

Near Infrared Spectroscopy and Food Authenticity

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1. Introduction

Developments in both hardware (eg, sensors, computers, instruments) and software (eg, new algorithms) have resulted in the growth of analytical and instrumental methods based in vibrational spectroscopy techniques such as Near Infrared (NIR) and Mid Infrared (MIR). These methods have been extensively used to qualitatively (eg, traceability and authenticity) and quantitatively (eg, measure composition) analyze several food matrices (Arvantoyannis et al., 2005; Karoui et al., 2010; Weeranantanaphan et al., 2011; Cozzolino, 2009, 2012; Downey, 2013).

Food authenticity is a well-established field of research, which involves input from different analytical techniques such as Gas Chromatography (GC), Mass Spectrometry (MS), NMR, DNA fingerprinting, and vibrational spectroscopy (NIR, MIR, Raman) (Cordella et al., 2002; Downey, 2013). However, there still remains a division over which analytical strategy to use in order to develop methods to target specific authenticity issues, identify adulterant in food products, or develop nontargeted methods with the aim of detecting deviations from the norm (Downey, 2013).

This chapter will provide a general introduction to NIR spectroscopy and chemometrics. In addition, examples on the applications and uses of NIR spectroscopy targeting issues related with authenticity and traceability in several food products such as fish, meat, honey, and wine will be discussed.

2. Traceability and Authenticity

The increasing market and consumer desire for quality of food have created a need for efficient and accurate analytical methods for the authentication and traceability of food. Several analytical methods have been or are currently in use to authenticate or trace food such as UV spectroscopy, High Performance Liquid Chromatography (HPLC), GC, Liquid Chromatography (LC), and MS (Cordella et al., 2002; Downey, 2013). Although these methods provide a high level of information about the chemistry of the compound measured, the sample requires different steps of preprocessing (eg, extraction, purification) before and during the analysis. Both NIR and MIR spectroscopy, with its intrinsic benefits such as being noninvasive and rapid and requiring almost no necessary sample preparation, have been able to determine physical and chemical parameters in different foods simultaneously.

The authentication of foods and agricultural products is of primary importance to keep consumers demands and to maintain the sustainable nature of the food industry. From a legislative point of view, quality standards were established by the industry and government in order to set the requirements for quality labels that specify the chemical composition of raw materials and foods. From an economic point of view, product authentication is essential in order to avoid unfair competition that can eventually create a destabilized market and disrupt regional or national economies (Arvantoyannis et al., 2005; Karoui et al., 2010; Weeranantanaphan et al., 2011; Cozzolino, 2009, 2012).

The authenticity of foods has two different aspects: (1) authenticity with respect to production (eg, geographical origin, organic vs nonorganic) and (2) authenticity with respect to the description (eg, adulteration issues) (Arvantoyannis et al., 2005; Weeranantanaphan et al., 2011). For example, geographical and botanical origins of food, such as “natural,” “organic,” “raw,” “unheated,” and “fresh versus frozen” have been analyzed using different methods and techniques (eg, volatile compounds, minerals, carbohydrates, and protein identification) (Arvantoyannis et al., 2005; Karoui et al., 2010; Weeranantanaphan et al., 2011; Cozzolino, 2009, 2012).

Food adulteration has been practiced since ancient times; however, it has become more sophisticated. The practice of adulteration commonly arises for two main reasons: firstly it can be profitable, and secondly, adulterants can be easily mixed and are subsequently difficult to detect (Karoui et al., 2010; Weeranantanaphan et al., 2011; Cozzolino, 2009, 2012). Foods or ingredients most likely to be targets for adulteration include those which are of high value or are subject to the vagaries of weather during their growth or harvesting (Karoui et al., 2010; Weeranantanaphan et al., 2011; Cozzolino, 2009, 2012). Adulteration can also take many forms, including the addition of sugars, acids, volatile oils, overdilution of concentrate, addition of juices of other fruits, use of concentrate in a “fresh” product, or the use of low quality product recovered from what are normally waste products of manufacture as premium products (Karoui et al., 2010; Weeranantanaphan et al., 2011; Cozzolino, 2009, 2012).

3. The Tools (Hardware and Software)

3.1 Near Infrared Spectroscopy

Infrared (IR) spectroscopy is originated from the absorption measurements of different IR frequencies by a sample positioned in the path of an IR beam (eg, NIR and MIR beams). When the frequency of a specific vibration is equal to the frequency of the IR radiation directed at the molecule, this molecule absorbs the radiation (Abbas et al., 2012; Woodcock et al., 2008; Karoui et al., 2010; Rodriguez-Saona and Alledendorf, 2011; Cozzolino, 2011; Cozzolino and Murray, 2012; Downey, 2013).

Absorption spectroscopy is based on the Beer–Lambert law stating that for a homogeneous and nonscattering liquid sample, the concentration of an absorber is proportional to the sample absorbance. Near IR is characterized by the overlapping of many different overtone and combination vibrations (vibrational modes of C—H, N—H, and O—H chemical bonds) resulting in broad bands that result in NIR spectra

data with low structural selectivity (compared with MIR spectra where fundamentals are more resolved, allowing the structure of a sample to be better elucidated). Overtones correspond to energy transitions that are higher than those for fundamentals (MIR), and in particular, the frequencies of the first and second overtones correspond to about two or three times that of the fundamentals observed in the MIR region. Combination bands result from transitions involving two or more different vibrational modes of one functional group occurring simultaneously: the frequency of a combination band is the sum or the multiples of the relevant frequencies. The absorption intensity decreases when the overtone level increases. However, the higher energy of NIR radiation and the implication of combination vibrations enable NIR spectroscopy to provide more complex structural information than in the MIR region. NIR spectroscopy is widely used to determine organic matter constituents in several types of samples (eg, liquids, solids, gas), as seen in Fig. 7.1. It is based on the absorption of electromagnetic radiation by a sample at wavelengths in the range between 800 and 2500 nm (Abbas et al., 2012; Woodcock et al., 2008; Karoui et al., 2010; Rodriguez-Saona and Alledendorf, 2011).

