Hyperspectral imaging techniques: an attractive solution for the analysis of biological and agricultural materials

Baeten, Vincent[1]; Fernández Pierna, Juan Antonio[2] & Dardenne, Pierre[1]

[1] Walloon Agricultural Research Centre, Department of Agricultural Products Quality, Chaussée de Namur, 24. 5030 Gembloux, Belgium

[2] Scientific collaborator F.N.R.S. Statistics and Informatics Department, University of Agronomical Sciences, Avenue de la Faculté 8, 5030 Gembloux, Belgium

Tel: +32 81 62 03 55; Fax: +32 81 62 03 88; E-mail: baeten@cra.wallonie.be

Content

Abstract

Recent developments in spectroscopy have led to the use of spectroscopic imaging instruments for the control and monitoring of agricultural, biological and environmental food and feed products. Spectral imaging techniques offer the possibility of collecting thousands of spectra of one sample or process. This can be done non-destructively and without interfering with the composition of the sample or the process. The studies considered in this chapter concern applications of hyperspectral imaging techniques, with a brief introduction on multispectral imaging studies performed on agro-food products.

1 Introduction

At the time of writing this chapter, the European probe Huygens was sending data from Titan, the largest of Saturn's moons. A huge amount of the data was coming from an imaging system installed on board the spacecraft. This instrument was set up several years ago and has travelled fixed to the Casini-Huygens spacecraft during its 7-year journey from Earth to that remote planet. During this period, hyperspectral imaging systems have been applied to biological and agricultural materials. An important evolution in this field is apparent when looking at the ABES (agricultural, biology and environmental sciences) current contents, which includes most of the scientific papers already published. As an example, for the keywords 'hyperspectral' and 'imaging' nine papers were referenced for the 1998–2001 period, but 40 papers were referenced for the 2002–2004 period. This change reflects a growing awareness of the potential of hyperspectral imaging systems to solve analytical challenges. *'Hyperspectral imaging history is at the early stage of its development and will occupy the analytical scene in the 21st century'* (Baeten and Dardenne, 2002; McClure, 2003).

For the first time in the scientific world, an analytical tool is available that allows researchers to acquire spatially resolved chemical information about biological and agricultural materials. With imaging systems working in the ultraviolet, visible, near-infrared (NIR), infrared and Raman spectral range of the electromagnetic spectrum, researchers are able to obtain information not only about composition, but also about its distribution. They are also able to obtain chemical information at the microscopic level as well as at the macroscopic level. For the research community, this is an important revolution. Spectral imaging techniques offer the potential to collect thousands of spectra of one sample or process. This can be done non-destructively and without interfering with the composition of the sample or with the process.

Several studies have shown the potential of spectral imaging systems for studying biological and agricultural materials. The studied applications include satellite and aircraft remote-sensing and macroscopic and microscopic imaging. For instance, agricultural applications relate to the use of imaging systems in the remote-sensing of crop production problems and to the use of this technology in the laboratory.

In the following sections, the perspectives offered by hyperspectral imaging for studying the composition and the distribution of chemical components and detecting defects and contamination of biological and agricultural materials are illustrated and discussed. In the final section of the chapter, other potential applications are reviewed briefly. Special emphasis is placed on instruments working in the near-infrared (NIR) region, as they appear to be very interesting for studying heterogeneous biological and agricultural samples. NIR radiation penetrates these samples rather well and gives diffuse reflectance information from both the outside and inside of the samples (Geladi *et al.*, 2004). Most of the studies considered here concern hyperspectral imaging techniques, but there are also brief references to multispectral imaging studies performed on agro-food products. The images shown in the following sections were collected at the Walloon Agricultural Research Centre (Gembloux, Belgium) with a Matrix NIR^{TM} instrument (Spectral Dimensions Inc., Olney, USA). This instrument includes an InGas array detector (240x320 pixels) active in the 900-1700 nm region of the electromagnetic spectrum.

2 Sample characterization and chemical species distribution

Hyperspectral imaging spectroscopy enables analysts to characterise the chemical species present in a sample and to study their distribution in the sample. It provides the analyst with a non-destructive and rapid technique that can be used to acquire simultaneously spatial and spectral information of heterogeneous biological and agricultural products. Hyperspectral imaging techniques, mainly NIR imaging, have the flexibility to tackle all samples, whatever their size, i.e. they are able to deal with microscopic particles, a single kernel or the whole sample (fruit) in order to study the distribution of a wide range of chemical compounds. For analysing biological and agricultural products in the field, imaging technology is rapidly replacing the time-consuming mapping techniques (Koehler *et al*., 2002). **Figures 1a and 1b** show score images of the transversal section of a banana.

Figure 1: NIR score images of the transversal section of a banana: (a) image reconstructed using the first principal component, (b) image reconstructed using the third principal component)

2.1 Analysis of fruit

One of the first studies in this field was conducted by Taylor and MacClure (1989). They used NIR imaging technology to undertake a plant physiology study. They worked with a CCD video camera with an effective sensitivity in the range 400-1100 nm and narrow-band interference filters. They showed the potential of this technique for studying the distribution of chlorophyll in plant tissues and water on different surfaces.

They also used this technique to study the damaged parts of tobacco leaves. Okumura *et al*. (1992) used the NIR imaging technology in the analysis of pears in a study of the correlation between water content and relative brightness. More recently, Tran and Grishko (2004) used this technology to determine the water content of olive leaves.

