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Authentication and Traceability of Agricultural and Food Products Using Vibrational Spectroscopy

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1. Introduction

Food and feed safety is an increasing concern for consumers following major crises related directly or indirectly to human health. EU has created a key tool, the Rapid Alert System for Food and Feed (RASFF 2015), for reacting quickly to food and feed safety emergencies and incidents. Currently, European foods are recognized globally for their high standards of production, labelling and safety. This is not yet the case, however, for the detection of food fraud or the enforcement of the relevant legislation. There

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is a clear need for an initiative that will link the major stakeholders, establish data-sharing tools and working practices, and provide rapid fit-for-purpose screening and verification methods. The European Food Integrity project (2014–2018) aims to address that need. It is not a single method based on a single technique that will address all the needs of farmers, producers, retailers, regulatory bodies and consumers, but rather a combination of methods and strict legislation. Farmers need analytical methods giving them the ability at the farm level to check that production matches the authenticity and quality criteria included in the product specifications. Food producers need analytical methods enabling them to define the authenticity criteria and check the compliance of the raw material produced. Retailers need tools enabling them to check that products reflect the criteria agreed with food producers. On the other hand, regulatory bodies need analytical methods for certifying the products in terms of the legislation on quality, safety and authenticity. Confirmatory methods are needed that provide indisputable information that could be used, if necessary, in court. The development of analytical tools is less important for consumers, who simply want enough information to feel reassured about the authenticity of a product and the likelihood of it meeting their expectations.

Currently, many methods based on various analytical techniques are used to authenticate agro-food products. Among them, vibrational spectroscopy techniques include NIR, MIR, Raman and Terahertz techniques. They are based on measuring the amount of electromagnetic radiation absorbed by a sample according to the Beer-Lambert law. They are techniques to consider when authenticity controls need to be established at the field level, at the point where products are delivered to factories or during the production process. Methods based on these techniques are indirect, rapid and do not require skilled staff. They are not confirmatory and are therefore seldom used in official control processes. Fingerprinting methods, however, are of interest to regulatory bodies because they allow rapid preventative action to be taken. It should be noted that, despite the many studies demonstrating their potential, the application of fingerprinting methods in routine analysis and food authenticity surveillance remains limited (Riedl et al. 2015). The second section of this chapter provides a general overview of the technology, main principles, instrumentation, sample presentation and new trends, as well as giving a brief overview of the chemometric tools used to extract chemically relevant information from the spectra.

In the third section of the chapter, several examples are discussed to illustrate the potential of vibrational spectroscopy in tackling authenticity challenges (e.g., the discrimination of cereal varieties), in identifying botanical origin, geographical origin and distillers' dried grains and solubles (DDGS) production process, in the traceability and authentication of fruits and in the early detection of fraud in food/feed ingredients. Several

examples come from European projects focusing on authenticity: Qsaffe (2011–2014), which looked at the early detection of fraud in feed and at DDGS authentication; PhotonFruit (2014–2015), which dealt with emergent spectroscopic techniques for the quality control and traceability of fruits and fruit-based products; FoodIntegrity (2014–2018), which aims to provide industries and regulatory authorities in the food and feed sectors with information on the analytical methods available, their use, performance and cost, as well as the availability of reference data, with links to literature and other databases.

2. Vibrational Spectroscopy Methods: NIR, MIR and Raman

This section gives the main principles of near-infrared, mid-infrared and Raman spectroscopy as well as new trends regarding instrumentation and sample presentation. It gives also a brief overview of the chemometric tools used through the examples presented in Section 3.

2.1 Principle

The term 'electromagnetic spectrum' refers to the collection of radiant energy sources, from gamma rays to radio waves. These waves are characterized by wavelength λ (the length of one wave, cm), frequency ν (the number of vibrations per unit time, Hz) and wave number ν (the number of waves per unit length, cm^{-1}). Spectroscopy can be defined as the study of the interaction between electromagnetic radiation and matter. The electromagnetic spectrum is divided into several regions, each of which induces specific molecular or atomic transition and is therefore suited to a specific type of spectroscopy. This chapter focuses on wavelengths in the 12,500–50 cm^{-1} range within which mid-infrared (MIR), near-infrared (NIR) and Raman spectroscopies are used for traceability and authentication.

Infrared radiation, lying between visible and microwave regions of the electromagnetic spectrum, is absorbed by organic molecules and converted into energy as molecular vibration. Vibrational transitions occur in the ground state of the molecule (Li-Chan 1996). Vibrational energy is quantized. Molecules can occupy discrete energy levels defined by whole numbers 0, 1, 2, and so on. Molecules, in nature, occupy the lowest energy level, 0. A transition from levels 0 to 1, in the MIR spectral region, is referred to as a fundamental transition. Transitions from levels 0 to 2 or 3, in the NIR spectral region, are defined as first and second overtones.

Although it is beyond the scope of this chapter to present the theory of vibrational spectroscopy, it is necessary to outline some of its basic principles in order to understand how each spectroscopic technique works.

The overall objective of vibrational spectroscopic techniques is to analyze a product in order to obtain qualitative and/or quantitative information from the interaction between the electromagnetic spectrum and its constituents (Abbas et al. 2012, Baeten et al. 2016).

2.1.1 Mid-infrared spectroscopy (MIRS)

MIRS refers to the absorption measurement of different MIR frequencies by a sample positioned in the path of an MIR beam (Baranska 1987). When the frequency of a specific vibration is equal to the frequency of the infrared radiation directed at a molecule, the molecule absorbs the radiation. The method is simple, non-destructive, rapid and environmentally friendly.

The MIR spectrum lies in the 400–4,000 cm^{-1} range of the electromagnetic spectrum. It is considered as a fingerprint of the sample in that no two molecular structures produce the same infrared spectrum, making infrared spectroscopy very interesting for traceability analysis (Coates 2000). MIRS allows the quality and authenticity of a sample, as well as the quantity of its components, to be determined. Using the Beer-Lambert law, it is possible to correlate the intensity of one band with the concentration of the active group of the product. MIRS is sensitive to functional groups and to highly polar bonds. Hydroxyl, amine and carbonyl groups are very active in the MIR region and produce high spectral signals.

2.1.2 Near-infrared spectroscopy (NIRS)

NIRS is based on the same absorption phenomenon described above for MIRS, but relates to the 12,500–4,000 cm^{-1} wavelength range (equivalent to 800–2,500 nm; nm is the unit usually used in NIRS) (Baranska 1987). This energy range is high enough to promote molecules from their fundamental vibrational energy levels to second or third excited vibrational states, but it is low enough not to reach the level of electron excitation in molecules. The method is simple, non-destructive and very fast (< 30 sec analysis time), but there is a greater penetration of the radiation than in MIRS. NIRS is suitable for in-line, on-line or at-line use, with minimum sample preparation requirements. It allows highly accurate and precise multi-component analysis (C-H, NH, S-H or O-H bonds).

NIRS can be used as a qualitative method. Due to its large bands being less resolved than in the case of MIRS (Luykx and van Ruth 2008), it is used mainly for quantifying sample properties, such as chemical composition (e.g., protein, glucose, humidity), bulk properties (e.g., density, viscosity, ripeness) and physical properties (e.g., temperature, particle size).

2.1.3 Raman spectroscopy

Unlike MIRS and NIRS, Raman spectroscopy is not concerned with an absorption phenomenon. It is based on irradiation with an intense monochromatic light source (usually a laser), which raises the energy of the system by inducing polarization in the chemical species. The polarized condition referred to a 'virtual state'. The vibrational energy levels in the molecules rise from the ground state to a short-lived, high-energy collision state, which returns to a lower-energy state by emitting a photon that has a lower frequency than the laser light (Stokes Raman scattering). A Raman spectrum between 4,000 and 50 cm^{-1} is a plot of the intensity of Raman scattered radiation as a function of its frequency difference from the incident radiation (Baranska 1987). This difference is called the Raman shift.

Raman spectroscopy has the advantage of requiring little or no sample preparation and allows samples to be measured through a glass container (samples can be analyzed directly inside a glass bottle or plastic bag). In addition, it is not affected by water band interference (ease of aqueous solutions analysis) or atmospheric perturbation, such as CO_2 and humidity (no need to purge the instrument).