Different instruments have been used or are under development in order to target traceability and authenticity issues in several food matrices. NIR spectrophotometers are conveniently classified according to the type of monochromators. For example, in a filter instrument, the monochromator is a wheel holding a number of absorption or interference filters, while its spectral resolution is limited. In a scanning monochromator instrument a grating or a prism is used to separate the individual frequencies of the radiation either entering or leaving the sample. The wavelength divider rotates, allowing radiation of the individual wavelengths to subsequently reach the detector. The development of Micro Electro Mechanical Systems (MEMS) combine mechanical parts, sensors, actuators, and electronics on a common substrate through the use of microfabrication technology. Other developments in instrumentation include focal plane array cameras in combination with liquid crystal tunable filter, acousto-optic

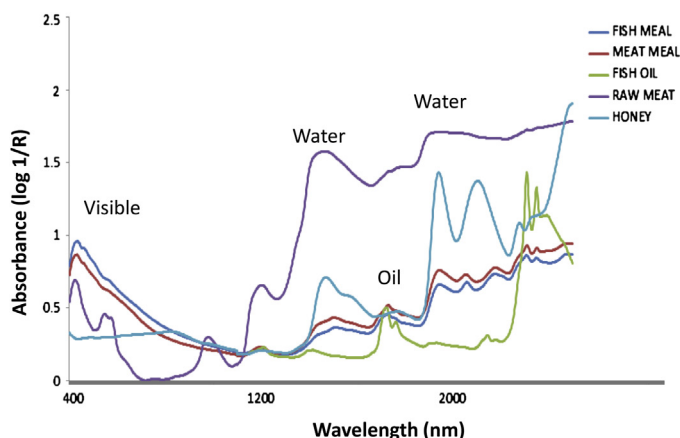


Figure 7.1 Figure shows typical NIR spectra of foods and agricultural products. The visible, water, and oil regions are also indicated.

tunable filters, the use of LEDs, or other monochromator principles which allow for much faster acquisition spectra. Multispectral and hyperspectral imaging systems are being incorporated to the myriad of instruments available. Multispectral (a few wavelengths) or hyperspectral (a continuous range of wavelengths) imaging systems produce a spectral data cube—a spectrum at every 2-D spatial position. A sequence of spatial images was acquired by means of an NIR camera and a set of band-pass filters. However, while the acquisition can be fast, the disadvantage of this approach is that only a limited number of wavelengths can be analyzed, and that they need to be known a priori. This technology represents a paradigm shift for industrial spectroscopy and enables a variety of new industrial applications (McClure, 2004; Cozzolino et al., 2003, 2009; Roggo et al., 2007; Nicolai et al., 2007; Huang et al., 2008; Manley, 2014). Table 7.1 summarizes and compares the advantages and disadvantages of NIR spectroscopy with other common methods and techniques used in authenticity.

3.2 Multivariate Data Methods

The combination of chemometrics or multivariate analysis with analytical instruments have the ability to determine more than one component at a time and can act as a support to establish links to other sample characteristics (McClure, 2003; Karoui et al., 2010; Weeranantanaphan et al., 2011; Cozzolino, 2009, 2012). Chemometrics covers quite a broad range of methods such as exploratory data analysis, Pattern Recognition (PR), and statistical Experimental Design (DoE) (Arvantoyannis et al., 1999; Naes et al., 2002; Downey, 1996, 1998).

The most commonly used techniques applied in the field of food authenticity are Principal Component Analysis (PCA), Partial Least Squares (PLS), and Principal Component Regression (PCR). Chemometrics, unlike classical statistics, considers multiple variables simultaneously and takes collinearity (the variation in one variable, or a group of variables, in terms of covariation with other variables) into account. Calibration development can mathematically describe the covariation (degree of association) between variables or find a mathematical function (regression model), by which

Table 7.1 Advantages and Disadvantages of NIR Spectroscopy Compared With Other Common Methods and Techniques Used in Authenticity

Advantages	Drawbacks
Nondestructive High reproducibility At, on, inline analysis Little or no sample preparation required Analysis of liquids, pastes, solids	Low sensitivity compared with other techniques such as NMR, MS Low selectivity compared with other methods

Adapted from Khakimov, B., Gürdeniz, G., Engelsens, S.B., 2015. Trends in the application of chemometrics to foodomics studies. *Acta Alimentaria* 44, 4–31.

the values of the dependent variables are calculated from values of the measured (independent) variables (Tzouros and Arvantoyannis, 2001; Naes et al., 2002; Reid et al., 2006; Oliveri et al., 2011; Blanco and Villaroya, 2002; Brereton, 2003, 2007).

PCA is used as a tool for screening, extracting, and compressing multivariate data. PCA employs a mathematical procedure that transforms a set of possibly correlated response variables into a new set of noncorrelated variables, called principal components (Fig. 7.2). PCA can be performed on either a data matrix or a correlation matrix depending on the type of variables being measured (Tzouros and Arvantoyannis, 2001; Naes et al., 2002; Reid et al., 2006; Oliveri et al., 2011; Blanco and Villaroya, 2002; Brereton, 2003, 2007). PCA produces linear combinations of variables that are useful descriptors or even predictors of some particular structure in the data matrix. However, according to some authors, PCA might not be the most adequate method to use (Downey, 2013).

Discriminant Analysis (DA), Linear Discriminant Analysis (LDA), and Partial Least Squares Discriminant Analysis (PLS-DA) can be considered qualitative calibration methods, and they are the most used methods in authenticity. Instead of calibrating for a continuous variable, one calibrates for group membership (categories). The resulting models are evaluated in terms of their predictive ability to predict the new and unknown samples (Standard Error of Prediction, SEP). Discrimination models are usually developed using PLS regression techniques, as described elsewhere. LDA is a supervised classification technique. The criterion of LDA for the selection of latent variables is maximum differentiation between the categories and minimal variance within categories. This method produces a number of orthogonal linear discriminant functions, equal to the number of categories minus one, that allow the samples to be classified in one or another category (Tzouros and Arvantoyannis, 2001; Naes et al., 2002; Reid et al., 2006; Oliveri et al., 2011).

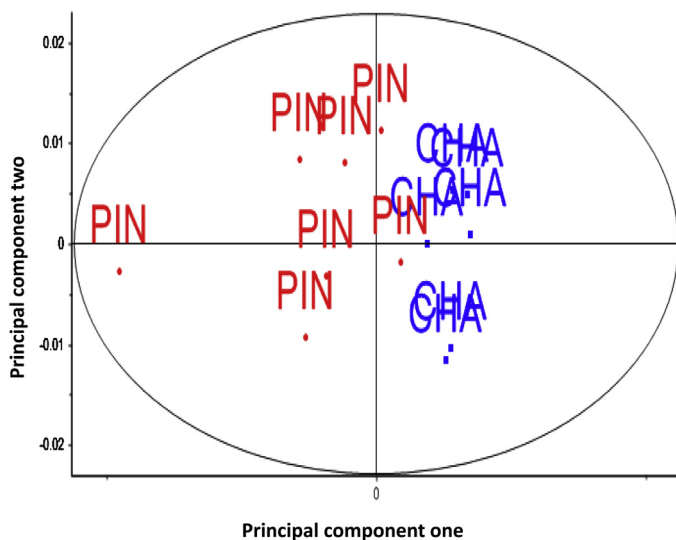


Figure 7.2 A typical example on the use of principal component score plots to visualize the patterns related with different wine styles. *PIN*, Pinot; *CHA*, Chardonnay.