Martinsen and Share (1998) and Martinsen *et al*. (1999) used an NIR hyperspectral imaging spectrometer to measure the spatial distribution of soluble solids across cut surfaces of kiwifruit. The spectral volumes were recorded as reflectance spectra from the illuminated surfaces of fruit cut near the longitudinal centre. This first study on fruit highlighted the potential of using imaging technology to study the spatial distribution of the samples' constituents. The hyperspectral imaging instruments used in this study included a diffraction grating system (i.e., a system involving scanning, step by step, the reflected light of a line of the sample) and a CCD array detector. The spectral volumes measured contained 150x242 pixels over a sample about 50 mm^2 with a spatial resolution of 1.2x2.0 line pairs per mm, corresponding to a horizontal spatial resolution of 0.5 mm and a vertical spatial resolution of 0.7. The spectra were measured between 650 nm and 1100 nm, with a resolution of 5 nm. In order to reduce the noise, the spectral volumes were smoothed. In addition, the spectra were corrected in order to compensate for the non-unity reflectance of the reference used, which was obtained from scans of photographic gray card collected at the beginning of each session. The partial least-squares (PLS) and iterative partial least-squares (ILS) methods applying different pre-treatments were used for data modelling, i.e. to build the calibration models. The best calibration model for determining soluble solids had a standard error of 1.2° Brix over a range of 4.7-14.1° Brix. The calibration was used to study the spatial distribution of the soluble solids in cut pieces of kiwifruit. This study would hardly have been possible using an traditional refractometer. It showed that the major limitation of this technique was the significant specular reflection due to the high water concentration (80-90%) of the samples. Similar work has been done by Sugiyama (1999) using multispectral imaging systems for the analysis and visualization of the sugar content in the flesh of a melon (Tsuta *et al*., 2002) and by Peng and Lu (2004) for predicting the firmness and soluble solids content of apples.

Hyperspectral spectroscopic techniques have been also used to study the ripeness of tomatoes (Padder *et al*., 2000, 2002, 2003). They used a hyperspectral imaging system which includes a prism-grating-prism (PGP) dispersive element, adapted transmission optics and a CCD detector that enabled them to collect spectra in the 430-900 nm spectral range. The spectral volumes were constructed step by step, one axis of the array detector being used as the spatial axis and the other as the spectral axis. In order to obtain the bi-dimensional image, the sample had to be presented step by step to the camera. The spectral resolution was 1.3 nm and the spatial resolution was about 30 μm. Principal component analysis (PCA) and Fisher's linear discriminant analysis (LDA; a classical classification method) were used, after normalization, to visualize the data and calibrate the instrument, respectively. LDA was used to establish classification rules, i.e. to define optimal boundaries between the various ripeness classes by maximising the difference between them. An unknown object was assigned to the class it most resembled. This study showed that hyperspectral images offer more discriminating power than RGB images for measuring the ripening stages in tomatoes. The classification error of individual pixels was reduced from 51% to 19%. This work also demonstrated the potential of the methodology for studying the total concentration of lycopene and chlorophyll, and the spatial distribution of this concentration.

Peirs *et al*. (2003) proposed a new technique based on hyperspectral NIR imaging to determine the maturity stage of pre-climacteric apples. They set up an instrument based on line-by-line scanning technology. The system consisted of a spectrograph, mounted on a camera, that dispersed the captured light of one spatial line into the individual spectral components. To construct the entire spectral volume, the sample was placed on a table allowing it to be moved step by step under the camera. The instrument was active in the 900-1700 nm region, with a spectral resolution of 2.2 nm and a spatial resolution of 1.5 mm. The data were first analysed by applying a Savitsky-Golay smoothing algorithm in order to reduce the noise level of the obtained images. Then a study using PCA was conducted to distinguish the starch concentrations within one apple and among several apples during maturation. The soluble solids content was predicted using PLS models involving all the pixels from the image. All the analyses were performed on cut apples. The results showed that hyperspectral imaging systems could be used to measure fruit quality parameters (starch index) during maturation and to study spatial starch degradation.

Hyperspectral imaging systems are also useful for assessing the firmness of peaches, avoiding the need to use the conventional destructive techniques (Lu and Peng, 2004; Lu, 2004). The authors used a CCD camera that enabled them to collect hyperspectral scattering images from peaches in the 500 and 1040 nm region of the electromagnetic spectrum, with a spectral resolution of 1.65 nm. In order to predict peach firmness, a procedure comprising outlier detection methods, space reduction techniques (PCA) and the neural network approach was applied. This procedure proved to be useful for assessing peach firmness with reasonable standard errors of prediction.

2.2 Analysis of kernels

The advantages of hyperspectral imaging techniques are also apparent in the studies undertaken to increase the understanding of plant physiology at the cellular level. Indeed, the ability of these imaging techniques to combine chemical and spatial information in a single analysis enables researchers to reach a new level of understanding of the analytical information and to conduct in-depth studies of the morphological structure of biological materials. In the case of wheat or corn kernels, for instance, qualitative and quantitative analytical results can be linked to morphological information (Budevska, 2002). One of the first microspectroscopic studies of agricultural products involved the use of the infrared microspectroscopic technique to analyse and characterise biological materials. The mapping strategy involving the successive acquisition of spectra sections of wheat was first used to generate functional group contour maps and surface plots. Generally, the maps and plots created were based on single wavelength intensity. Infrared microspectroscopic mapping instruments were also used to study cellular and sub-cellular chemical heterogeneities (Wetzel *et al*., 1998). The use of microscopic mapping techniques was limited because of the complexity and heterogeneity of biological materials and the difficulty of performing a complete analysis easily. The introduction of focal plane array (FPA) detectors helped improve the microspectroscopic technique by facilitating the collection of images of plant materials. The first study involving an FPA detector concerned the analysis of a wheat section in order to show the structure of different parts of the kernel (Marcott *et al*., 1999). False colour images were constructed using the information at dedicated frequencies. The hyperspectral microspectroscopic imaging techniques were used, for instance, to study the endosperm/aleurone/pericarp area of a mature corn kernel (Budevska, 2002). In this study, the authors used the absorbance at different frequencies to study the distribution of the various constituents, such as proteins, carbohydrates and lipids, of the kernel section. This and other studies have shown that hyperspectral imaging in combination with sophisticated chemometric tools present an important opportunity for rapid chemical imaging of biological and agricultural products. At the cellular level, the ability to fingerprint the chemical composition in a space-resolved manner makes this a valuable tool for characterising genetically altered materials (Budevska, 2002).