As in the case of MIRS, Raman spectroscopy provides structural information and can be considered as a fingerprint. It also provides information from backbone structures and symmetric bonds (e.g., carbon double and triple bonds, and aromatic groups), as well as qualitative and quantitative information. No two molecules have exactly the same Raman spectrum, and the intensity of the scattered radiation is proportional to the amount of material present.

A brief overview of the three techniques is given in Table 1. For each technique, the table shows the spectral region, radiation source, excitation conditions, origin of bands, vibrational modes, band shape, particle size, drawbacks and a selection of applications.

2.2 Instrumentation

Because of the increasing use of infrared and Raman spectroscopies as screening and quality control methods, spectrometers are evolving rapidly at the laboratory, industry, field and farm levels.

2.2.1 Mid-infrared spectrometers

Dispersive spectrometers were the first infrared instruments. The energy emitted from an infrared source is separated into individual frequencies by the use of a prism or grating system. The detector measures the quantity of energy at each frequency that has passed through or been reflected from a sample, resulting in a spectrum that is a plot of intensity vs. frequency.

Table 1. Some Characteristics of NIR, MIR and Raman Spectroscopies.

	NIR	MIR	Raman
Spectral region	4,000–12,500 cm^{-1} (800–2,500 nm)	400–4,000 cm^{-1}	50–4,000 cm^{-1}
Radiation source	Polychromatic near-infrared light from globar tungsten light source	Polychromatic mid-infrared light from globar tungsten light source	Monochromatic visible or near-infrared light from a laser
Excitation conditions	Change in dipole moment	Change in dipole moment	Change in polarizability
Band origin	Radiation absorption	Radiation absorption	Radiation scattering
Vibrational modes	Overtones and combinations of vibrational modes	Stretching and bending fundamental vibrations	Stretching and bending fundamental vibrations
Band shape	Broad peaks arising from overlapping absorption bands	Well resolved, assignable to specific chemical groups	Well resolved, assignable to specific chemical groups
Particle size	Dependent	Dependent	Independent
Interference	Water	Water	Fluorescence
Main applications	Quantification	Structural elucidation and compound identification	Structural elucidation and compound identification

Due to the limitations of these dispersive instruments, however (e.g., slow scanning and lack of reproducibility), they were replaced by Fourier transform (FT) spectrometers.

FT spectrometers enable all infrared frequencies to be measured rapidly and simultaneously. They are equipped with a simple optical device called an 'interferometer'. It contains a beam splitter, which divides an incoming infrared beam into two parts. One beam is reflected off a fixed mirror and the other off a moving mirror, and then both beams are recombined at the beam splitter. Due to changes in the position of the moving mirror in relation to the fixed one, an interference pattern is generated that results in a signal called an 'interferogram'. This signal, a function of time, cannot be interpreted directly. It is converted mathematically by FT, resulting in a frequency spectrum. The detectors used are deuterated-triglycine sulfate (DTGS), based on measuring temperature changes, and the nitrogen-cooled Mercury cadmium telluride (MCT) photon detector.

The advantages of MIR spectrometers include their speed (all frequencies are scanned simultaneously), sensitivity (high optical throughput and sensitive detectors), mechanical simplicity (only one mirror of the interferometer moves) and internal calibration (an internal wavelength calibration standard using a HeNe reference laser), making FT infrared analysis very accurate and reproducible. On-line MIR spectrometers are used for quantification at low analyte levels because they are very

sensitive, the main issue being the strong absorption of water (Bellon-Maurel et al. 1994).

2.2.2 Near-infrared spectrometers

The characteristics of near-infrared instruments make them ideal for industrial applications, especially because of their robustness, simplicity and humidity resistance. Their main elements are a radiation source (thermal or non-thermal) (Osborne et al. 1993, McClure 2001), wavelength selectors, sampling accessories and detectors (Single-channel or Multichannel) (McClure 2001).

Pasquini (2003) classified spectrometers according to the technology used for wavelength selection.

- Filter-based instruments using filters as wavelength selectors.
- Light-emitting diodes (LED)-based instruments using LED as a source of narrow bands of NIR radiation or to produce a polychromatic, highly stable source in which radiation is dispersed by using a monochromator based on gratings or filter optics.
- Acousto-optical tunable filters (AOTF)-based instruments using AOTF, which allows constructing instruments that have no moving parts and can reach very high scan speeds over a broad NIR spectral range. The wavelength precision is about ± 0.05 nm and the resolution depends on the wavelength, with typical values of 5–15 nm for a wavelength range of 1,000–2,500 nm.
- Dispersive optics-based instruments using diffraction gratings. Initially, these instruments suffered from slow scan speed, lack of wavelength precision and presence of moving parts, making them difficult to use. In the past decade, the construction of dispersive optics based on the concave grating and sensor array usually used in spectrophotometers with non-moving parts has meant that spectra can now be collected in a few milliseconds.
- FTIR using interferometer technology. These spectrometers combine most of the best characteristics in terms of wavelength precision and accuracy (wavelength accuracy is higher than 0.05 nm), high signal-to-noise ratio and scan speed.

On-line NIRS is well developed and widely implemented. Huang et al. (2008) conducted a review of NIR on-/in-line analysis of foods such as meat, fruit, grain, dairy products and beverages, covering the previous 10 years of research in this field. The tendency is now to use miniaturized spectrometers adapted to specific conditions of measurement in fields, greenhouses and on-line agro-food industrial production. They are flexible enough for a wide range of optical fibers and measurement accessories to be

connected. These spectroscopic devices are currently the subject of extensive research and development (Crocombe 2013) aimed at the improvement of detector technologies, microelectro-mechanical systems (MEMS) and high-precision optical components. Some of the new instruments are based on MEMS technology, on the Fabry–Perot-Based MidWave InfraRed (MWIR) microspectrometer (Ebermann et al. 2009) and others such as the NIR grating spectrometer for mobile phone applications (Pügner et al. 2016).

2.2.3 Raman spectrometers

Raman spectroscopy measures the shift in frequency from the photons emitted by the excitation laser. Because it can be performed using any range from UV to NIR, there are two types of Raman instruments, dispersive Raman spectrometers and FT-Raman spectrometers, each one with advantages for specific types of analysis.

With dispersive instruments, the scattered light is collected through a filter and focused onto a monochromator, which allows the separation of the different energies of the Raman scattering. The radiation is directed onto a silicon charge-coupled device (CCD). Visible laser excitation is usually done with these instruments (lasers emitting at 473 nm, 532 nm, 633 nm and 780 nm). Irradiation at these wavelengths enables obtaining of high Raman signals because the intensity of the Raman scatter is proportional to the fourth power of the Raman excitation frequency. A problem that can occur here is the fluorescence phenomenon, which saturates the CCD detector and makes it difficult to conduct Raman measurements.

The near-infrared laser radiation range corresponding to less energy can provide a solution to the fluorescence problem, using FT-Raman spectrometers. These instruments have a neodymium-doped yttrium aluminium garnet (Nd3+:YAG) laser irradiating at 1,064 nm and sensitive, single-element, near-infrared detectors, such as indium gallium arsenide (InGaAs) or liquid nitrogen-cooled germanium (Ge) detectors. FT-Raman spectrometers use an interferometer that functions in the same way as FT-IR spectrometers and has the same advantages.

Depending on the nature of the sample and the objective of the analysis, dispersive or FT-Raman spectrometers can be used. Although the Raman instrument market is growing rapidly, the use of these devices in the agro-food industry remains limited.

2.3 Sample presentation

MIR, NIR and Raman instruments enable the analysis of a great variety of feed and food samples in their liquid or solid form. In order to obtain the best quality spectrum and have confidence in the results, it is important to use the best handling technique for the analyzed sample. Table 2 lists the most

Table 2. Comparison of Sample Presentation and Most Common Measurement Modes for MIRS and NIRS.