The application of Artificial Neural Networks (ANN) is a technique for data and knowledge processing that is characterized by its analogy with a biological neuron (Otto, 1999; Naes et al., 2002). Unlike linear regression, PCR, and PLS, ANN can deal with nonlinear relationships between variables. Both PLS and PCA are the two most powerful tools for data analysis which require instrumental methods to extract the information about quality attributes hidden in the data (“model calibration”) (Tzouros and Arvantoyannis, 2001; Naes et al., 2002; Reid et al., 2006; Oliveri et al., 2011).

4. Examples and Applications

Near infrared spectroscopy has been used as tool to authenticate, trace, or discriminate several foods and agricultural products. This section illustrates, with examples, different uses and applications of NIR spectroscopy to authenticate or trace fish, meat, wine, and honey products.

4.1 Fish and Fish By-Products

4.1.1 Geographical Origin

The proximate chemical composition of tilapia fillets sourced from four distinctive geographical regions of China (Guangdong, Hainan, Guangxi, and Fujian Province) was assessed using NIR spectroscopy. The method proposed by Liu et al. (2006) provided an example of fish traceability. The tilapia origin traceability model was developed using NIR spectroscopy coupled with Soft Independent Modeling of Class Analogy (SIMCA). The authors reported that when classifying tilapia by means of SIMCA, more than 80% of samples from the Guangdong, Hainan, and Fujian regions and 75% of fillets from the Fujian region were correctly and exclusively assigned to the corresponding clusters. Overall, only 1% to 2% of the fish samples were classified incorrectly. The results derived from this study indicated that NIR spectroscopy coupled with pattern recognition methods such as SIMCA were a feasible way to determine the origin and traceability of export tilapia fillets (Liu et al., 2006).

4.1.2 Fresh Versus Frozen

The potential of Visible (VIS) and NIR (400–1000 nm) hyperspectral imaging as a rapid and noninvasive method was investigated to differentiate freshness in prawn samples. Unfrozen and frozen groups, as well as two different levels of freshness, were used for classification. Spectral data were obtained from whole prawn samples and preprocessed using derivatives (first and second). The Successive Projections Algorithm (SPA) was used to select the individual wavelengths for classification. Several other algorithms were also used, such as Least Squares-Support Vector Machine (LS-SVM), Adaptive Boosting (AdaBoost), and Back-Propagation Neural Networks (BP-NN). The results from this study demonstrated that SPA-LS-SVM achieved satisfactory average correct classification rates (98.33% and 95%) for the prediction of

samples in the unfrozen and frozen groups, respectively. The overall results showed that the hyperspectral imaging technique is promising for the rapid and noninvasive freshness classification of prawns (Dai et al., 2015).

The substitution of fresh fish with frozen-thawed fish is a typical fraud that not only damages consumers from an economical point of view, but also causes safety issues. It is in this context that fish authentication is important for correct product labeling, as promoted by recent regulatory actions. The results of the authentication of fresh West African goatfish (*Pseudupeneus prayensis*) fillets using a portable VIS-NIR instrument, a compact digital camera, and a texture analyzer were reported by Ottaviano et al. (2014). The classification performance of the different analytical technologies and how the fusion of different technologies can be effectively used to improve the classification accuracy was reported by the authors. The combination of VIS-NIR and the digital camera was considered as very promising for cost-effective online/at-line applications, as both sensors are rapid and noninvasive and do not require sample preparation (Ottaviano et al., 2014).

The rapid and nondestructive measurement of freshness is essential for the control of fish and the quality and safety of its products. The prediction of freshness in silver chub samples were developed using fourier transform near infrared (FT-NIR) spectroscopy using several algorithms: PLS, interval-PLS, synergy interval-PLS, and SVM. The results reported by these authors indicated that FT-NIR spectroscopy together with SVM could be a reliable method for the detection of fish freshness (Ding et al., 2014).

4.1.3 Authentication

The potential use of NIR for the authentication of raw and cooked freeze-dried rainbow trout (*Oncorhynchus mykiss*) fillets was evaluated using different classification techniques. Latent variable models applied on the spectral data were developed and used to estimate proximate composition, fatty acid profile, fillet yield, and cooking loss, and to classify the available dataset by the rearing farm and genetic strain of each sample. The results from this study showed that NIR spectra can be used both to accurately estimate several chemical properties and to classify samples by rearing farm and by processing method (raw vs cooked) (Zotte et al., 2014).

The use of NIR spectroscopy was investigated for the analysis of whole fish and fish fillets from different species. O'Brien et al. (2013) have evaluated a miniaturized, handheld instrument to discriminate between high-quality and expensive from lower-quality, less expensive fillets. According to these authors, such issues related with authenticity can occur in practice due to the mislabeling of products in the harvesting or processing plant or to the fraudulent substitution of high-quality and low-quality species at the restaurant level. To test the possibility of distinguishing superior from lower-quality fish species, NIR spectra were measured in diffuse reflection from the skin and meat of the investigated fish. Subsequently, the spectra were evaluated using PCA and further classified using SIMCA. The authors reported good classification results in order to distinguish between skin and meat and to discriminate between different fish species, such as salmon trout versus samlet, cod versus winter cod, and between mullet versus red mullet, as well as to differentiate between the different fish origins (France, Senegal, Norway, Iceland, and Italy) (O'Brien et al., 2013).

4.2 Meat and Meat By-Products

More than ever, consumers today demand clear and reliable information about the food they consume. This issue has a great impact on the economy since the consumer's choice is greatly influenced by the food composition detailed in the label. In the case of processed meat products, this is going to be especially important because a simple visual inspection would not allow us to discriminate between the different components so easily (as in the case of fresh meat). In order to assure fair trade, food safety, and freedom of choice, honest and accurate food labels are a requisite that must be assured by legal authorities. In order to achieve this, robust and reliable analysis methodologies must be implemented in control laboratories. Many consumers place particular emphasis on noncompositional aspects of meat as food that are not related to composition (eg, protein, fat, color), and they include geographical origin, production methods, rearing, or feeding systems (Weeranantanaphan et al., 2011; Sentandreu and Sentandreu, 2014; Kamruzzaman et al., 2015).