Hyperspectral imaging systems have also been used to develop single kernel methods to determine quality parameters. Cogdill *et al*. (2002, 2004) proposed an NIR hyperspectral imaging technique for the quality analysis of a single corn kernel (Stervener *et al.,* 2003). They focused on calibrating the hyperspectral imaging instrument to predict the constituent concentrations of single kernels from NIR hyperspectral images. The instrument worked in transmission in the 750-1090 nm. PLS and PCR algorithms were applied to develop mathematical calibrations for determining moisture and oil content. The moisture and oil calibrations had a standard error of cross-validation (SECV) of 1.20% and 1.738% respectively. They showed that reference chemistry contributed more than 50% of the total variance of the oil calibration. The authors also developed an automated kernel analysis and sorting system to single out maize kernels and place them in the field of view (FOV) of the NIR imaging system.

Hyperspectral imaging techniques have also proved to be a valuable tool for analysing the wheat fraction. Robert *et al.* (1991) constructed one of the first NIR imaging systems and demonstrated its potential for wheat fraction analysis. Discriminant analysis methods were used to construct discriminant models in order to classify ingredients according to their sources. The instrument enabled the authors to collect spectra in the range of 900-1900 nm, with a resolution of 50 nm. In order to test the instrument, the authors studied its ability to discriminate isolated wheat fractions (pellets made by brand, gluten and carbohydrate) (Bertrand *et al*., 1996; Bertrand and Dufour, 2000). Using the absorbances at six wavelengths (900, 950, 1000, 1450, 1500 and 1600 nm), they were able to discriminate the different fractions with a level of error between 0 and 10 %, depending on the fraction considered.

2.3 Analysis of food and feed mixtures

Baeten *et al.* (2001a, 2001b, 2004) proposed the use of NIR microscopic techniques for the complete screening of animal feed. Their research was in the support of the Proposal for a European Parliament and Council Directive amending Directive 79/373/EC on the marketing of compound feed. In this document, the EC highlights the advantages of the labelling requirements for compound feed for livestock in order to facilitate the tracing of compound feed. FPA NIR imaging spectroscopy was proposed as a more efficient method than those currently available, which tend to be time-consuming and to require significant analytical expertise. Fernández *et al*. (2004a, 2005) applied chemometric classification strategies to automate the method and to reduce the need for constant expert analysis of the data. They compared the performance of various discrimination methods for multivariate data. Support Vector Machines (SVM) were shown to be the best technique for classifying feed particles as either meat-and-bone meal (MBM) or vegetal, using the spectra from NIR images.

These imaging techniques could also be very useful for studying the distribution of products in a mixture (Fernández *et al*., 2004b). An example is the development of techniques for rapid, precise and reliable screening of compound feed. One possible way of conducting this study is to construct a classification tree by arranging the various products in a dichotomist way, where each node of the tree constitutes a discriminating chemometric model. In the case of food products, imaging techniques could be useful for discriminating between salt and sugar, as shown in Figure 2. Similarly, various spices could be discriminated using data obtained with the hyperspectral imaging technique.

Figure 2: Images reconstructed from the first principal component for a pure sample of sugar and a mixture 50/50 sugar and salt, respectively.

3 Detecting contamination and defects in agro-food products

In efforts to improve the quality and safety of food, rapid and non-invasive methods, which can be implemented to assess hazardous conditions in agricultural and food production, are essential. Vibrational spectroscopy is one of the techniques chosen by the agro-food sector to evaluate rapidly and at a reasonable cost the quality and safety of food products. Conventional vibrational spectroscopic methods have the disadvantage that the analysis focuses on only a relatively small part of the material analysed, while the imaging spectroscopic approach allows researchers to characterise the whole spatial variability. This approach is particularly suitable for detecting localized defects in agro-food products, as well as detecting contamination of feed.

3.1 Detecting contamination in meat products

Multispectral imaging system has been proposed for the quality control of many food products. It has been used for the inspection of poultry carcasses during processing in order to identify cadaver diseases and tumours and to detect surface contaminants. The contamination of meat products is an important concern because bacterial pathogens are a major cause of illness and death. The contamination of poultry carcasses with bacterial food-borne pathogens can occur through exposure to ingesta or faecal material during the processing of the food product. Recently, hyperspectral imaging instruments have been used to identify faecal and ingesta contamination of poultry carcasses (Park *et al*., 2002a, 2002b). In this study, the authors used a visible NIR hyperspectral imaging system working in the range of 400-900 nm. The spectral resolution of the hyperspectral images was approximately 0.9 nm. The instrument was a line-scan imaging instrument and the time required to scan a whole carcass was about 34 s. They used hyperspectral images at key wavelengths, as well as wavelength ratio images to highlight carcass contamination. Background noise was eliminated by using a masking process and contamination was separated from the carcass using a stretching procedure. In another study by Lawrence *et al*. (2003), the authors demonstrated the usefulness of PCA for distinguishing the contaminants inside poultry carcasses. From their analysis of 80 carcasses they showed, with this limited sample, the potential of imaging systems for detecting faecal and ingesta contamination. More than 96% of the contaminants were detected. The authors felt that more work was needed on vibrational spectroscopy using hyperspectral data. The drawback of the hyperspectral imaging approach in this application was that it was too slow for real-time on-line processing. But hyperspectral imaging systems can be more widely used to select the most adapted frequencies for a multispectral system used for routine analysis.

