial processing. The class of adulteration obtained an overall correct classification, mean per-class recall and precision of 83.3%, 83.3%, and 84.5%, respectively. The classification error for 98.48% of the samples was, at most, 10% for LDA. The overall correct classification, mean per-class recall and precision of pure pork, adulterated and pure beef was over 94% (mean recall, precision and overall correct classification was

94.4%, 99.4%, and 98.5%, respectively). The PLS-R/DA showed a correct classification of 98.5% using 12 PLS-R components after cross-validation.

In a study by Ma et al. (2015), VIS-NIR HIS was used to classify the fresh and frozen-then-thawed pork meats. The pork *Longissimus dorsi* muscles were obtained from a local market. The first group of fresh samples were divided without any freezing treatment, the second group of meat samples were frozen at −18°C for 24 h and then thawed at 20°C for 2 h, and the third group were frozen and then thawed twice. The correct classification rate was applied to assess the performance of the PLS-R/DA classifier for model establishment. The correct classification rate of 97.7% was achieved, confirming the high potential of textures for fresh and frozen-then-thawed meat discrimination. The PCA with three components explained 99.9% of variance and the first three PC images (the optimal GLGCM images) explained 98.1%, 1.3%, and 0.4%, respectively. This method is a powerful tool and allows the analysis of the quality of food and its authenticity.

In a 2016 study, chicken adulteration in minced beef was detected with VIS-NIR HIS (400–1000 nm) and HIS was acquired in the reflectance mode (RM) (Kamruzzaman et al. 2016). The pure minced beef and minced chicken were collected from a local supermarket. The minced beef samples were adulterated by mixing minced chicken in the range of 0% at 50%. Hyperspectral images were transformed into absorbance (A) and used the Kubelka-Munk (KM) function (Nobbs 1985). The performance of PLS-R developed using raw and pre-treatment spectra (1st derivative, 2nd derivative, MSC, and SNV). The percentage of chicken adulteration in minced beef was predicted with R^2_c of 0.97, 0.97, and 0.95 with the corresponding RMSEC values of 2.5%, 2.6%, and 3.3% for RM-PLS-R, A-PLS-R, and KM-PLS-R, respectively. When applied to an independent validation set, they were capable of predicting adulteration with R^2_p of 0.97, 0.97, and 0.96 and the corresponding RMSEP of 2.67%, 2.45%, and 3.18%, for RM-PLS-R, A-PLS-R, and KM-PLS-R, respectively. The ratio of percentage deviation values obtained were 5.84, 6.24, and 4.81 for RM-PLS-R, A-PLS-R, and KM-PLS-R, respectively.

Multispectral and hyperspectral imaging are quick techniques that allow a large number of samples to be analyzed at the same time and provide spectral data on the chemical, biological, and physical characteristics of samples. However, the instrumentation is costly and data processing can limit the use of this method in real time.

6.3 Spectral Data Processing

Spectroscopic methods associated with chemometric methods are tools for the identification of species and foodstuffs that are not on the label. However, identifying regions of interest and features in the spectrum, sometimes

called regions of interest, of the tested substances is important for the optimization of the methodologies (De Jong 1990).

Univariate methods that can be used, namely, the averages and standard deviations, descriptive statistics, box plots, analysis of variance (ANOVA), pair-wise comparisons of mean values with Fisher's LSD test, and t–test. These methods were applied, for example, by Pillonel et al. (2005) in the study of geographic origin of European Emmental cheese. A comparison between univariate and multivariate methods was performed by Moustafa et al. (2015) for evaluating the efficiency of spectral resolution when manipulating ratio spectra applied to ternary mixtures in common cold preparations. Multivariate regression techniques have been widely used to study food authentication. A comprehensive introduction and review on multivariate regression procedures can be found in Higgins (2005), van den Hout et al. (2007), and Cruyff et al. (2016) and references therein.

The application pre-treatment may remove the effects of unsystematic spectral data and eliminate variations, light scattering, random noise, external factors, and base line changes (Rinnan et al. 2009). The most common pre-treatment methods are the standard normal variate (SNV) (Barnes et al. 1989), the multiplicative scatter correction (MSC) (Dhanoa et al. 1994), the Norris-Williams derivation (Rinnan et al. 2009), and the Savitzky-Golay 1st and 2nd derivatives (Savitzky and Golay 1964).

The SNV is an accurate and reliable method for ranking in the spectroscopic field. The SNV is also an ideal technique for classification and validation of the results of PCA (Alamprese et al. 2013, Schmutzler et al. 2015, Ropodi et al. 2015). The MSC method is a simple processing step that attempts to account for scaling effects and offset (baseline) effects. This correction is achieved by regressing a measured spectrum against a reference spectrum and then correcting the measured spectrum using the slope (and possibly intercept) of this fit (De Jong 1990). The Norris-Williams derivation is a basic method developed to avoid the noise inflation in finite differences. This technique was elaborated on by Norris and Williams in 1984 as a way to calculate the derivative of NIR spectra. The NW derivation includes two steps, smoothing of the spectra and first-order derivation (Norris and Williams 1984). The Savitzky-Golay method reveals a larger structure of spectral data resulting in an easy interpretation of the chemical basis of the observed signals. The derivatives can also be used in conjunction with SNV (Press and Teukolsky 1990).