4.2.1 Raw Versus Processed Meats

A nondestructive method based on VIS-NIR spectroscopy to rapidly and accurately discriminate between raw meat and meat injected with water was evaluated. Water-injected meat samples were built by injecting water into nondestructured meat samples consisting of pigskin, fat layers, and muscle layers. The VIS-NIR spectra of raw meat and meat samples injected with water were collected and analyzed. Effective spectral NIR bands were extracted from the preprocessed spectral data and used to classify samples according to raw meat or water-injected meat, as well as different levels of water-injected meat. These results showed that the overall recognition accuracy to distinguish between raw and water-injected meat was higher than 90%, using SVM. For water-injected meat samples with different water injection rates, the recognition accuracy showed a positive correlation with the water injection rate difference. The results also demonstrated that the proposed method can be effectively used for discriminating water-injected meat from raw meat (Hag et al., 2015).

4.2.2 Fresh Versus Frozen

Fresh and frozen-thawed pork meats were classified using VIS-NIR hyperspectral imaging using eight wavelengths from the VIS range as follows: 624, 673, 460, 588, 583, 448, 552, and 609 nm. The first three Principal Components (PCs) obtained using PCA accounted for over 99.98% of the variance. Classification models were developed using PLS-DA with the reflected spectra at full wavelengths and those at the optimal wavelengths, the extracted textures based on the PC images, and the fused variables combining spectra at the optimal wavelengths and textures. The results showed that the best classification rate (97.73%) was achieved, confirming the high potential of textures for fresh and frozen-thawed meat discrimination (Ma et al., 2015).

4.2.3 Adulteration

Multispectral imaging supported by multivariate data analysis was used to detect minced beef fraudulently substituted with pork and vice versa. Multispectral images

in 18 different wavelengths of 220 meat samples in total from four independent experiments (55 samples per experiment) were acquired for this study. The appropriate amount of beef and pork-minced meat was mixed in order to achieve nine different proportions of adulteration and two categories of pure pork and beef. After an image processing step, data from the first three experiments were analyzed using PLS-DA and LDA. The results reported by [Ropodi et al. \(2015\)](#) showed very good discrimination between pure and adulterated samples, for PLS-DA and LDA, yielding 98.48% overall correct classification. Additionally, 98.48% and 96.97% of the samples were classified within a $\pm 10\%$ category of adulteration for LDA and PLS-DA, respectively. Lastly, the models were further validated using the data of the fourth experiment for independent testing, where all pure and adulterated samples were classified correctly in the case of PLS-DA, while LDA was proved to be less accurate ([Ropodi et al., 2015](#)).

Donkey meat samples from different parts of a donkey body (neck, rump, and tendon), beef, pork, and mutton samples were used to develop classification models based in NIR spectroscopy. The accuracies of classification models were developed using Mahalanobis distances, SIMCA, and LS-SVM, respectively, in combination with pretreatment such as derivatives (first and second), Multiplicative Scatter Correction (MSC), and Standard Normal Variate (SNV). The optimal models for intact samples obtained by Mahalanobis distances correctly classified 100% of the samples in the calibration set and 98.96% in the prediction set. The authors of this study also examined different minced samples with different accuracies using different algorithms where donkey meat samples were correctly classified with 100% rate in either calibration or prediction. Overall, these results showed that it is feasible that NIR spectroscopy combined with chemometrics discriminate donkey meat from other sources of meat ([Niu et al., 2014](#)).

The potential of VIS-NIR spectroscopy to discriminate between dark cuts from normal cuts of beef samples was examined using commercial meat samples. One hundred and twenty beef carcass sides were selected from a slaughter plant by experienced graders according to their carcass grade, such as 60 A grade carcasses (normal) and 60 B4 grade carcasses (dark cuts) were classified using PLS-DA. Two different instruments were evaluated delivering similar classification rates (90–95%). According to [Prieto et al. \(2014\)](#), further work remains to be carried out to develop robust VIS-NIR models to be implemented online in the abattoir, where portable equipment applied directly on the carcass could objectively assist in dark-cutting carcass segregation.

4.3 Meals and Feeds

4.3.1 Identification of Animal Species

Since the bovine spongiform encephalopathy (BSE) crisis, the use of animal proteins in animal feed has been prohibited by the European Union (EU) ([Cozzolino and Murray, 2004, 2012](#)). NIR microscopy was used to identify different classes of animal by-products. Samples of fish meals and meals of land animal origin were ground, sedimented, and analyzed using an Auto Image Microscope connected to an FT-NIR. Sediment fraction particles were spread on a Spectralon plate, presented to the NIR microscope, and scanned in the region between 1112 and 2500 nm. The Support Vector

Machine (SVM) algorithm was used to construct models to identify class origin. Models correctly classified 100% of the samples in the calibration set and between 95% and 95.5% in the validation set. The results demonstrated the potential of FT-NIR microscopy as a rapid method for distinguishing between fish and land animal particles (De la Haba et al., 2007).

The use of NIR spectroscopy combined with chemometrics was used to identify and authenticate fishmeal batches made with different fish species. Samples from a commercial fishmeal factory were scanned in the NIR region (1100–2500 nm) in reflectance. Several methods such as PCA, DPLS, and LDA based on PCA scores were used to identify the origin of fishmeal produced using different fish species. The DPLS modes correctly classified 80% and 82% of the fishmeal samples while LDA models correctly classified >80% of fishmeal samples according to fish species (Cozzolino et al., 2002, 2005; Cozzolino and Murray, 2012).

Current and future legislation regarding the use of processed animal proteins in animal nutrition requires the availability of robust analytical methods that allow for the proper implementation of corresponding legal restrictions. The use of NIR microscopy (NIRM) is a method that allows for the differentiation between meat and bone meal and fishmeal, and it is assumed that the different content and composition of the fat is one of the factors responsible for the observed differences. The use of NIRM has been evaluated in order to check for the influence of intentionally introduced reduction of the fat content on the capability of the NIRM method to correctly classify defatted samples. This has practical implications, since processed animal proteins may be defatted by solvents under real-world conditions. The results reported by Tena et al. (2014) confirmed that the scope of the NIRM method could be successfully extended to samples that have been previously extracted with non-polar solvents. Only after the use of stricter techniques, such as extraction with chlorinated solvents or hydrolysis, the NIRM method produced a higher portion of wrong classifications. However, since these extraction techniques are not often used under real-world conditions, the impact upon the use of the NIRM method in the feed sector for the specific application of the differentiation between meat and bone meal and fishmeal is minor (Tena et al., 2014).