The same hyperspectral imaging technique has been also used to detect poultry skin tumours (Chao *et al.,* 2002). Vibrational spectroscopic imaging showed potential for detecting localized poultry diseases/defects, including include skin tumours and inflammatory process. Poultry skin tumours are ulcerous lesions surrounded by a rim of thickened skin and dermis. The authors used a hyperspectral imaging system to select wavelengths for designing a multispectral imaging system. The imaging system worked in the range of 420-850 nm and included a CCD camera equipped with an imaging spectrograph. The instrument enabled them to capture the spectral information from one of the spatial dimensions. The second spatial dimension was achieved through successive scans of samples moved along on a conveyor belt. For this study, they analysed eight poultry carcasses with tumours. The image processing included masking in order to separate the chicken image from the background. The spatial region of interest, containing skin defects, was then selected and PCA was applied to select useful bands for detecting tumorous regions. This information was used to set up a multispectral instrument that was able to collect the images simultaneously at the three narrow-band wavelength regions. Feature selection was then performed in order to apply the fuzzy logic classifiers technique. The features included mean, standard deviation, skewness, coefficient of variation and kurtosis. As the results showed, they were able to separate normal from tumorous skin areas with increasing accuracy as more features were included. In the best result, 91 and 86% of normal and tumorous tissues, respectively, were detected.

3.2 Detecting contamination and defects in fruit

Kim *et al.* (2001) proposed a hyperspectral reflectance and fluorescence imaging system for assessing the quality and safety of food commodities. The instrument used a line-by-line scanning technique for a wide range of sample sizes and included a CCD detector and various lighting peripherals. It was active in the 430-930 nm range and had a spectral resolution of 10 nm and a spatial resolution of about 1 mm. This imaging resolution was considered adequate to detect the features or small anomalies that may be encountered in many food commodities. Calibrations and image correction procedures were proposed and discussed. The authors demonstrated the versatility of hyperspectral imaging systems using sample fluorescence and reflectance images of apples to assess their safety and quality (Kim *et al.,* 2002; Chen and Kim, 2004). Apples are an important agricultural commodity and special attention needs to be paid to bacteria contamination. They can be contaminated with bacteria from animal faecal material or ingesta of an animal's gastrointestinal tracts. Material with diseased or fungal-contaminated surfaces or with open skin cuts and bruises may become sites of decay and bacterial growth. There is therefore a need to develop technologies and methodologies to detect fruit defects and contamination in the post-harvesting pre-processing stage.

For this reason, Kim *et al*. (2002) proposed using a set of reflectance and fluorescence image data. One of their studies involved discriminating between a healthy apple and an apple with fungal contamination and bruising. In a second study, they tried to evaluate spatial and spectral responses of hyperspectral reflectance images of faecal-contaminated apples. In the first study, a complete examination of the spectra was done, providing a detailed elucidation of spectral features of apples and their variations caused by contamination. From this study, they concluded that multispectral imaging could become an integral part of food production industries for automated on-line application. In the second study, apple surfaces exhibited flat reflectance responses, while the thick faeces treatment showed a monotonic increase in intensities in the NIR region from approximately 730 to 850 nm. In the spectra of thin faecal treatment on shaded and sun-exposed sides of certain apple varieties, a slight slope change was observed in the same region. The spectral changes were less apparent when the faecal contamination was thinly applied; in these cases, the apple skin was visible through the thin smear. The authors paid special attention to the inherent surface morphology and skin coloration that can significantly affect the performance of the methodology if they are not properly addressed (Chen and Kim, 2004).

Spectral imaging techniques have been also proposed by other authors for detecting contamination and defects in apples. Mehl *et al.* (2002) proposed the use of the hyperspectral analysis to detect defects on selected apple cultivars. They used hyperspectral images to develop a multispectral technique. They worked with three apple cultivars selected for their shape and spectral differences, and used normal apples (i.e. without defects) as well as abnormal apples affected by bruising, diseases and contamination. The hyperspectral imaging system allowed them to determine scabs, fungal and soil contamination and bruising using either PCA or the absorption intensities at a specific frequency. It allowed also them to select three spatial bands capable of separating normal from contaminated apples. These spectral bands were used to develop a multispectral imaging system with specific band pass filters. The performance of the multispectral imaging system was tested on a set of 153 normal and contaminated and/or damaged apples. The correct classification of the apples was found to vary from 76% to 95%, depending on the cultivars analysed.

Also with the objective of detecting bruising, Lu (2003) developed an automated system to help the fruit industry provide better fruit for consumers and reduce potential economic losses. This study used an NIR hyperspectral imaging system active in the range of 900-1700 nm, with a spectral resolution of 4.3 nm. The system had a spatial resolution of 3 mm and 2 mm, depending on the axis. A complete three-dimensional hyperspectral cube was obtained by scanning, step by step, the entire surface of the fruit. The author's objective was to develop an imaging system for bruising detection and to identify and segregate new and old bruises from the normal tissues of apples. For this, hyperspectral images of individual apples were acquired over a period of 47 days, at various intervals, to evaluate the changes in bruising over time. The bruise-free apple images were acquired before the study started and used as a reference to identify bruise evolution during storage. A bruise detection algorithm was developed. Using this algorithm, the original samples were first corrected by removing the background, and then normalization was performed to reduce the variations of reflectance caused by an illumination source in the image. A PCA (the author also proposed a minimum noise fraction [MNF]) was then applied to enhance bruising features and reduce data dimensionality. The study showed that the spectral region between 1000 nm and 1340 nm was the most appropriate for detecting apple bruising. It was also observed that bruising features changed over time and that the rate of the change varied with the variety and fruit sample.

More recently, Mehl *et al.* (2004) proposed using an hyperspectral imaging approach to detect apple surface defects and contamination. They used an instrument working in the range of 424-899 nm which scanned the samples line by line.