Mode	Principle	Type of samples	Accessories	Advantages	Drawbacks
Transmission	IR beam passes through the sample, and the transmitted energy is measured	Powders, liquids and gases	No accessory, but need to prepare a sample in a pellet or film	Low cost of cells Qualitative and quantitative measurements	Difficulty of ensuring good repeatability of deposited films or thickness of KBr pellets
Attenuated total reflection (ATR)	IR radiation enters the crystal in which it is reflected. It penetrates the sample a finite amount via the 'evanescent' wave. At the output end of the crystal, the beam is directed out of the crystal and back into the normal beam path of the spectrometer	Strongly absorbing and thick sample solids that can be ground into powders; Flat materials liquids	Crystal with a high refractive index	No sample preparation (no need to heat, press or grind samples) Fast and easy clean-up	Difficulty of analyzing heterogeneous samples
Diffuse reflection	IR radiation interacts with the particles and then reflects off their surface. Light is diffused or scattered as it moves throughout the sample	Samples that can be ground into a fine powder; Granular samples	Reflection accessory	Little sample preparation (grinding the sample, no need to press) Fast and easy clean-up	Difficulty of defining the path length
Transflection	The IR radiation collected results from light reflected by the measuring cell and light transmitted from the sample	Partially transparent samples	Cells with reflective surface Probes		Difficulty of obtaining the same repeatable optical path between samples

widely used infrared measurement modes and their principles, types of samples, accessories, advantages and drawbacks. Raman analysis requires only a glass container for liquids or a rotation sample holder for solids.

Combining NIRS, MIRS and Raman spectroscopy with imaging technology enables obtaining spectral and spatial information simultaneously. Analyses are achieved in a very short time by recording sequential images of the analyzed sample with each image plane being collected at a single wavelength band. Taking the example of NIR imaging, the compilation of the reflected energy images, taken sequentially at each wavelength, produces a hyperspectral cube. For each pixel, the compilation of absorbances at each wavelength produces a spectrum (Abbas et al. 2012). It should be noted that for feed and food applications, NIR hyperspectral imaging is far more widely used than MIR or Raman imaging, which are more suited to polymer and pharmaceutical applications.

MIRS and Raman spectroscopy, and to some extent NIRS, have evolved from interesting research techniques and now provide valuable analytical tools that can be used on farms, in industries and at production sites. Using them for authentication purposes is of great interest.

2.4 Chemometrics

As these methods are indirect, they require the use of chemometrics to extract chemically relevant information from spectra with statistical, mathematical and computer tools (Massart et al. 1988). In the authenticity and traceability examples presented in the third section, several multivariate techniques were used to explore data patterns (principal component analysis, PCA) or build discrimination models for correctly identifying the products of different functional classes of any given food ingredient (linear discriminant analysis, LDA; soft independent method of class analogy, SIMCA; partial least squares discriminant analysis, PLS-DA). More sophisticated indicators/tools (e.g., GH for calculating Mahalanobis distance; weighted principal components analysis, WPCA) can be used (see Section 3.5). The results of the discrimination models can be expressed in different ways. Sensitivity refers to the percentage of samples from the class studied that have been correctly classified by the model. Specificity refers to the percentage of samples not from the class studied that have been correctly classified by the model. Classification error is the sum of the false positive results (percentage of samples predicted to belong to the class studied, when they do not) and false negative results (percentage of samples predicted not to belong to the class studied, when they do).

3. Traceability and Authenticity: Food and Feed Examples

This section gives several examples to illustrate the potential of vibrational spectroscopy regarding authenticity challenges such as the discrimination of barley varieties, the origin identification of distillers' dried grains and solubles (DDGS), the authentication of fruits and finally, the early detection of fraud in food/feed ingredients.

3.1 Discrimination among varieties: the case of barley

Breeders, farmers and consumers are showing increasing interest in *Triticum* species other than common wheat (*T. aestivum* L.), such as emmer (*T. dicoccum* L.) and spelt (*T. spelta* L.), which are ancestral hulled wheat species characterized by higher resistance to stress and by specific nutritional qualities (Escarnot et al. 2012). Effective species discrimination among grains is increasingly important not only for the food industry in terms of the characteristics and qualities required, but also for the protection of Protected Geographical Indication (PGI)-certified species (e.g., farro della Garfagnana emmer). Discussions with representatives of the food industry involved in the cereal chain has shown that NIRS is undertaken in many cereal producer sites in order to rapidly check quality and safety parameters, with the potential to use fingerprinting approaches for detecting possible fraud (Suman 2016). In order to solve some authentication issues, the discrimination of species/varieties is needed at the kernel level. For instance, only 3% of common wheat (*T. aestivum* L.) and durum wheat (*T. durum* L.) crops are authorized to produce pasta in Italy. As the durum wheat price is generally higher than the price of common wheat (up to 35% higher), there is a considerable risk of economic fraud by mixing common and durum wheat. Food production companies also often need to discriminate species/varieties at the particle level in the flour. For example, oats (*Avena sativa* L.) are widely used in breakfast cereals because of its high nutritional value. It is economically profitable to add wheat flour to oat flour, which can lead to health problems because wheat contains gluten, whereas oats are gluten-free (Wang et al. 2014).

A previous review (Vermeulen et al. 2010) showed that the use of NIRS in wheat analysis in the 1980s focused on discriminating among wheat varieties based on flour quality. In the decade following the year 2000, more sophisticated NIR hyperspectral imaging systems (NIR-HIS) were developed, combining spatial and spectral information. The initial studies showed that applying chemometric tools in NIR-HIS offered new prospects to the agro-food industry for classifying kernels according to quality criteria (e.g., bread-baking quality, hardness) (Williams 2009a,b). The major advantages of NIR-HIS are that recognition does not depend on

the expertise of the analyst and that it is possible to automate all procedures and analyze a large number of samples.

An original discrimination study of 176 barley samples representing 24 varieties tested in trials for registration in the Belgium catalogue conducted over 3 years (2004–2006) in eight Belgium locations (Monfort et al. 2006) was performed by the Walloon Agricultural Research Centre (CRA-W) research team (Vermeulen et al. 2007). The study sought to develop a fast and reliable method for varietal discrimination, essential for the efficient traceability and quality control system required by the seed sector as well as by the food and feed sectors. In this study, the use of NIR imaging technology was investigated in order to classify barley varieties at the single kernel level. The results were compared with those obtained using classical NIR methods based on bulk analysis or classical field data (e.g., earliness, yield) and technological data (e.g., kernel size, thousand kernel weight [TKW], specific weight, protein, humidity) collected by breeders.

The 24 varieties were grouped into three classes: 6-row winter barley class (6RW); 2-row winter barley class (2RW); and 2-row spring barley class (2RS). The samples were selected to represent variations in climate, geographical location and agronomy in Belgium. Two sets of samples were selected for the study:

- Sample set 1 was used for discriminating the three barley classes (6RW, 2RW, 2RS). A set of 96 samples, including 24 varieties tested at four Belgian locations (Enghien, Gembloux, Havelange and Poperinge) and obtained from the 2005 harvest, was created. This dataset contained 11 varieties of 6RW, seven varieties of 2RW and six varieties of 2RS.
- Sample set 2 included only 6RW samples and was used for discriminating the 6RW varieties. A set of 112 samples, including eight varieties tested at seven Belgian locations (Enghien, Gembloux, Havelange, Poperinge, Leffinge, Dommartin and Bassevelde) and obtained from the 2004 and 2005 harvests, was created. The varieties studied were Carola, Nikel, Seychelles, Sumatra, Palmyra, Jolival, Mandy and Pelican.

In this study, two types of NIR instruments were used: the classical NIR spectrometer and the NIR imaging spectrometer.

The classical NIR spectrometer was an NIR TECATOR Infratec 1241 (FOSS, Hillerod, Denmark) working in the 850–1,050 nm range and used mainly at the cereal collection sites. With this instrument, one spectrum by sample (bulk of kernels) was obtained. Initially, the spectra were used to predict some technological parameters (protein content and humidity) by using the calibration equations developed from barley databases built at CRA-W over more than 30 years. The mean spectra per sample were then used to build the discrimination equations among barley classes or varieties.

The NIR imaging spectrometer used in this experiment was a MatrixNIR™ Chemical Imaging System (Malvern, Olney, USA) described by Fernandez et al. (2005). Reflectance images on 10 kernels by barley sample were collected in the 900–1,700 nm window. The spectrum of each kernel was the average of the spectra acquired from the total surface of the kernel.