4.4 Wine

4.4.1 Identification of Grape Variety

Verification of authenticity of wine and other alcoholic beverages by means of NIR spectroscopy has become a field of increase expansion in both research and in the wine industry. Examples on the use of VIS-NIR spectroscopy to discriminate between wines made from several grape varieties (eg, Riesling, unwooded Chardonnay, Shiraz, Sauvignon Blanc, Tempranillo) or produced from different geographical origins (Australia, New Zealand, Spain, Italy) where different classification accuracies were reported by several authors (Fig. 7.3). Different multivariate data methods were used including PCA, LDA, PLS-DA, and SIMCA (Cozzolino et al., 2003, Liu et al., 2008; Arvantoyannis, 2010; Riovanto et al., 2011; Casale et al., 2010).

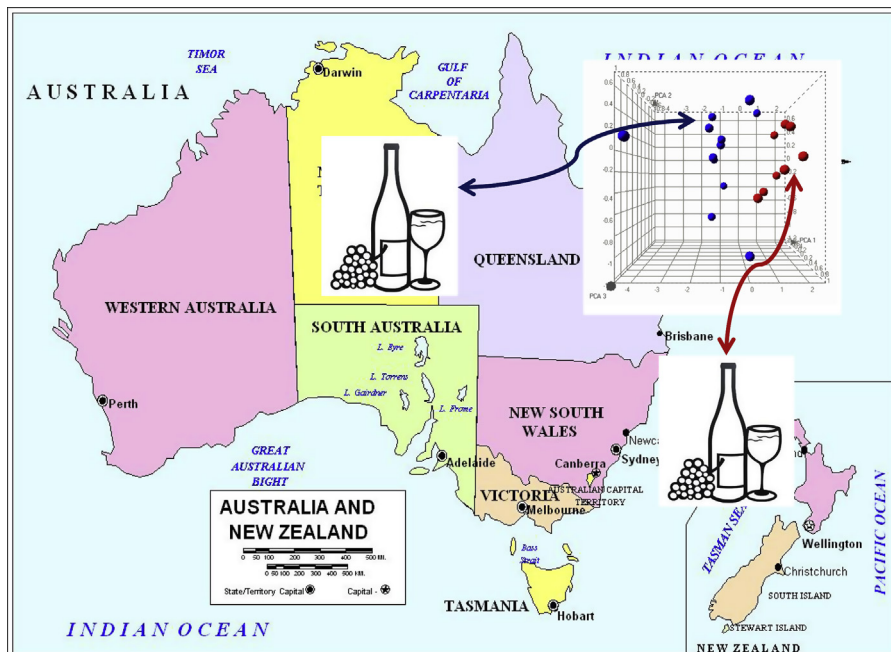


Figure 7.3 Schematic representation of the use of NIR spectroscopy to target wine authenticity. The use of a principal component score based on the NIR spectra of wines allows the identification of the origin of wines from either Australia or New Zealand.

4.4.2 Denomination, Designation, and Geographical Origin

Chemometrics combined with ultraviolet (UV) and VIS-NIR spectroscopy were evaluated to classify wines from different Spanish-controlled Designation of Origin (DO), namely, Rías Baixas (North Spain). Wine samples made solely from the Albariño cultivar from subzones Condado, Salnés, Rosal, and Ribeira do Ulla were analyzed in transmittance mode using the UV, VIS, and NIR ranges alone or in combination. PCA was used to explore and determine patterns in the data where SIMCA, LDA, and SVM classification methods were used to classify the samples. According to the authors the different combinations of spectral ranges and classification methods yield different rates of classification depending on the region. For example, wines originating from the Condado region were completely classified using SIMCA and combinations of the UV/VIS/NIR, NIR, or VIS/NIR spectral ranges, while wines from Salnés and Ribeira do Ulla were completely classified using SIMCA and the UV/VIS spectral range. These results showed that combinations of UV/VIS/NIR spectra and chemometric methods can be used as a rapid technique to classify subzones into a DO such as Rías Baixas. This technique may also assist authentication because the models also discriminated wines from outside Rías Baixas (Martelo Vidal et al., 2013).

The use of NIR spectroscopy was evaluated to distinguish the wines produced in two close provinces of the Abruzzo region of Italy. A collection of 32 wines was considered, 18 of which were produced in the province of Chieti, while the other 14 were

from the province of Teramo. A conventional dual-beam spectrophotometer was used for absorption measurements in the 1300–1900 nm spectroscopic range. Score maps in the PC1-PC2 or PC2-PC3 spaces were obtained, which successfully grouped the wine samples in two distinct clusters, corresponding to Chieti and Teramo provinces, respectively. A modeling of dual-band spectroscopy was also proposed, making use of two LEDs for illumination and a PIN detector instead of the spectrometer. These data were processed using Linear Discriminant Analysis, which demonstrated satisfactory classification results (Mignani et al., 2014).

Geographical origins of wine grapes are significant factors affecting wine quality and wine prices, and it is important to discriminate different wine original regions quickly and accurately. The determinations of wine regions based on Bayesian information fusion that fused NIR transmission spectra information and MIR spectra information of wines were reported. In this method, Xiang et al. (2014) aimed to improve the determination results by expanding the sources of analysis information (combination of NIR and MIR). The four different regions are Huailai, Yantai, Gansu, and Changli, which are all typical geographical originals for Chinese wines. NIR and MIR discriminant models for wine regions were established using PLS-DA based on NIR spectra and MIR spectra separately. The discriminant models reported yield in average accuracy rates of calibration sets between 78.1% and 82.7%, respectively. These results suggested that Bayesian information fusion of NW and MW spectra is feasible for fast identification of wine original regions (Xiang et al., 2014).