Monochromatic images and second difference analysis methods were used to sort wholesome and contaminated apples. The authors worked with samples of four apple cultivars collected after harvesting and before processing. Wholesome and contaminated samples were analysed. The results of direct monochromatic observation indicated that there were no single waveband images that allowed normal apples to be differentiated from all apples that are defective or contaminated. Using the second difference method, the contaminated and damaged portions of the apples were more distinguishable from the normal portions. The results showed that the bruised parts and soil contamination could be easily determined. An important output was that there were no differences in the observations of the various apple cultivars using the appropriate data treatment procedure.

3.3 Detecting contamination and defects in cereals

Hyperspectral imaging techniques also facilitate the development of methods for assessing the level of contamination during cereal production, storage and processing. Cereal contamination includes adulteration by other cereal species or seeds from other crops, decayed and damaged grains (e.g. mouldy grains), animal faeces (e.g. from birds and rodents), seed contaminated with mycotoxins and insect infestations. Cereal contamination reduces the quality of the product, which results in financial loss if it is not detected at the early stage. Ridgway and Chambers (1998) demonstrated the potential of the multispectral NIR imaging technique for detecting insects inside wheat kernels (see also Ridgway *et al.,* 2001). Figure 3 shows the score images reconstructed with (a) the third principal component of four lupin seeds and (b) the sixth principal component of four coffee beans. The image was collected using a hyperspectral imaging system active in the 900-1700 nm range. PCA allowed the insect infestation of the seed in the upper right part of the image to be enhanced. The same instrument was also used to acquire an image of wheat kernels in order to detect the presence of insects. Figure 4 is the reconstruction image based on the three first principal components, highlighting the contamination of the sample by an adult weevil.

Figure 3: Images reconstructed from the first and the third principal component for four intact and infested lupin seeds and coffee beans, respectively.

Figure 4: Reconstruction image based on the three first principal components of an image of a wheat kernel, including an adult weevil.

3.4 Detecting the contamination of compound feed

Hyperspectral imaging techniques have been used for developing a new method to detect MBM in compound feed. This is crucial in order to enforce legislation banning the use of animal proteins in compound feed enacted after the European 'mad cow' crisis (Gizzi *et al.,* 2003). Piraux *et al.* (1999) proposed using FT-NIR microscopy, i.e. optic microscopy coupled with a classic Fourier-transformed spectrometry in the NIR spectral region. Baeten *et al.* (2001) applied stepwise linear discriminant analysis (SLDA), PLS and artificial neural networks (ANN) on a large spectral library of more than 10,000 spectra from different animal and vegetal meals obtained with an NIR microscope. These studies demonstrated the relevance of the NIR spectra of particles for detecting the presence of low levels of MBM. However, the microscopic method proposed was time consuming. This led Baeten and Dardenne

(2002) and Fernández *et al.* (2005) to propose using NIR imaging spectroscopy to accelerate the process. The method proposed consisted of using an NIR camera that allowed the simultaneous detection of a larger number of ingredients in a single analysis, and was faster than classical and NIR microscopy because it collected thousands of spatially resolved spectra in a highly parallel fashion.

In their study, reflectance images were collected in the 900-1700 nm range, with a spectral resolution of 10 nm. The imaging spectrometer included an InGaAs FPA with 240 x 320 pixels (76 800 spectra per scan), along with a liquid crystal tuneable filter (LCTF) for wavelength selection. The effective field of view (FOV) covered approximately 5 cm^2, allowing simultaneous analyses of 300-400 particles. The spectral images obtained were background-corrected and converted to absorbance units prior to further analysis. Chemometric techniques were applied in order to discriminate according to the origin of the samples. Michotte *et al*. (2004) applied PLS and ANN as discrimination methods. They demonstrated that the combination of an NIR camera and chemometrics could be a powerful tool for detecting animal particles in feed. They also showed that the detection limit can reach a value of 0.1%, depending on the number of particles analysed. Fernández *et al*. (2005) compared the performance of different chemometric techniques for discrimination applied to data obtained with the NIR camera. In this work, the performance of a new method for multivariate classification, Support Vector Machines (SVM), was compared to classical chemometric methods such as PLS and ANN for classifying feed particles as either MBM or vegetal using the spectra from NIR images. They showed that while all the classification algorithms tested performed well, the ANN and SVM models showed superiority over PLS. In their paper, SVM is preferred to ANN because of the reduced amount of data that contribute to the solution, the much lower rate of false-positive detection and the fact that SVM leads to one global solution. Figure 5 presents the results of the best SVM model for detecting MBM in a sample that has been artificially spiked.

Figure 5: MBM particles detection using an SVM model.

4 Other agronomic and biological applications

The application of hyperspectral imaging techniques to microscopic and macroscopic studies of biological and agricultural materials have been outlined and discussed. But the story of the use of imaging systems started with a demonstration of the potential of these technologies for satellite and aircraft remote-sensing. Satellite-based images generally cover large areas over fixed-time intervals and with a relative low spatial resolution, while aircraft-based images cover smaller areas, are more flexible in acquiring the images and have a high spatial resolution. Various instruments have been developed for observations of Earth. These include the well-known Landsat 1, SPOT (Satellite Pour l'Observation de la Terre) and AVHRR (Advanced Very High Resolution Radiometer) satellites; and the AIS (Airborne Imaging System), AVIRIS (Airborne Visible and Infrared Imaging Spectrometer) and CASI (Compact Airborne Spectrographic Imager) aircraft equipment (Curran, 1989; Yao *et al*., 2001)

The different equipments used for satellite and aircraft remote-sensing are active in the visible and infrared range and can be classified according to their use of multispectral or hyperspectral technology. The multispectral instruments (e.g. Landsat, SPOT) allow the spectral information of a limited number of bands to be collected and are generally dedicated instruments. In contrast, the hyperspectral instruments (e.g. AVIRIS, CASIS) allow the spectral information from narrow bands from a wide spectral range to be collected. These instruments seem to be able to identify most biological materials (Vane and Goetz, 1993; Jacobsen, 2000). It is generally accepted that the reflectance data extracted from hyperspectral instruments are more sensitive and have extended the application of the spectroscopic remote-sensing approach. Remote-sensing has proved to be a very valuable tool for obtaining essential data for a wide range of problems. One of the most well-known applications of remote-sensing is its use in environmental and ecological research, such as its use for the remote observation of ecosystems, the management of natural resources and the detection of pollution. (Santos, 2000; Curran, 2001; Ker and Ostrovsky, 2003).