For each sample set (sets 1 and 2), the same methodology was applied to the agronomic and technological data, the classical NIR spectra and the NIR-HIS spectra. PLS-DA models were built in order to assess whether or not the differences observed among varietal classes or among varieties in term of agricultural and technological (agro-technological) data could also be identified with the spectral data. Models were built using samples with known classes and were validated by cross-validation using the leave-one-out method, where each sample was successively left out of the model formulation and independently predicted once. The models were also validated with a test set selected by splitting each sample set into two groups, one for training (model construction) and the other for testing (representing about 15% of the samples). The splitting was done by selecting, at random, four samples by group (6RW, 2RW, 2RS) or two samples by variety, representing the different locations and years. In order to compare the performance of the PLS-DA models with the three sets of data (agro-technological data, classical NIRS spectra and NIR-HIS spectra), the same varieties were selected for the test sets.

In the first step, PLS-DA was used to build models for classifying the three barley classes 6RW, 2RW, 2RS (Sample set 1). Table 1 (left side) shows the sensitivity and specificity of each of the three groups at the calibration, cross-validation and test stages. The classification errors in cross-validation varied between 5 and 9% based on NIR imaging data, as opposed to 0–14% based on classical NIR or 12–17% based on agro-technological data. The performance of the models with the test set was better with NIR-HIS in terms of classification errors (3–14%), as opposed to classical NIR (6–25%) and agro-technological (6–25%) data.

In the second step, PLS-DA was used to build models for classifying the six RW varieties (Sample set 2). Table 3 (right side) shows the sensitivity and specificity for each of the eight varieties at the calibration, cross-validation and test stages. The classification errors in cross-validation varied between 23 and 38% based on NIR imaging data, as opposed to 1–34% based on classical NIR and 5–45% based on agro-technological data. The performance of the models with the test set was poorer, with high classification errors: 7–43% using agro-technological data, 0–50% with classical NIR and 23–54% with NIR-HIS.

In brief, PLS-DA classification models showed that NIR imaging had a classification accuracy of 91% for the three classes (2RS, 2RW, 6RW), as opposed to 87% for classical NIR and 83% for agro-technological data. With

Table 3. Performance of PLS-DA Discrimination Models for Sample Sets 1 and 2 on Agro-Technological Data, Classical NIR Spectra and NIR-HIS Spectra.

	SAMPLE SET 1			**SAMPLE SET 2**							
	Agro-technological data			**Agro-technological data**							
	2RS	2RW	6RW	Carola	Nikel	Seychelles	Sumatra	Palmyra	Jolival	Mandy	Pelican
Calibration	**84 samples/21 varieties: 20 2RS, 24 2RW, 40 6RW**			**96 samples, 12 by variety**							
Sensitivity	85.0	91.7	87.2	91.7	100	83.3	100	100	83.3	91.7	91.7
Specificity	90.5	89.8	81.8	86.9	86.9	64.3	83.3	78.6	39.3	98.8	91.7
Classification error	12.3	9.3	15.5	10.7	6.6	26.2	8.3	10.7	38.7	4.8	8.3
Cross-validation	**84 samples/21 varieties: 20 2RS, 24 2RW, 40 6RW**			**96 samples, 12 by variety**							
Sensitivity	85.0	91.7	84.6	91.7	91.7	83.3	91.7	75.0	75.0	91.7	91.7
Specificity	87.3	84.7	81.8	83.3	82.1	64.3	82.1	76.2	35.7	98.8	90.5
Classification error	13.8	11.8	16.8	12.5	13.1	26.2	13.1	24.4	44.6	4.8	8.9
Test	**12 samples/3 varieties: 4 2RS, 4 2RW, 4 6RW**			**16 samples, 2 by variety**							
Sensitivity	100	100	75.0	50.0	50.0	100	50.0	100	100	100	100
Specificity	87.5	62.5	75.0	64.3	85.7	71.4	85.7	71.4	42.9	85.7	85.7
Classification error	6.2	18.7	25.0	42.9	32.1	14.3	32.1	14.3	28.5	7.1	7.1
Classical NIR spectra				**Classical NIR spectra**							
	2RS	2RW	6RW	Carola	Nikel	Seychelles	Sumatra	Palmyra	Jolival	Mandy	Pelican
Calibration	**84 spectra/21 varieties: 20 2RS, 24 2RW, 40 6RW**			**96 spectra, 12 by variety**							
Sensitivity	100	95.8	89.7	91.7	75.0	83.3	83.3	100	75.0	100	100
Specificity	100	96.6	83.7	89.0	65.9	79.3	74.4	95.1	62.2	98.8	94.0
Classification error	0.0	3.8	13.3	9.7	29.6	18.7	21.1	2.4	31.4	0.6	3.0

Cross-validation	**84 spectra/21 varieties: 20 2RS, 24 2RW, 40 6RW**			**96 spectra, 12 by variety**							
Sensitivity	100	91.7	89.7	91.7	66.7	83.3	83.3	91.7	75.0	100	90.9
Specificity	100	91.4	81.4	86.6	64.6	74.4	72.0	90.2	61.0	97.6	94.0
Classification error	0.0	8.5	14.4	10.9	34.3	21.1	22.4	9.0	32.0	1.2	7.5
Test	**12 spectra/3 varieties: 4 2RS, 4 2RW, 4 6RW**			**16 spectra, 2 by variety**							
Sensitivity	50.0	100	100	100	100	50.0	50.0	100	100	100	0.0
Specificity	100	87.5	87.5	100	42.9	64.3	71.4	100	57.1	100	100
Classification error	25.0	6.2	6.2	0.0	28.6	42.9	39.3	0.0	21.4	0.0	50.0
NIR-HIS spectra				**NIR-HIS spectra**							
	2RS	2RW	6RW	Carola	Nikel	Seychelles	Sumatra	Palmyra	Jolival	Mandy	Pelican
Calibration	**840 spectra/21 varieties: 200 2RS, 240 2RW, 400 6RW**			**960 spectra, 120 by variety**							
Sensitivity	96.0	91.2	91.2	72.3	76.3	74.8	84.0	78.8	63.0	67.8	77.8
Specificity	95.1	93.0	92.0	57.2	76.5	61.7	75.2	75.3	75.4	72.0	80.6
Classification error	4.4	7.9	8.4	35.2	23.6	31.7	20.4	23.0	30.8	30.1	20.8
Cross-validation	**840 spectra/21 varieties: 200 2RS, 240 2RW, 400 6RW**			**960 spectra, 120 by variety**							
Sensitivity	96.0	91.2	90.7	67.2	71.2	68.9	79.8	76.3	60.5	66.1	73.5
Specificity	94.3	91.9	91.3	56.3	75.9	61.2	73.6	74.3	74.6	71.4	79.4
Classification error	4.8	8.4	9.0	38.2	26.5	34.9	23.3	24.7	32.4	31.2	23.5
Test	**120 spectra/3 varieties: 40 2RS, 40 2RW, 40 6RW**			**160 spectra, 20 by variety**							
Sensitivity	97.5	89.7	83.8	78.9	70.0	50.0	40.0	60.0	70.0	75.0	15.0
Specificity	97.4	88.3	87.3	49.3	83.5	42.4	56.1	82.0	84.2	72.7	77.7
Classification error	2.6	11.0	14.4	35.9	23.3	53.8	51.9	29.0	22.9	26.2	53.7

regard to classifying varieties in the 6RW class, the results obtained were lower, with a classification accuracy of 78% for agro-technological data, 77% for classical NIR spectra and 63% for NIR-HIS. The classification of some varieties, such as Mandy, however, was better, with rates of 93%, 100% and 74%, respectively. Mandy was clearly different from the other 6RW varieties, being a late variety and having a lower TKW.

A recent study by Zhu et al. (2012) indicated that NIR-HIS could differentiate three types of wheat: strong gluten wheat, medium gluten wheat and weak gluten wheat. The classification accuracy of six wheat cultivars reached 93%. Similarly, Kong et al. (2013) showed the possibility of classifying four rice seed cultivars with a classification accuracy of 100%.

In order to improve classification accuracy, the trend now is to combine several techniques to examine the potential of sensor fusion and data fusion. Zhang et al. (2012) developed classification models to discriminate six maize seed varieties using HIS in the visible and near-infrared (380–1,030 nm) region (VIS-NIR). They showed that by combining textural variables and spectral data, they could achieve a classification accuracy of 98.9%. Yang et al. (2015) achieved a classification accuracy of 98.2% for four varieties of maize seeds by combining morphological, textural and spectral features extracted from VIS-NIR HIS (400–1,000 nm). Teye et al. (2014) showed that the single sensor NIRS and electronic tongue (ET) used to discriminate five cocoa bean varieties had a classification accuracy of 83–93%, whereas data fusion had a classification accuracy of 100%.