4.5 Honey

4.5.1 Floral Origin

Similar to wine, the use of vibrational spectroscopy to authenticate honey has been expanded (Ruoff et al., 2006; Woodcock et al., 2007, 2009; Cozzolino et al., 2011). The feasibility of NIR spectroscopy and multivariate analysis as tools to classify Chinese honey samples according to their different floral origins was explored. Five kinds of honey, namely, acacia, linden, rape, vitex, and jujube, were analyzed using an NIR spectrophotometer with a fiber optic probe. Classification models based on the NIR spectra were developed using Mahalanobis-Distance Discriminant Analysis (MD-DA) and a Back Propagation Artificial Neural Network (BP-ANN). By the MD-DA model, total correct classification rates of 87.4% and 85.3% were observed for the calibration and validation samples, respectively, while the ANN model resulted in total correct classification rates of 90.9% and 89.3% for the calibration and validation sets, respectively. By ANN, the respective correct classification rates of linden, acacia, vitex, rape, and jujube were 97.1%, 94.3%, 80.0%, 97.1%, and 85.7% in calibration, and 100%, 93.3%, 80.0%, 100%, and 73.3% in validation. The results indicated that NIR combined with a classification technique could be a suitable technology for the classification of Chinese honeys from different botanical origins (Chen et al., 2012).

4.5.2 Adulteration With Sugars

The implementation of VIS-NIR spectroscopy for the detection of glucose concentration in a mixture of Saudi and imported honey samples were adulterated by

glucose syrup using five concentrations: 0, 5, 12, 19, and 33 g/100 g, respectively. The results obtained by these authors suggested that NIR spectroscopy is a powerful technique for the quantification of glucose adulteration in Saudi honey ([Mouazen and Wallan, 2014](#)).

The combination of NIR spectroscopy and chemometrics has been evaluated to detect adulteration in honey samples. Several algorithms and preprocessing methods were used such as wavelet transformation (WT), PCA, LS-SVM, SVM, BP-ANN, LDA, and *K* nearest neighbors. These authors reported that WT was the most effective method for variable selection, and the best classification models were achieved using LS-SVM (accuracy of 95.1%) ([Zhu et al., 2010](#)).

4.5.3 Geographical Origin

The information contained in the NIR spectra of honey samples sourced from Protected Geographical Indication (PGI), namely, “Mel de Galicia,” was processed by means of different chemometric techniques to develop an authentication system for this type of sample. According to these, authors classification based on SIMCA achieved the best classification model with 93.3% of sensitivity and 100% of specificity. The main conclusion from these authors was that the combination of NIR information data with SIMCA allowed the development of a single and fast method to differentiate between genuine PGI-Galician honey samples and other commercial honey samples from other origins ([Herrero Latorre et al., 2013](#)).

4.5.4 Brand Identification

The discrimination of four commercial brands of Portuguese honey samples according to their botanical origin using a combination of sensors such as fusion of impedance Electronic Tongue (E-Tongue) and optical spectroscopy (UV–VIS–NIR) with PCA and Cluster Analysis (CA) was explored. A new technique for variable selection through one-dimensional clustering, which proved very useful for data fusion, was also evaluated. The results were validated against the standard sample identification method using melissopalynology analysis. Both the electronic and optical spectra were fitted to analytical models, and the model coefficients were used as new variables for PCA and CA. According to [Ulloa et al. \(2013\)](#), this approach has improved honey classification by the E-Tongue but not by the optical methods. The use of Multiway PCA (MPCA) was the best option for data fusion, yielding 100% classification success. The authors have demonstrated that the use sensor fusion (electronic and optical spectroscopy) data and variable selection methods significantly improved the classification of the honey samples through multivariate statistical analysis ([Ulloa et al., 2013](#)).

5. Concluding Remarks

This chapter has shown how NIR spectroscopy has been developed as one of the most versatile methods to target issues associated with authenticity, discrimination, or traceability in several foods. The main advantages of NIR spectroscopy

over traditional chemical and chromatographic methods (eg, HPLC, GC, GC–MS) are the speed, minimal sample preparation, and ease to use in an industrial setting or routine operations. However, adapting and applying this method to efficiently and consistently monitor authenticity, we need to increase our understanding about the chemical and biochemical basis associated with origin/authenticity/traceability derived from the NIR spectra, in order to maintain a sustainable food production and to guarantee to the consumers the origin of the foods. However, some of the disadvantages and limitations of NIR spectroscopy are related with the lack of sensitivity and selectivity compared to other tools such as MIR and Raman spectroscopy. NIR absorption bands are generally broad and overlapping therefore to extract useful information from NIR spectral data, and multivariate data analysis tools such as PCA and PLS are necessary. In addition, the use of the multivariate data analysis makes the method validation very challenging.

New approaches, such as the use of new algorithms, data preprocessing, the combination of different sensors (sensor fusion), and the developments in hyperspectral spectroscopy proved to be an alternative to the “classical” use of NIR spectroscopy in order to improve the classification accuracy of the models.

In the near future, we can imagine that portable and easy to use NIR instruments (eg, instruments and apps in mobile phones) will allow consumers to verify the authenticity of their foods as well as to trace the origin of the product at the supermarket, acting as the first line of defense against food fraud.

References

- Abbas, O., Dardenne, P., Baeten, V., 2012. Near-infrared, mid-infrared, and Raman spectroscopy. In: *Chemical Analysis of Food: Techniques and Applications*. Elsevier Science, Pico Y. Burlington, pp. 59–91.
- Arvantoyannis, I.S., Chalhouh, C., Gotsiou, P., Lydakis-Simantiris, N., Kefalas, P., 2005. Novel quality control methods in conjunction with chemometrics (multivariate analysis) for detecting honey authenticity. *Critical Reviews in Food Science and Nutrition* 45, 193–203.
- Arvantoyannis, I., Katsot, M.N., Psarra, P., Soufleros, E., Kallinthraka, S., 1999. Application of quality control methods for assessing wine authenticity: use of multivariate analysis (chemometrics). *Trends in Food Science & Technology* 10, 321–329.
- Arvantoyannis, I., 2010. Wine Authenticity, traceability and safety monitoring. In: Reynolds, A.G. (Ed.), *Managing Wine Quality*. CRC Pres, Boca Raton, USA.
- Blanco, M., Villaroya, I., 2002. NIR spectroscopy: a rapid-response analytical tool. *Trends in Analytical Chemistry* 21, 40–50.
- Brereton, R.G., 2003. *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*. John Wiley & Sons, Ltd, Chichester, UK.
- Brereton, R.G., 2007. *Applied Chemometrics for Scientist*. John Wiley & Sons, Ltd, Chichester, UK.
- Casale, M., Oliveri, P., Armanino, C., 2010. NIR and UV-vis spectroscopy, artificial nose and tongue: comparison of four fingerprinting techniques for the characterisation of Italian red wines. *Analytica Chimica Acta* 668, 143–147.