Other applications concern the study of vegetation indices, the monitoring of crop and its use in precision agriculture. Vegetation indices are calculated using spectroscopic remote-sensing imaging data in order to study crop characteristics. The vegetation indices include leaf area index, wet biomass, plant height, grain yield and red-edges index that are affected by climate, soils, cultural practices, management and technological inputs. Traditionally, multispectral techniques have been applied to these problems, and combinations of reflectance at different wavelength bands have been used to minimise the effect of the differences in soil background and atmospheric conditions. Recent developments have shown that hyperspectral imaging data could improve the calculation of the vegetation indices (Wessman *et al*., 1997; Clevers, 1999; Tenkabail *et al.,* 2000; Clevers and Jongschaap, 2001).

AIS has also demonstrated the use of hyperspectral imaging data for characterising grassland canopy, which is an important component of the agricultural landscape. Buffet and Oger (2003a, 2003b) tried to demonstrate this performance based on spectral remote-sensing and field observations of representative meadows in south-east Belgium. An interesting aspect of their study was that it showed the potential of combining information from sensors in different regions of the electromagnetic spectrum. Two sensors were used to acquire the hyperspectral data used in their study: a CASI sensor working in the 400-950 nm region, with a ground resolution of 2.5 x 2.5 m and a spectral resolution of 6 nm; and a SASI sensor working in the 850-2500 nm region, with a ground resolution of 2 x 2 m and a spectral resolution of 10 nm. They also showed the different relationships existing between physico-chemical parameters and hyperspectral data in order to assess grassland canopy and the possibility of discriminating between different types of meadows.

Precision agriculture is an important area for satellite and aircraft remote-sensing. Spectral information collected using remote-sensing systems provides information about the use of water, pesticides or fertilizers to meet crop needs. Seelan *et al*. (2003) showed that these technologies used with geographical information systems (GIS) and global positioning systems (GPS) may provide tools that will enable farmers to maximise the economic and environmental benefits of precision agriculture The spectral information obtained provides most of the information needed on plant stress and health. This information is combined with the spatial resolution, which is valuable for monitoring crop appearance. An important application is the possibility of the early detection of crop infestations. In this regard, Hamid and Larsolle (2003) proposed employing agricultural remote-sensing systems using imaging spectroscopy for the characterisation and determination of fungal diseases in wheat. Their aim was to discriminate between healthy and diseased areas in a spring wheat crop suffering from fungal infestation. The plant-cover damage level in the affected areas was also studied in real-time. They used hyperspectral crop reflectance data consisting of 164 bands in the 360-900 nm region.

Another application in this area is the work by Yang *et al.* (2001, 2004). They have been using airborne hyperspectral imagery to estimate grain sorghum yield variability and also to map variable growing conditions and yields of cotton and corn.

5 Conclusion

We have shown in this chapter that hyperspectral imaging techniques provide an attractive solution for the microscopic and macroscopic analysis of biological, agricultural and environmental materials. The various applications outlined show the benefits of these techniques for sample characterization and chemical species distribution, for fruit and kernels and for food and feed mixtures. We also reviewed the advantages of NIR imaging methods for detecting contamination and defects in agro-food products such as meat, fruit, cereals and compound feed.

In the final part of the chapter we looked at the most recent findings from studies on hyperspectral imaging techniques, mainly those based on remote-sensing. These have proved to be a very valuable tool for a wide range of applications in environmental and ecological research. The use of hyperspectral imaging in the biological and agricultural sciences has increased in recent years because it provides an attractive analytical solution to meeting the justified consumer demand for quality.

6 Bibliography

Baeten, V. and Dardenne, P. (2001a) The contribution of near infrared spectroscopy to the fight against the mad cow epidemic, *NIRS news*, 12 (6), 12-13.

Baeten V., Michotte Renier A., Sinnaeve G. & Dardenne, P. (2001b) Analyses of feedingstuffs by near-infrared microscopy (NIRM): detection and quantification of meat and bone meal (MBM). *Proceedings of the sixth International symposium on food authenticity and safety*, 28–30 November, Nantes, France.

Baeten, V. and Dardenne, P. (2002). Spectroscopy: Developments in instrumentation and analysis, *Grasas y Aceites,* 53 (1), 45-63.

Baeten, V., Michotte Renier, A., Fernández Pierna, J. A., Fissiaux, I., Fumière, O., Berben, G. and Dardenne P. (2004) Review of the possibilities offered by the near infrared microscope (NIRM) and near infrared camera (NIR Camera) for the detection of MBM. *Proceedings of the International Symposium "Food and feed safety in the context of prion diseases"*. 16-18 June, Namur, Belgium.

Bertrand, D. and Dufour E. (2000) *La spectroscopie infrarouge et ses applications analytiques*. Editions Tec & Doc, Paris.

Bertrand, D., Robert, P., Novales, B. and Devaux, M. (1996). Chemometrics of multichannel imaging in *Near Infrared Spectroscopy: the Future Waves* (Eds; Davies AMC and Williams P.). NIR publications, Chichester, UK, pp. 72-90.

Budevska, B.O. (2002). Vibrational spectroscopy imaging of agricultural products. John Wiley & Sons Ltd.

Buffet, D. and Oger, R. (2003a) Hyperspectral Imagery for Environmental Mapping and Monitoring: "Case Study of Grassland in Belgium", Geographical Information Systems and Remote Sensing: Environmental Applications, *Proceeding of the International Symposium*, Volos, Greece, 7-9 November.