3.2 Distillers' dried grains and solubles fraud in relation to botanical origin, geographical origin and production process

The ban on using processed animal protein in the feed led the feed sector to look for other possible protein sources. Among the various possibilities and apart from soybean meal, which is the main source of proteins in feed, distillers' dried grains and solubles (DDGS) could also be an important source. In the USA, 30% of corn is used for ethanol production and most of the DDGS obtained as a residue of the process are exported to Europe. The use of antibiotics or fermentation supplements to improve ethanol production process poses risks to the feed chain. Usually, the product labelling of an affected feed lot shows origin and the paper documentation shows traceability. Incorrect product labeling is common in embargo situations and alternative analytical strategies for ensuring feed authenticity are therefore needed.

Within the framework of the European QSaffe project (2011–2014), a study was conducted on authenticating the origin of DDGS. A total of 191 DDGS samples were collected from reliable sources in Canada, China, Europe and the USA. They were produced from corn (*Zea mays*) and wheat

(*Triticum aestivum* L.) and obtained during the industrial production of bio-ethanol or alcoholic beverages. Various analytical techniques were used in this study: NIRS (Zhou et al. 2014), NIR microscopy (NIRM) (Tena et al. 2015), MIRS (Nietner et al. 2013, Vermeulen et al. 2015a) and Raman spectroscopy (Haughey et al. 2013), as well as MS-based approaches such as proton transfer reaction–mass spectrometry (PTR-MS) (Tres et al. 2014), DART-Orbitrap MS and liquid chromatograph quadrupole time-of-flight MS (LC/Q-TOF/MS) (Novotna et al. 2012). Two proven techniques in food authenticity, isotope ratio mass spectrometry (IRMS) (Nietner et al. 2014) and DNA analysis using polymerase chain reaction (PCR) (Debode et al. 2012), were also among the methods that could identify the DDGS origin. The methods developed were able to determine the botanical origin of the DDGS (corn vs. wheat), and several of them were able to determine the geographical/production origin of the DDGS. These techniques were compared in terms of their complementarities, and an overall strategy for tracing and confirming DDGS origin was described by Vermeulen et al. (2015b).

The results presented below illustrate another approach for studying variability in DDGS samples based on their composition. The 191 DDGS were initially analyzed using a FOSS XDS NIR spectrometer active in the 400–2,500-nm range. Quality parameters such as moisture, protein, fat, fiber and ash were estimated using equations constructed with historical NIRS databases (Fernandez et al. 2010). Samples were described in detail by Vermeulen et al. (2015b). For this study, some samples were removed from the initial dataset because of doubt about their botanical origin based on IRMS analyses. In order to characterize the variability of the retained 181 DDGS samples in terms of production origin, PCA was performed using the five quality parameters, with normalization and autoscale pre-processing applied to the data.

Figure 1 shows the PC1 vs. PC2 scores plot that allowed DDGS samples from corn, wheat, rice and a mixture of wheat and corn to be distinguished. Wheat DDGS from several companies in various European countries were characterized by higher protein content (33.1%) and lower fat content (4.9%) than corn DDGS (28.7% and 8.3%, respectively). Mixtures of wheat and corn DDGS were characterized by medium protein content (30.3%) and low fat content (5.3%). The rice DDGS group was represented by only one sample; it differed considerably from wheat and corn and had a high ash content.

Figure 2 shows the same PC1 vs. PC2 scores plot where the sample marks are coloured according to information on geographical origin and process. Several corn DDGs groups were identified. One group of DDGS samples, residues from a bio-ethanol production company in China (Corn China Origin 1), was characterized by medium protein content (31.1%) and very low fat content (3.2%), which could be explained by fat

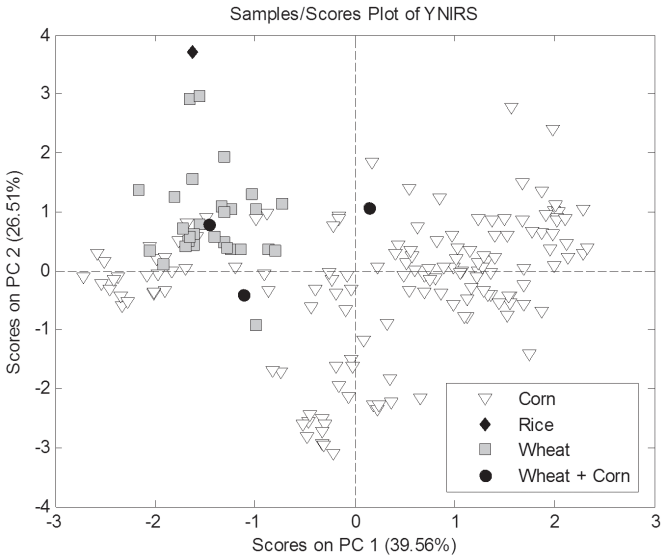

Figure 1. PCA on Predicted Technological Values for Wheat, Corn, Rice and Wheat + Corn Mixtures DDGS Groups: Scores Discriminating the Botanical Groups.

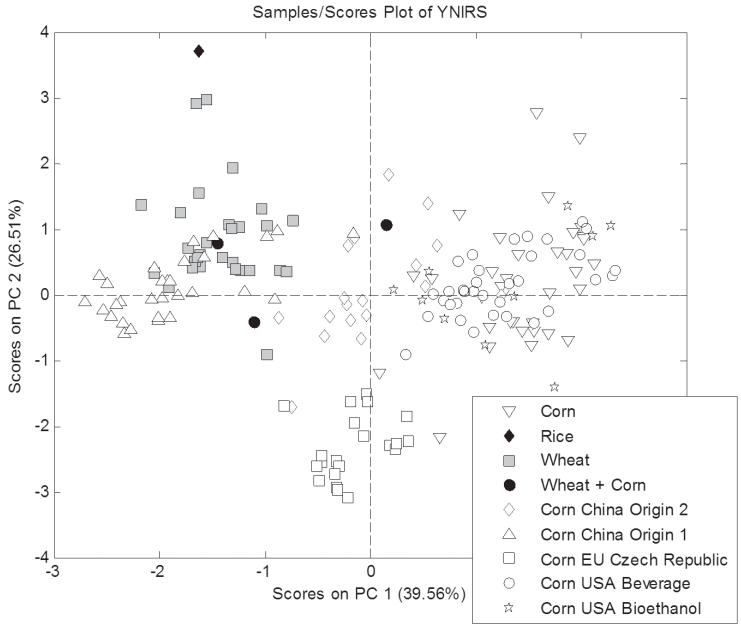

Figure 2. PCA on Predicted Technological Values for Wheat, Corn, Rice and Wheat + Corn Mixtures. DDGS Groups: Scores Discriminating the Industrial Processes.

extraction in the production process. A second group of DDGS samples from another bio-ethanol production company in China (Corn China Origin 2) was characterized by medium fat (8.0%), protein (29.4%), fiber (7.3%) and ash (4.7%) content. A third group of DDGS from a bio-ethanol production company in the Czech Republic (Corn EU Czech Republic) was characterized by medium fat (8.4%) and protein (29.7%) content, but high fiber content (7.9%) and low ash content (3%). DDGS groups from several bio-ethanol or alcoholic beverage production companies in the USA (Corn USA bio-ethanol and Corn USA beverage) were characterized by low protein content (27.3% and 26.7%, respectively) and high fat content (9.8% and 10.1%, respectively).

Figure 3 shows the PC1 and PC2 loadings of PCA. PC1 and PC2 explain 39.6% and 26.5% of the variation, respectively, which are related mainly to protein and fat content (PC1) and ash and fiber content (PC2).

This study showed that PCA gave acceptable results for determining botanical and geographical origin based on compositional profiles. It enabled corn DDGS from three bio-ethanol plants (two in China, origins 1 and 2, and one in the Czech Republic) to be visually distinguished from corn DDGS emanating from bio-ethanol and alcoholic beverage plants in the USA, indicating the potential of each ethanol plant to produce DDGS with consistent compositional characteristics.