- Cordella, Ch, Moussa, I., Martel, A.-C., Sbirrazzuoli, N., Lizzani-Couvelier, L., 2002. Recent developments in food characterisation and adulteration detection: technique-oriented perspectives. *Journal of Agricultural and Food Chemistry* 50, 1751–1764.
- Cozzolino, D., 2009. Near infrared spectroscopy in natural products analysis. *Planta Medica* 75, 746–757.
- Cozzolino, D., 2011. Infrared methods for high throughput screening of metabolites: food and medical applications. *Combinatorial Chemistry & High Throughput Screening* 14, 125–131.
- Cozzolino, D., 2012. Recent trends on the use of infrared spectroscopy to trace and authenticate natural and agricultural food products. *Applied Spectroscopy Reviews* 47, 518–530.
- Cozzolino, D., Murray, I., 2012. A review on the application of infrared technologies to determine and monitor composition and other quality characteristics in raw fish, fish products, and seafood. *Applied Spectroscopy Reviews* 47, 207–218.
- Cozzolino, D., Corbella, E., Smyth, H.E., 2011. Quality control of honey using infrared spectroscopy: a review. *Applied Spectroscopy Reviews* 46, 523–538.
- Cozzolino, D., Murray, I., 2004. Analysis of animal by-products. In: Roberts, C.A., Workman, J., Reeves III, J.B. (Eds.), *Near Infrared Spectroscopy in Agriculture*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, USA, pp. 647–662.
- Cozzolino, D., Chree, A., Murray, I., Scaife, J.R., 2002. The assessment of the chemical composition of fishmeal by near infrared reflectance spectroscopy. *Aquaculture Nutrition* 8, 149–155.
- Cozzolino, D., Chree, A., Scaife, J.R., Murray, I., 2005. Usefulness of near infrared reflectance (NIR) spectroscopy and chemometrics to discriminate fishmeal batches made with different fish species. *Journal of Agricultural and Food Chemistry* 53, 4459–4463.
- Cozzolino, D., Smyth, H.E., Gishen, M., 2003. Feasibility study on the use of visible and near-infrared spectroscopy together with chemometrics to discriminate between commercial white wines of different varietal origins. *Journal of Agricultural and Food Chemistry* 51, 7703.
- Chen, L., Wang, J., Ye, Z., Zhao, J., Xue, X., Vander Heyden, Y., Sun, Q., 2012. Classification of Chinese honeys according to their floral origin by near infrared spectroscopy. *Food Chemistry* 135 (2), 338–342.
- Dai, Q., Cheng, J.-H., Sun, D.-W., Pu, H., Zeng, X.-A., Xiong, Z., 2015. Potential of visible/near-infrared hyperspectral imaging for rapid detection of freshness in unfrozen and frozen prawns. *Journal of Food Engineering* 149, 97–104.
- De la Haba, M.J., Pierna, J.A.F., Fumiere, O., Garrido-Varo, A., Guerrero, J.E., Perez-Marin, D.C., Dardenne, P., Baeten, V., 2007. Discrimination of fish bones from other animal bones in the sedimented fraction of compound feeds by near infrared microscopy. *Journal of Near Infrared Spectroscopy* 15, 81–88.
- Ding, R., Huang, X., Han, F., Dai, H., Teye, E., Xu, F., 2014. Rapid and nondestructive evaluation of fish freshness by near infrared reflectance spectroscopy combined with chemometrics analysis. *Analytical Methods* 6, 9675–9683.
- Downey, G., 1996. Authentication of food and food ingredients by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 4, 47–61.
- Downey, G., 1998. Food and food ingredient authentication by mid-infrared spectroscopy and chemometrics. *Trends in Analytical Chemistry* 17, 418–424.
- Downey, G., 2013. Vibrational spectroscopy in studies of food origin. In: Brereton, R.G. (Ed.), *New Analytical Approaches for Verifying the Origin of Food*. Woodhead, Publisher.

- Hao, D.-M., Zhou, Y.-N., Wang, Y., Zhang, S., Yang, Y.-M., Lin, L., Li, G., Wang, X.-L., 2015. Recognition of water-injected meat based on visible/near-infrared spectrum and sparse representation. *Spectroscopy and Spectral Analysis* 35, 93–98.
- Herrero Latorre, C., Pena Crecente, R.M., Garcia Martin, S., 2013. A fast chemometric procedure based on NIR data for authentication of honey with protected geographical indication. *Food Chemistry* 141, 3559–3565.
- Huang, H., Yu, H., Xu, H., Ying, Y., 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. *Journal of Food Engineering* 87, 303–313.
- Kamruzzaman, M., Makino, Y., Oshita, S., 2015. Non-invasive analytical technology for the detection of contamination, adulteration, and authenticity of meat, poultry, and fish: a review. *Analytica Chimica Acta* 853, 19–29.
- Karoui, R., Downey, G., Blecker, C., 2010. Mid-infrared spectroscopy coupled with chemometrics: a tool for the analysis of intact food systems and the exploration of their molecular structure–quality relationships – a review. *Chemical Reviews* 110, 6144–6168.
- Khakimov, B., Gürdeniz, G., Engelsen, S.B., 2015. Trends in the application of chemometrics to foodomics studies. *Acta Alimentaria* 44, 4–31.
- Liu, L., Cozzolino, D., Cynkar, W.U., Gishen, M., Colby, C.B., 2006. Geographic classification of Spanish and Australian Tempranillo red wines by visible and near infrared spectroscopy combined with multivariate analysis. *Journal of Agricultural and Food Chemistry* 54, 6754.
- Liu, L., Cozzolino, D., Cynkar, W.U., Damberg, R.G., Janik, L., O'Neill, B.K., Colby, C.B., Gishen, M., 2008. Preliminary study on the application of visible–near infrared spectroscopy and chemometrics to classify Riesling wines from different countries. *Food Chemistry* 106, 781.
- Ma, J., Pu, H., Sun, D.-W., Gao, W., Qu, J.-H., Ma, K.-Y., 2015. Application of Vis-NIR hyperspectral imaging in classification between fresh and frozen-thawed pork Longissimus Dorsi muscles. *International Journal of Refrigeration-Revue Internationale du Froid* 50, 10–18.
- Manley, M., 2014. Near-infrared spectroscopy and hyperspectral imaging: non-destructive analysis of biological materials. *Chemical Society Reviews* 43, 8600.
- Martelo-Vidal, M.J., Domínguez-Agis, F., Vázquez, M., 2013. Ultraviolet/visible/near-infrared spectral analysis and chemometric tools for the discrimination of wines between subzones inside a controlled designation of origin: a case study of Rías Baixas. *Australian Journal of Grape and Wine Research* 19, 62–67.
- McClure, W.F., 2003. Review: 204 years of near infrared technology: 1800–2003. *Journal of Near Infrared Spectroscopy* 11, 487–518.
- Mignani, A.G., Ciaccheri, L., Gordillo, B., Mencaglia, A.A., Gonzalez-Miret, M.L., Heredia, F.J., Cichelli, A., Vo-Dinh, T., Lieberman, R.A., Gauglitz, G.G., 2014. Near-infrared spectroscopy and pattern-recognition processing for classifying wines of two Italian provinces. In: *Advanced Environmental, Chemical, and Biological Sensing Technologies XI. Proceedings of SPIE*, 9106.
- Mouazen, A.M., Al-Walaan, N., 2014. Glucose adulteration in Saudi honey with visible and near infrared spectroscopy. *International Journal of Food Properties* 17, 2263–2274.
- Naes, T., Isaksson, T., Fearn, T., Davies, T., 2002. *A User-Friendly Guide to Multivariate Calibration and Classification*. NIR Publications, Chichester, UK.
- Nicolai, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. *Postharvest Biology and Technology* 46, 99–118.
- Niu, X.-Y., Shao, L.-M., Dong, F., Zhao, Z.-L., Zhu, Y., 2014. Discrimination of donkey meat by NIR and chemometrics. *Spectroscopy and Spectral Analysis* 34, 2737–2742.