Buffet, D. and Oger, R. (2003b) Characterisation of grassland canopy using CASI-SASI hyperspectral imagery, Presented at the *CASI-SWIR2002 Workshop*, Bruges, 4 September.

Chao, K., Mehl, P.M. and Chen, Y.R. (2002) Use of Hyper- and Multi-Spectral Imaging for Detection of Chiken Skin Tumors, *Applied Engineering in Agriculture*, **18** (1), 113-119.

Chen, Y.R. and Kim, M.S. (2004). Visible/NIR imaging spectroscopy for assessing quality and safety of agrofoods. *Near infrared spectroscopy: Proceedings on the 11th International Conference*.

Clevers, J.G.P.W. (1999) The use of imaging spectrometry for agricultural applications, *ISPRS Journal of Photogrammetry & Remote Sensing,* **54**, 299-304.

Clevers, J.P.G.W. and Jongschaap, R. (2001) Imaging Spectrometry for Agricultural Applications, in *Imaging Spectrometry* (eds F.D. van der Meer and S.M. de Jong), Kluwer Academic Publishers, NL, pp. 157-199.

Codgill, R.P., Hurburgh, C.R., Jr, Jensen, T.C. and Jones, R.W. (2002) Single-Kernel Maize Analysis by Near-Infrared Hyperspectral Imaging in *Proceedings of the 10th International Conference on Near-Infrared Spectroscopy* (Eds A.M.C. Davies and R.K. Cho), Korea, 2001. pp 243-247.

Codgill, R.P., C.R. Hurburgh, Jr. and G.R. Rippke. 2004. Single kernel maize analysis by near-infrared hyperspectral imaging. *Trans. ASAE* **47** (1), 311-320.

Curran, P.J. (1989) Remote sensing of foliar chemistry, *Remote Sensing of Environment*, **29**, 271-278.

Curran, P.J. (2001) Imaging spectrometry for ecological applications, *JAG*, **3 (**4), 305-312.

Fernández Pierna J.A., Baeten V., Michotte Renier A. and Dardenne P. (2004a) NIR camera and Chemometrics: the winner combination for the detection of MBM in *book of abstracts of the International Symposium on Food and feed safety in the context of prion diseases*, 6-18 June 2004, Namur, Belgium. pp 119-120.

Fernández Pierna J.A., Didelez G., Baeten V., Michotte Renier A. and Dardenne P. (2004b) Chemometrics for multispectral and hyperspectral data: application to screening of compound feeds, *Proceedings of the International Conference of Chemometrics in Analytical Chemistry (CAC-2004)*, 20-23 September, Lisbon, Portugal.

Fernández Pierna J.A., Baeten V., Michotte Renier A., Cogdill R.P. and Dardenne P. (2005) Combination of Support Vector Machines (SVM) and Near Infrared (NIR) imaging spectroscopy for the detection of meat and bone meat (MBM) in compound feeds, *Journal of Chemometrics*, accepted for publication.

Geladi, P., Burger, J. and Lestander, T. (2004). Hyperspectral imaging: calibration problems and solutions. *Chemometrics and Intelligent Laboratory Systems* 72, 209-217.

Gizzi G., van Raamsdonck L.W.D., Baeten V., Murray I., Berben G., Brambilla G., von Holst C. (2003). An overview of tests for animal tissues in animal feeds used in the public health response against BSE *in risk analysis of prion diseases in animals*. (Eds C.I. Lasmézas & D.B. Adams) *Sci. Tech. Rev.*, **22** (1), 311-331.

Hamid Muhammed, H. and Larsolle, A. (2003) Feature-vector based analysis of hyperspectral crop reflectance data for discrimination quantification of fungal disease severity in wheat, *Biosystems Engineering*, **86** (2), 125-134.

Jacobsen, A. (2000) Analysing Airborne Optical Remote Sensing Data from a Hyperspectral Scanner and Implications for Environmental Mapping and Monitoring – results from a study of *casi* data and Danish semi-natural, dry grasslands. *PhD thesis*. National Environmental Research Institute, Denmark. 148pp.

Kerr J.T. and Ostrovsky M. (2003) From space to species: ecological applications for remote sensing, *Trends in Ecology and Evolution*, **18** (6), 299-305.

Kim, M.S., Chen, Y.R. and Mehl, P.M. (2001) Hyperspectral Reflectance and Fluorescence Imaging System for Food Quality and Safety, *Transactions of the ASAE,* **44** (3), 721-729.

Kim, M.S., Lefcourt, A.M., Chao, K., Chen, Y.R., Kim, I. and Chan, D.E. (2002) Multispectral Detection of Fecal Contamination on Apples Based on Hyperspectral Imagery: Part I. Application of Visible and Near-Infrared Reflectance Imaging, *Transactions of the ASAE*, **45** (6), 2027-2037.

Koehler IV, F.W., Lee, E., Kidder, L.H. and Lewis, E.N. (2002). Near infrared spectroscopy: the practical chemical imaging solution. *Spectroscopy Europe* 14/3, 12-19.

Lawrence, K.C., Windham, W.R., Park, B. and Buhr, R.J. (2003) A hyperspectral imaging system for identification of faecal and ingesta contamination on poultry carcasses, *Journal Near Infrared Spectroscopy,* **11**, 269-281.

Lu, R. (2003) Detection of Bruises on Apples Using Near-Infrared Hyperspectral Imaging, *Transactions of the ASAE,* **46** (2), 523-530.

Lu, R. (2004) Multispectral imaging for predicting firmness and soluble solids content of apple fruit, *Post harvest Biology and Technology*, **31** (2), 147-157.