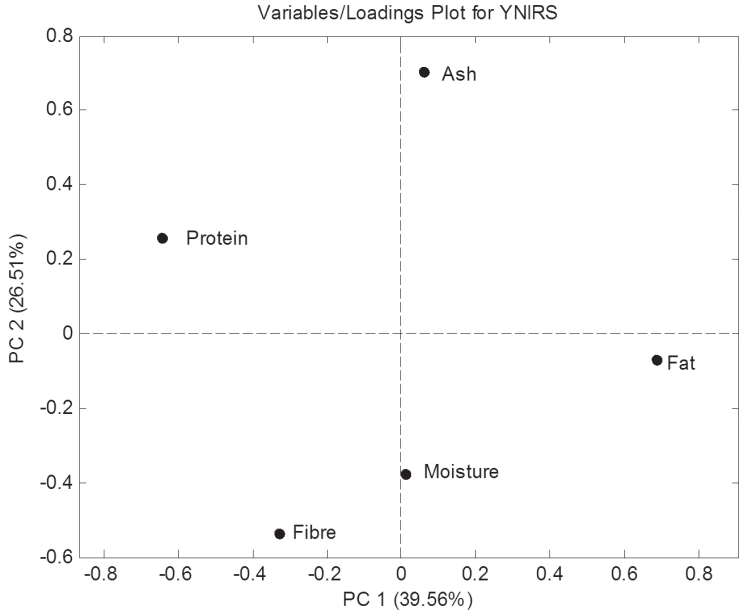

Figure 3. PCA on Predicted Technological Values for Wheat, Corn, Rice and Wheat + Corn Mixtures. DDGS Groups: Loadings Discriminating the Botanical Groups and Industrial Processes.

The study also showed that established analytical approaches in food analysis can be applied to DDGS and could be used for authenticating other materials in the animal feed sector.

3.3 Authentication of fruits and fruit-based products

The authentication and traceability of fruit and fruit-based products pose challenges that need solutions. The authenticity issues relating to these products include assessment of fruit varieties used, assessment of origin of production, assessment of process applied and absence of adulteration with unexpected fruits varieties or exogenous compounds (e.g., sugar, additives). These challenges required fast and reliable methods that can be applied at the level of field production (orchard), transformation unit (artisanal plant or industrial plant) and retailers (local retailer, open market and supermarket). NIRS-based methods could meet these requirements. The authentication and traceability of açai fruits (*Euterpe oleracea*) and cocoa beans (*Theobroma cacao*), both from the Amazonian basin were selected as examples to illustrate the potential of NIRS methods.

3.3.1 Study case 1: açai fruits

More than 200 edible fruits are consumed in the Amazonian basin. Fruits are usually eaten fresh, as juice or puree, and are included in numerous desserts. Fruit production in the region is based on a mixture of extractivism and cultivation. Amongst the Amazonian fruit, açai has a special status. It is produced from a tall, multi-stemmed palm that can reach heights of 3–12 m and is indigenous to the Amazonian basin. The fruit is a dark, spherical berry with a diameter of about 0.7–1.5 cm, and a stone representing 85–90% of the fruit weight (Bichara and Rogez 2011). Fruit production has doubled over the past ten years, being actually over one million tons (IBGE 2016), Most açai production is for export to the USA and Europe, where açai juice is seen as an energy-enhancing drink rich in lipids, fiber and phenolic compounds (Schauss et al. 2006). The palm is native of floodplains ecosystem and has been extensively planted thanks to important irrigation systems in other Amazonian and non-Amazonian lands. In order to ensure traceability and authenticity of açai fruit from this region, it was necessary to develop technological tools for the agricultural industry in post-harvest monitoring and quality improvement. Results from a study conducted by the Federal University of Para (UFPA) in Brazil and CRA-W in Belgium showed that NIRS is an efficient, rapid and non-destructive analytical tool and therefore suitable for the post-harvest monitoring (Amaral 2015a,b). One of the experiments involved assessing the ability of NIRS to determine the geographical origin of açai fruits at a regional level as well as at the level of

the entire Amazon basin. In order to conduct this assessment, 106 samples of açai fruit were collected from three municipalities in the north of Pará state in Brazil, all bordering the Amazon river or its tributaries. Twenty fruits of each sample were randomly chosen for the NIR readings, using a handheld device (Phazir, from Polychromix, USA) to collect spectra in the 1,596–2,396 nm range. PCA and LDA were applied to the mean spectra in order to study the potential of NIR to discriminate açai fruits according to their origin. PC-3 and PC-5 managed to partially discriminate Ponta de Pedras fruit samples from those from the other municipalities (Abaetetuba and Muana) (Fig. 4). With LDA permitted to construct discriminant models that could discriminate sample origins with a success rate of 71–90% for locality of origin. A repeatability study showed that NIR has a coefficient of variation < 5%. The first study indicated that NIRS could be used to determine the geographical origin of açai fruits (Amaral et al. 2015a). Another study sought to discriminate açai fruit samples from two agronomically different areas (i.e., floodplains or irrigated lands) based on NIRS combined with chemometrics. The results showed that the methodology was suitable for quality control in the açai industry and could also be used for traceability and authenticity purposes (Amaral et al. 2015b).

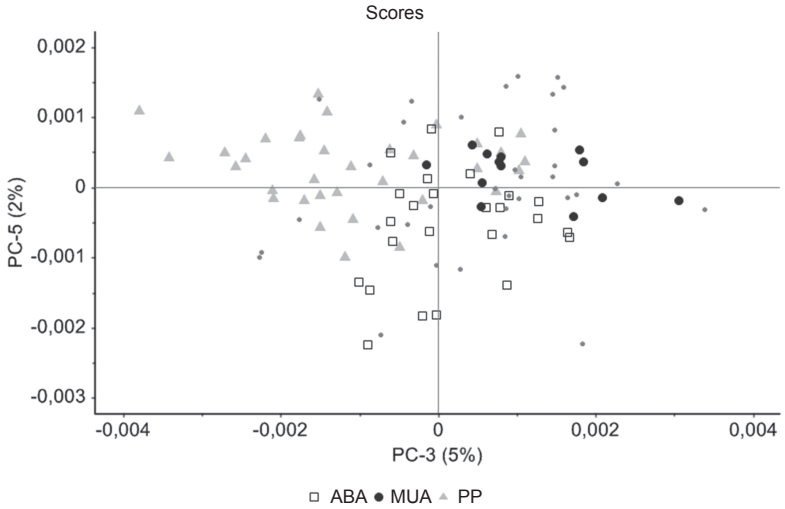

Figure 4. PCA for the Three Municipalities (PP = Ponta de Pedras, ABA = Abaetetuba, MUA = Muana).

3.3.2 Study case 2: cocoa beans

Cocoa (*Theobroma cacao*) is a native fruit from Amazonia widely cultivated in many tropical countries. Cocoa fruits, called pods, are collected by the farmers then the beans and the pulp are extracted by breaking the pods and fermented for about six days. The fermented beans are dried before

being sold and processed. Cocoa is the basis of all chocolate products and the flavor of the beans depends on variety, cultivation method and conditions (including soil and climate) and post-harvest treatment, which consists mainly of fermentation and drying. In the context of international markets, cocoa-based companies need to manage the supply chain from producers to consumers carefully. Two broad categories of cocoa beans are distinguished by the International Cocoa Organization (ICCO) on the world cocoa market: "fine or flavor" cocoa beans, and "bulk" or "ordinary" cocoa beans. Fine or flavor cocoa beans are produced from Nacional, Criollo and Trinitario cocoa-tree varieties, while bulk cocoa beans come from Forastero trees, normally originating from the lower Amazon region. Currently, only 5% of world cocoa is classified as fine or flavor. Fine or flavor cocoa beans are traded in a niche market that is relatively small but highly specialized and distinct from the bulk cocoa beans market. The unique organoleptic (flavor and aroma) characteristics of fine or flavor cocoa generally attract premium prices for this cocoa. Thus, there is a need for faster, cheaper and more accurate real-time traceability and authentication methods. Traceability to cocoa farms enables marketers to impose liability costs on farms, thereby creating incentives for farms to supply safe and high-quality cocoa beans. Cocoa beans that have been collected safely and have been well fermented and properly dried will attract a higher price. Some studies have described the use of vibrational spectroscopy for determining key quality parameters such as caffeine, theobromine and epicatechin content (Davrieux et al. 2006, 2007a,b, 2009, Hue et al. 2014a,b, Alvarez et al. 2012), as well as for discriminating cocoa beans in terms of variety, genotype or fermentation level.