After pre-treatment of the spectral data a few simple statistic methods allow extraction of information from the spectral data.

The PCA method is applied to spectral data to reduce the dimensionality, to classify samples, and to identify outliers. The original variables are transformed into new uncorrelated variables called PC that are a linear combination of the original variables. The main components are linearly independent and represent variations in the dataset in descending order with PC1 describing the largest variance, PC2 the second largest variance, and so on.

The LDA method is a probabilistic classification technique that allows for maximum separation of samples between categories. The number of samples must be greater than the number of variables. This method allows the recognition of supervised patterns where the number of categories and the samples belonging to each category defined above is based on the assumption that samples of the same group are more similar than samples belonging to different groups. This method also allows a linear transformation maximizing the variance between classes and minimizing the variance within the class (Morsy and Sun 2013b, Uddin et al. 2005, Alamprese et al. 2013).

The PLS method permits an associate set of independent variables (predictors, X) to response variables (observations, Y) by reducing the original number of descriptors to a new set of data based on a reduced number of orthogonal factors called latent variables. The PLS-DA method accounts for the maximum separation between the classes in the data where the variable is dependent and categorized (Morsy and Sun 2013b). PLS-R is used to reduce the original predictors to a new variable which has better predictive power (Sun et al. 2012a, Morsy and Sun 2013b).

The SIMCA method provides a useful classification of high dimensional variations and incorporates PCA to reduce the dimensions of spectral information. The computing speed of SIMCA with PCA can be increased by calculating the covariance matrices and the indices. The MIR ATR spectroscopy with SIMCA makes it possible to successfully detect and quantify adulterants(Meza-Márquez et al. 2010, Zhao et al. 2015). The mean difference, standard deviation of difference, RMSECV, and R^2 are used for validation. Generally, a good model should have high R^2 and RPD and low RMSEC and RMSEP.

The PNN method consists of establishing decision limits in feature space with distinct patterns belonging to different classes. This method improves the standards of classification and enables faster speed training (Cheng et al. 2015). The PNN method showed potential for the analysis of the NMR data technique. PNN can be used as a classifier and to find variables with the highest impact in classification (Standal et al. 2010). For the MLR, it is necessary to establish the wavelength that can relate two or more explanatory variables and the response variable (Kamruzzaman et al. 2013). The GLCM method is an image processing method for resource collection textural analyzing the relationship of levels and slope between 2 pixels (Karoui et al. 2006).

6.4 Discussion and Conclusions

Authentication of foodstuffs is crucial due to design food with adulteration by substitution of species, geographical origin, or freshness. NIR spectroscopy detects the number of bands of smaller molecules OH, CH, and NH compared to mid-infrared spectroscopy which detects a greater number of

the molecules in the food matrix in more detail. This involves stretching, bending, and shaking movements of functional groups, such as CC, CH, OH, C = O and NH (Mamani-Linares et al. 2012, Alamprese et al. 2013, Zhao et al. 2014).

MIR spectroscopy in conjunction with Fourier transform and chemometric methods proves to be a promising technique for the analytical determination of adulteration of Halal food (Rahmania et al. 2015). However, the results of the ATR technique are affected by water content contained in food producing noise (signal). The method is fast, non-destructive and does not involve a lot of sample preparation, giving sufficiently reliable results. MIR spectroscopy requires dilution of samples unlike NIR, however this technique has difficulty in reading samples with large amounts of water, such as fish (Uddin et al. 2005, Zhao et al. 2014). NIR spectroscopy is a powerful technique for rapid analysis in line applied to inspections of foodstuffs and discrimination of linear and nonlinear allowing adulteration to be detected with ease (Morsy and Sun 2013b). FS reduces the time and cost of the measurements and analysis of enzymatic bio-analytic chemistry. This method can identify fish and detect fresh from frozen-then-thawed samples (Karoui et al. 2006). In a study by Jakes et al. (2015), investigators used a simple, quick, and inexpensive basic extraction with chloroform to obtain triglycerides in NMR spectra and found that 60 MHz ^{1}H NMR is a viable approach in high yield for the determination of adulteration in meat.

To obtain vibrational spectroscopic results, it is necessary to use chemometric models. The PCA, LDA, SIMCA, and PLS-R/DA methods demonstrated that, combined with spectroscopy methods, these techniques are useful tools for authentication and detection of adulteration in food (Kamruzzaman et al. 2013, Meza-Márquez et al. 2010, Zhao et al. 2014). RS is a promising technique in providing specific information on the identification of sample matrices based compounds (lipids, proteins, carbohydrates), it is sensitive to smaller components such as microorganisms responsible for spoilage and it provides detailed information on molecular vibrations and the chemical structure of molecules without causing damage to the small sample required for analysis (Boyaci et al. 2014, Argyri et al. 2013, Al Ebrahim et al. 2013).