- O'Brien, N., Hulse, C.A., Pfeifer, F., Siesler, H.W., 2013. Technical note near infrared spectroscopic authentication of seafood. *Journal of Near Infrared Spectroscopy* 21, 299–305.
- Oliveri, P., Di Egidio, V., Woodcock, T., Downey, G., 2011. Application of class-modelling techniques to near infrared data for food authentication purposes. *Food Chemistry* 125, 1450–1456.
- Ottavian, M., Fasolato, L., Serva, L., Facco, P., Barolo, M., 2014. Data fusion for food authentication: fresh/Frozen-thawed discrimination in west African goatfish (*Pseudupeneus prayensis*) filets. *Food and Bioprocess Technology* 7, 1025–1036.
- Otto, M., 1999. *Chemometrics: Statistics and Computer Application in Analytical Chemistry*, second ed. Wiley-VCH, Federal Republic of Germany, p. 343.
- Prieto, N., Lopez-Campos, O., Zijlstra, R.T., Uttaro, B., Aalhus, J.L., 2014. Discrimination of beef dark cutters using visible and near infrared reflectance spectroscopy. *Canadian Journal of Animal Science* 94, 445–454.
- Reid, L.M., O'Donnell, C.P., Downey, G., 2006. Recent technological advances for the determination of food authenticity. *Trends in Food Science & Technology* 17, 344–353.
- Riovanto, R., Cynkar, W.U., Berzaghi, P., Cozzolino, D., 2011. Discrimination between shiraz wines from different Australian regions: the role of spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry* 59, 10356–10359.
- Rodriguez-Saona, L.E., Allendorf, M.E., 2011. *Annual Review of Food Science and Technology* 2, 467.
- Roggo, Y., Chalus, P., Maurer, L., Lema-Martinez, C., Edmond, A., Jent, N., 2007. A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies. *Journal of Pharmaceutical and Biomedical Analysis* 44, 683–690.
- Ropodi, A.I., Pavlidis, D.E., Mohareb, F., Panagou, E.Z., Nychas, G.-J.E., 2015. Multispectral image analysis approach to detect adulteration of beef and pork in raw meats. *Food Research International* 67, 12–18.
- Ruoff, K., Luginbuhl, W., Bogdanov, S., 2006. Authentication of the botanical origin of honey by near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry* 54, 6867–6872.
- Sentandreu, A.M., Sentandreu, E., 2014. Authenticity of meat products: tools against fraud. *Food Research International* 60, 19–29.
- Tena, N., Fernandez Pierna, J.A., Boix, A., Baeten, V., von Holst, C., 2014. Differentiation of meat and bone meal from fishmeal by near-infrared spectroscopy: extension of scope to defatted samples. *Food Control* 43, 155–162.
- Tzouros, N.E., Arvantoyannis, I.S., 2001. Agricultural produces: synopsis of employed quality control methods for the authentication of foods and application of chemometrics for the classification of foods according to their variety or geographical origin. *Critical Reviews in Food Science and Nutrition* 41, 287–319.
- Ulloa, P.A., Guerra, R., Cavaco, A.M., Rosa da Costa, A., Figueira, A.C., Brigas, A.F., 2013. Determination of the botanical origin of honey by sensor fusion of impedance e-tongue and optical spectroscopy. *Computers and Electronics in Agriculture* 94, 1–11.
- Woodcock, T., Downey, G., O'Donnell, C.P., 2008. Better quality food and beverages: the role of near infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 16, 1–29.
- Woodcock, T., Downey, G., O'Donnell, C.P., 2009. Near infrared spectral fingerprinting for confirmation of claimed PDO provenance of honey. *Food Chemistry* 114, 742–746.
- Woodcock, T., Downey, G., Kelly, J.D., 2007. Geographical classification of honey samples by near-infrared spectroscopy: a feasibility study. *Journal of Agricultural and Food Chemistry* 55, 9128–9134.

- Weeranantanaphan, J., Downey, G., Allen, P., Da-Wen, S., 2011. A review of near infrared spectroscopy on muscle food analysis: 2005–2010. *Journal of Near Infrared Spectroscopy* 19, 61–104.
- Xiang, L.-L., Li, M.-H., Li, J.-M., Li, J.-H., Zhang, L.-D., Zhao, L.-L., 2014. Determination of wine original regions using information fusion of NIR and MIR spectroscopy. *Spectroscopy and Spectral Analysis* 34, 2662–2666.
- Zhu, X., Li, S., Shan, Y., Zhang, Z., Li, G., Su, D., Liu, F., 2010. Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics. *Journal of Food Engineering* 101, 92–97.
- Zotte, A.D., Ottavian, M., Concollato, A., Lorenzo, S., Martelli, R., Parisi, G., 2014. Authentication of raw and cooked freeze-dried rainbow trout (*Oncorhynchus mykiss*) by means of near infrared spectroscopy and data fusion. *Food Research International* 60, 180–188.