Recently, the potential of using NIRS to discriminate cocoa genotypes from Ecuador has been assessed (Davrieux et al. 2013). The Ecuadorian cocoa production is 190,000 T per year. Two main clones are grown: Nacional and CCN-51. The Nacional accounts for 80% of the production and is recognized as fine cocoa. This variety is probably indigenous to Ecuador while the CCN-51 cacao variety (hybrid) became widely planted in Ecuador since 1997. Nowadays this variety represents 20% of the production and is classified as of poor quality.

In this study conducted by the Instituto Nacional de Investigaciones Agropecuarias (INIAP, Ecuador) and CIRAD (France), 641 samples were collected over 3 years (2009–2012), representing six different cocoa producing zones in Ecuador and the two genotypes (i.e., Nacional and CCN51). Roughly ground unshelled beans (nibs) were prepared just before analysis using a Seb 810004-Prepline grinder (Seb, Ecully, France). Cocoa samples were analyzed for their NIR diffuse reflectance spectrum using a XDS monochromator spectrometer (Foss NlRSystems, Silver Spring, USA) with rectangular cells and moving RSA system (Rapid Solid Analyser). About 100 g of cocoa were analyzed per sample. A principal component

analysis was done on the spectral matrix (n = 641), using centered data and variance/covariance matrix as metric, then the H Mahalanobis distances to the mean average spectra were calculated for each sample on the base of calculated PCs. According to H distance, five samples, presented H > 3, were considerate as spectral outliers and removed.

Then, the remaining sample set was separated in two sub-sets: learning set and validation set. To be representative of the original repartition, 30% of the samples were selected randomly per year for both genotypes. Doing this way, 191 samples (81 CCN-51 and 110 Nacional) were selected as validation samples and the remaining 445 samples (186 CCN-51 and 259 Nacional) were used for calibration. Different classification methods (LDA, Mahalanobis distance discrimination, and SIMCA) were tested using WinISI (Infrasoft, Port Matilda, USA), Xlstat (Addinsoft, Paris, France) and Pirouette (Infometrix Bothel, USA) software.

The best results, expressed as correct classification rates, were observed using SIMCA method. The correct classification rate for the learning set was 94.4% with 10 CCN51 and 15 Nacional misclassified and the correct classification rate was 94,8% for the validation set. The error in validation was about 5%, with only nine samples out of 191 misclassified: six CCN51 (out of 81) and three Nacional (out of 110). One sample (CCN51) was unclassified.

The scatter plot of validation samples distances to each group centroids defined by the model, showed that few samples were close to the separation line (Fig. 5).

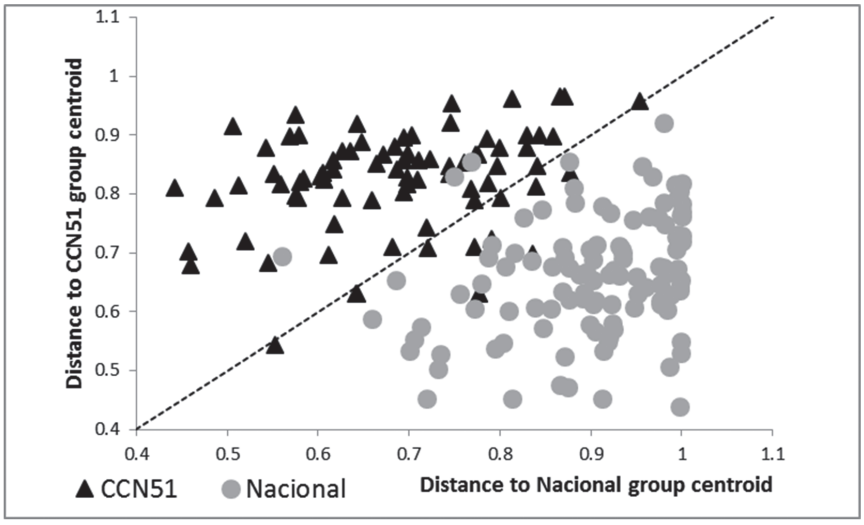

Figure 5. NIRS Discrimination of Cocoa Samples in Terms of Genotype (Nacional Versus CCN51).

The discriminating power calculation highlighted that fat -CH_2 absorptions bands (1724 nm and 2308 nm) were prominent in the discrimination.

The potential of NIR-HIS has been also studied for analyzing whole cacao beans from the Amazon basin (Rogez et al. 2015). The objective of the study conducted by UFPA and CRA-W was to discriminate individual beans in terms of their geographical origin and fermentation time. More than 2,000 cocoa beans from 147 samples from Para state were collected. Samples that had been fermented or dried and came from different areas and producers over two harvest years (2012–2013) were tested. Hyperspectral images were collected using an NIR hyperspectral line scan or push-broom imaging system combined with a conveyor belt (Burgermetrics, Latvia). Each image consisted of 320-pixel lines acquired in the 1,100–2,400 nm wavelength range. Figure 6 gives the PCA results (PC1 versus PC4), showing the discrimination of the beans in terms of process applied (fermented vs. non-fermented; sun-dried vs. dark-dried).

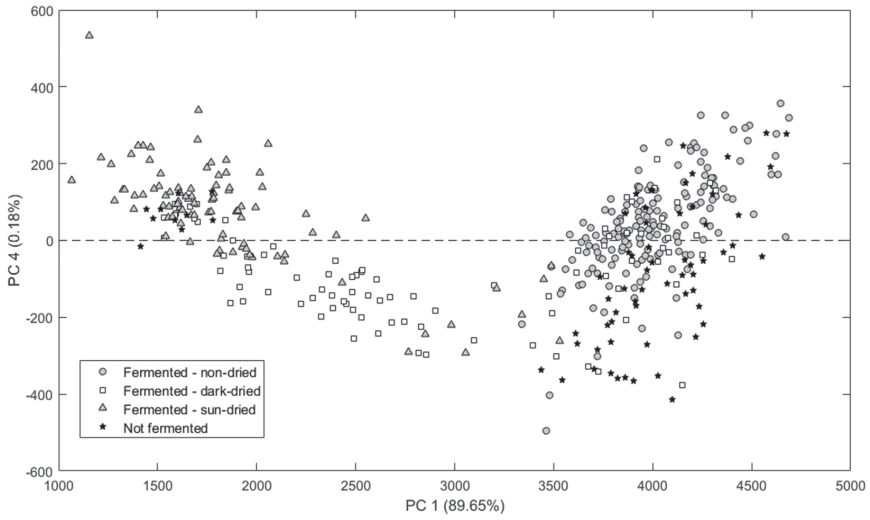

Figure 6. PCA plot (PC1 vs. PC4) of the 147 Cocoa Beans from Tome-Açu Municipality (Para State, BR) Analyzed, Showing the Discrimination of the Beans in Terms of Process Applied (fermented vs. non-fermented; sun-dried vs. dark-dried).

3.4 Early detection of fraud in food/feed ingredients: the case of unapproved protein enhancement with melamine

Among the many crises in the food and feed industries in recent years, one of the most serious in terms of health and economic effects was the use of melamine (WHO/FAO 2008, Tyan et al. 2009). In 2007, the US Food and Drug Administration (FDA) found melamine in pet food and in samples