Water interference does not occur with RS and results in water samples can be analyzed by glass or polymer packaging. However, analyzing samples using FS can hide the impurities and heat from the intense laser radiation can destroy the sample or hide the spectrum. To alter this effect, the use of a NIR laser reduces or prevents the fluorescence of the samples. It is a method with a high potential for identification purposes (Lohumi et al. 2015). Although NMR is a technique that allows the detection and analysis of different types of fat, it is an expensive technique, can yield spectra with too many peaks, and requires very concentrated solutions. While NIR, MIR and RS are well-established techniques, they are based on a sample point, a relatively small area of a species, which is not capable of providing the composition gradients yielding mean results compared with the multispectral

TABLE 6.1

Detection Techniques of Different Types of Meat and Fish Species

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Type of Adulteration	Food Products	Detection Method	Chemometric Method	References
Substitution or removal of ingredients	Halal and non-Halal Chinese Ham sausages	FTIR	PLS-R/DA and PCA	(Xu et al. 2012)
	Veal	Fourier transform -NIR	PCA	(Schmutzler et al. 2015)
	Lamb	NIR hyperspectral imaging	PCA and PLS-R	(Kamruzzaman et al. 2013)
	Iberian pork sausages	NIR	PCA and MLSD	(Ortiz-Somovilla et al. 2005)
	Beef or bovine meat	UV–VIS, NIR and MIR	PCA, LDA and PLS-R	(Alamprese et al. 2013)
		Raman	PCA	(Boyaci et al. 2014)
		Raman	PCA and PLS-R	(Ebrahim et al. 2013)
		60 MHz 1H NMR	Naïve Bayes classification model, PCA	(Jakes et al. 2015)
		FTIR	PLS-R and PCA	(Kurniawati et al. 2014)
		Mid-infrared	PLS-R and SIMCA	(Meza-Márquez et al. 2010)
		ATR-FTIR	PLS-R/DA	(Nunes et al. 2016)
		FTIR	PLS-R and PCA	(Rahmania et al. 2015)
		FTIR	PLS-R	(Rohman et al. 2011)
		Multispectral imaging	LDA and PLS-R/DA	(Ropodi et al. 2015)
		TD-NMR	SIMCA, KNN and PLS-R/DA	(Santos et al. 2014)
		Fourier transform-Raman		(Zając et al. 2014)
		Mid-infrared ATR	SIMCA, PCA and PLS-R/DA	
	Carp fish fillets	Hyperspectral imaging	SIMCA and PNN	(Cheng et al. 2015)
	Chicken	VIS NIR hyperspectral imaging	PLS-R	(Kamruzzaman et al. 2016)

(*Continued*)

TABLE 6.1 (CONTINUED)

Detection Techniques of Different Types of Meat and Fish Species

Type of Adulteration	Food Products	Detection Method	Chemometric Method	References
Fresh vs. thawed meat	Porcine	NIR hyperspectral imaging	PLS-R/DA	(Barbin et al. 2013)
		VIS–NIR hyperspectral imaging	PCA	(Ma et al. 2015)
		Hyperspectral imaging		(Pu et al. 2015)
		VIS and NIR hyperspectral imaging	PNN	(Pu et al. 2015)
	Beef	NIR spectroscopy	LDA and PLS-R/DA	(Morsy and Sun 2013a)
		Mid-infrared ATR spectroscopy	PCA and LDA	(Zhao et al. 2014)
	Fish	Front-face fluorescence	PCA and FDA	(Karoui et al. 2006)
		NIR	PCA and PLS-R/DA	(Ottavian et al. 2013)
Classification of species or origin	Gadoid fish species	13C NMR	PCA and BBN	(Standal et al. 2010)
	Lamb	NIR	LDA, PCA and PLS-R/DA	(Jakes et al. 2015)
	Different meat species	Raman spectroscopy	PCA	(Boyaci et al. 2014)
		VIS and NIR	PLS and PCA	(Cozzolino and Murray 2004)
		Hyperspectral imaging	PCA and PLS-R/DA	(Kamruzzaman et al. 2012)
		VIS and NIR spectroscopy	PCA and PLS-R	(Mamani-Linares et al. 2012)
	Deer	Shifted excitation Raman difference spectroscopy	PCA	(Sowoidnich and Kronfeldt 2012)

image. HIS and MS are important methods for food inspection as analysis is more convenient, fast and they analyze a larger number of samples simultaneously. However, the high initial costs and difficulties in data acquisition have limited the use of this real-time technology (Kamruzzaman et al. 2012) (Table 6.1).

Within this research area of the food industry, the future perspective is the application of multispectral imaging to many foodstuff samples to determine

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the quantity of contaminants and the application of spectroscopy techniques to determine adulteration of food (Raman).

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