of wheat gluten imported from China (FDA 2007). In 2008, almost 300 metric tons of soybean meal destined to organic chickens in France were withdrawn from the market after the authorities discovered melamine levels in them that were 50 times higher than the permitted standard (Adams 2008). Also in 2008, milk and infant formula in China was found to be adulterated with melamine, affecting more than 300,000 children, with six infants dying from kidney stones or other kidney damage (Branigan 2008). Melamine was deliberately added at milk-collecting stations to diluted raw milk, ostensibly to boost its protein content. Subsequently, melamine was detected in many milk and milk-containing products, as well as other food and feed products, exported to many countries worldwide. These crises illustrated the need for a sensitive, reliable and rapid procedure for detecting melamine in both food and feed products (Chan et al. 2008, Dobson et al. 2008, Chen 2009, Gossner et al. 2009). With this aim, in recent years public and private researchers have been focusing on the development of suitable screening methods. Currently, most of the available procedures use LC or gas chromatographic (GC) methods combined with MS. Alternative methods available include the use of antibodies, molecularly imprinted polymers, capillary electrophoresis or gold nanoparticles (Ai et al. 2009, Yan et al. 2009, Sun et al. 2010, Rovina et al. 2015). A complete list of methods was presented by Lin et al. (2009) and Liu et al. (2012). Most of these methods, however, are expensive, matrix dependent, destructive and time-consuming, and require extensive sample preparation. A possible alternative could be the use of vibrational spectroscopy techniques, such as NIRS, which has been used for many years as a quality control tool in the food and feed sectors (Norris et al. 1976, Murray 1986, 1993, Barton and Windham 1988, Shenk and Westerhaus 1995). Only recently, with the development of multivariate calibration procedures, NIRS has been used to detect melamine adulteration in food/feed matrices (Dong et al. 2009, Lu et al. 2009, Mauer et al. 2009, Balabin et al. 2011, Smirnov 2011, Haughey et al. 2012, Abbas et al. 2013, Fernández Pierna et al. 2014, 2015, Baeten et al. 2016). In most of these studies, information from the spectra was obtained using classical and innovative chemometric tools in a targeted way (i.e., knowing in advance the fraudulent substance [melamine] to be detected). More studies are now focusing on the development of non-targeted procedures for characterizing certain products and detecting the presence of possible known or unknown contaminants or fraudulent substances, before the food/feed chain is reached (Baeten et al. 2014, de Jong et al. 2016, Fernández-Pierna et al. 2016). The use of statistical tools to interpret multivariate data obtained from spectra should lead to the establishment of rules for checking compliance against product specifications. In industries already equipped with NIR technology for quality control, it would be easy to adapt it in order to simultaneously check possible contamination at both the start and

end of the production chain. Spectra could be combined with chemometric tools to simultaneously check whether or not a product adheres to fixed specifications in terms of composition and quality parameters. Classical chemometric tools could be useful for both tasks, but one tool will usually not be enough for characterizing a product because most of these tools are problem-oriented, meaning that it would be difficult to create thresholds that are useful in tackling future problems. Fernández Pierna et al. (2015) proposed a combination of chemometric techniques with individual characteristics and orientations. This combination includes pattern-recognition techniques that provide adequate differentiation, as well as regression methods evaluated according to their ability to handle the available dataset and predict the status of new samples. The combination therefore facilitates decision-making about product acceptance or rejection. A new technique known as local window PCA (LWPCA), based on a moving window criterion, was proposed and considered as an untargeted method. In this method, a moving window was selected along the wavelength axes in vibrational spectroscopic data and then individual PCA analyses are performed. A calibration set was selected in a localized way from a historical data in order to characterize products that are the most spectroscopically similar to the one to be predicted. Spectral score residuals in this calibration set were extracted and used to build thresholds applied to spectral score residuals of the sample to be predicted. When a residual, at a certain wavenumber, did not meet the defined thresholds, the sample was viewed as abnormal, indicating the possible presence of unusual ingredients and therefore allowing non-targeted analysis. In the case of melamine contamination of milk, this technique was successfully applied (Fernández Pierna et al. 2016). The work was based on the FT-MIR spectra of milk contaminated with melamine. A dataset of 300 samples of UHT liquid milk was used as an historical and clean dataset. Another 12 UHT liquid milk samples were contaminated with melamine at various levels ranging from 0.01% to 1% (100–10,000 ppm). Visual observation of the spectra did not enable clear conclusions to be drawn. GH values allowed abnormalities detection at levels higher than 500 ppm. LWPCA, however, allowed contamination at levels up to 100 ppm to be detected, but at those levels, the detection of melamine in milk became unstable, suggesting that the technique had probably reached its limit of detection. LWPCA technique can also be used for detecting adulterants in soybean meal. Fernández Pierna et al. (2015) devised a complete procedure based on chemometrics and the use of NIRS at the entrance of a feed mill in order to provide early evidence of non-conformity and unusual ingredients. The study focused on the characterization of pure soybean meal with the aim of creating an early control system for detecting and quantifying any unusual ingredients that might be present in the soybean meal, such as melamine, cyanuric acid

or whey powder (milk serum). Results showed that the use of NIR, combined with some simple chemometric tools based on distances and residuals from regression equations, was appropriate for authenticating important feed products (soybean meal) and detecting the presence of abnormal samples or impurities in the laboratory and at the feed mill. LWPCA can also be used to address this problem. Table 4 shows the results of the various criteria used to determine the presence of abnormal samples in the data. The first three datasets, collected directly at the feed mill (Fernández Pierna et al. 2015), contained 75, 66 and 57 samples of pure soybean meal, respectively, as well as 59 and 43 mixtures of soybean meal and whey for datasets 1 and 2, respectively, and 48 mixtures of soybean meal and DDGS for dataset 3. A fourth dataset contained five samples of pure soybean meal and 60 mixtures of soybean meal, melamine and cyanuric acid at varying concentrations. In Table 4, the results are presented in terms of classification accuracy, with black for pure soybean meal and red for the different mixtures. The methods used corresponded to the application of PLS regression models on historical data for protein and fat, the calculation of GH values and the application of LWPCA. Most of the methods applied enabled the soybean meal to be characterized. When detecting a possible contaminant, higher percentages of samples correctly detected were obtained with the LWPCA method. Applying a combination of the four techniques to the NIR data at the start of a production chain could lead to

Table 4. Classification Accuracy Percentage for Datasets of Soybean Meal and Soybean Meal Contaminated with Whey, DDGS, Melamine and/or Cyanuric Acid Using the PLS Model (Protein, fat), GH and LWPCA.

		Protein (%)	**Fat (%)**	**GH (%)**	**LWPCA (%)**
Dataset 1	Soybean meal (75 samples)	93.3	100	94.7	96.0
	Soybean meal + whey (59 samples)	91.5	81.4	94.9	96.6
Dataset 2	Soybean meal (66 samples)	98.5	98.5	92.4	93.9
	Soybean meal + whey (43 samples)	95.3	95.3	95.3	95.3
Dataset 3	Soybean meal (57 samples)	93.0	100	100	94.7
	Soybean meal + DDGS (48 samples)	43.7	14.6	14.6	72.9
Dataset 4	Soybean meal (5 samples)	100	100	100	100
	Soybean meal + melamine/cyan acid (60 samples)	63.3	66.7	95.0	95.0
Mean	Soybean meal detection	96.2	99.6	96.8	96.2
	Contaminant detection	73.5	64.5	75.0	90.0

important cost-savings by detecting non-conformity and authenticating important food/feed products (in this case, soybean meal). A possible limitation would be the low sensitivity of NIR to minor constituents, which is probably not a major drawback when dealing with significant contamination crises.

4. Conclusions

The application of vibrational spectroscopy methods to agricultural and food product examples has shown the important potential of these analytical tools in traceability and authentication. NIRS is already widely accepted in the food and feed sectors for determining, in a unique analysis, a large variety of quality control parameters. Strategies using analytical NIR techniques combined with dedicated statistical data analysis tools could be easily implemented in both routine laboratories and in industries to address authentication issues. The ability to use this technique on-line in production plants and the possibility of building a network of spectrometers make NIRS a very attractive screening tool for the food and feed sectors. As shown in the species/varieties discrimination examples, the use of sensors and data fusion to identify varieties at the kernel level opens up new analytical approaches to be investigated. Such approaches could be used to improve the potential of grain sorters, depending on the quality required. The example of DDGS authentication at the international level illustrated the analytical tools available for the feed sector. With the complexity of industrial processes used in plant feed companies and the tendency to promote both regional and organic feed production, more work is needed on feed authentication order to ensure animal feed safety. The examples of açai fruit and cocoa bean authentication and traceability demonstrated the potential of NIRS methods using miniature hand-held instruments as well as NIR-HIS. Using the example of melamine fraud, the description of the development of new chemometric tools such as LWPCA showed the possibility of using simple tools for NIR spectral data treatment in order to authenticate food and feed products and detect abnormal samples at an early stage. New initiatives at the European level, such as the FoodIntegrity and Authent-Net projects (2016–2018), enable analytical experts and funding bodies to provide Europe with an up-to-date and integrated ability to detect fraud and ensure the integrity of the food chain, as well as to coordinate inter-disciplinary research aimed at protecting consumers against fraud.

Keywords: Near infrared, mid infrared, raman spectroscopy, vibrational spectroscopy, authenticity, traceability, food, feed

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