Lu, R. and Peng, Y. (2004). Hyperspectral scattering for assessing peach fruit firmness. *Proceedings on the ASAE/CSAE Annual International meeting*, Ottawa, Ontario, Canada, August 1-4.

Marcott, C., Reeder, R.C., Sweat, J.A., Panzer, D.D. and Wetzel, D.L. (1999). FT-IR Spectroscopic Imaging Microscopy of Wheat Kernels Using a Mercury-Cadmium-Telluride Focal-Plane Array Detector, *Vibr. Spectrosc*. 19, 123-129.

Martinsen, P. and Schaare, P. (1998) Measuring soluble solids distribution in kiwifruit using near-infrared imaging spectroscopy, *Postharvest Biology and Technology* **14**, 271-281.

Martinsen, P., Schaare, P. and Andrews, M. (1999) A versatile near infrared imaging spectrometer, *Journal Near Infrared Spectroscopy,* **7**, 17-25.

McClure, W.F. (2003). 204 years of near infrared technology: 1800-2003, *J. Near Infrared Spectrosc*. 11, 487-518.

Mehl, P.M., Chao, K., Kim, M. and Chen, Y.R. (2002). Detection of defects on selected apple cultivars using hyperspectral and multispectral image analysis. *Applied Engineering in Agriculture*, 18 (2), 219-226.

Mehl, P.M., Chen, Y.R., Kim, M.S. and Chan, D.E. (2003) Development of hyperspectral imaging technique for the detection of apple surface defects and contaminations, *Journal of Food Engineering*, **61** (1), 67-81.

Michotte Renier, A., Baeten, V., Sinnaeve, G., Fernández Pierna, A. and Dardenne P. The NIR camera: a new perspective for meat and bone meal detection in feedingstuffs in *Proceedings of the 11th International Conference on Near-Infrared Spectroscopy* (Eds A. Garrido *et al.*), April 2004, Cordoba, Spain.

Okamura, K., Sato, H., Muraki, H., Hiramatsu, M. and Shimomoto, Y. (1992). Imaging by using near infrared absorption. *Proceedings on the 7th Symposium on the Imaging and sensing technology in industrial fields*, Tokyo, July 7-8.

Park, B., Windham, W.R., Lawrence, K.C., Buhr, R.J. and Smith, D.P. (2002a) Imaging spectrometry for detecting faeces and ingesta contamination on poultry carcasses in *Proceedings of the 10th International Conference on Near-Infrared Spectroscopy* (Eds A.M.C. Davies and R.K. Cho), Korea, 2001. pp 457-461.

Park, B., Lawrence, K.C., Windham, W.R., Chen, Y.R. and Chao, K. (2002b). Discriminant analysis of dual-wavelength spectral images for classifying poultry carcasses. *Computers and Electronics in Agriculture* 33, 219-231.

Peirs, A., Scheerlinck, N., De Baerdemaeker, J. and Nicolaï, B.M. (2003) Starch index determination of apple fruit by means of a hyperspectral near infrared reflectance imaging system, *Journal Near Infrared Spectroscopy*, **11**, 379-389.

Peng, Y. and Lu, R. (2004). Predicting apple fruit firmness by multispectral scattering profiles. *Proceedings on the ASAE/CSAE Annual International meeting*, Ottawa, Ontario, Canada, August 1-4.

Piraux, F. and Dardenne P. (1999). Feed authentication by near-infrared microscopy in *Proceedings of the 9th International Conference on Near-Infrared Spectroscopy* (Eds Giangiacomo R. et al.), Verona, Italy, June 1999.

Polder, G., van der Heijden, G.W.A.M. and Young, I.T. (2000) Hyperspectral Image Analysis for Measuring Ripeness of Tomatoes, *Presented at the 2000 ASAE International Meeting,* Paper number 003089, Milwaukee, Wisconsin, July 9-12, 2000.

Polder, G., van der Heijden, G.W.A.M. and Young, I.T. (2002). Spectral image analysis for measuring ripeness of tomatoes. *Transactions of the ASAE*, 45 (4), 1155-1161.

Polder, G., van der Heijden, G.W.A.M. and Young, I.T. (2003). Tomato sorting using independent component analysis on spectral images, *Real-Time Imaging* 9, 253-259.

Ridgway, C. and Chambers, J. (1998) Detection of insects inside wheat kernels by NIR imaging, *Journal Near Infrared Spectroscopy,* **6**, 115-119.

Ridgway, C., Davies, R. and Chambers, J. (2001) Imaging for the High-Speed Detection of Pest Insects and Other Contaminants in Cereal Grain in Transit, *Presented at the 2001 ASAE International Meeting,* Paper Number : 013056, Sacramento, California, USA, July 30-August 1, 2001.

Robert, P., Bertrand, D., Devaux, M.F. and Sire, A. (1991). Identification of chemical constituents by multivariate near infrared spectral imaging. *Anal. Chem.*, 64: 664-667.

Santos, A.M.P. (2000) Fisheries oceanography using satellite and airborne remote sensing methods : a review, *Fisheries Research*, **49 (**1), 1-20.

Seelan, S.K., Laguette, S., Casady, G.M. and Seielstad, G.A. (2003) Remote sensing applications for precision agriculture : A Learning community approach, *Remote Sensing of Environment*, **88** (1-2), 157-169.

Stevermer, S.W., Steward, B.L., Codgill, R.P. and Hurburgh, C.R. (2003) Automated Sorting and Single Kernel Analysis by Near-Infrared Hyperspectral Imaging, *Presented at the 2003 ASAE International Meeting,* Paper Number: 036159, Las Vegas, Nevada, USA, July 27-30.

Sugiyama, J. (1999) Visualization of sugar content in the flesh of a melon by near infrared imaging, *Journal of Agricultural and Food Chemistry*, **47**, 2715-2718.

Taylor, S.K. and McClure, W.F. (1989) NIR Imaging Spectroscopy: Measuring the Distribution of Chemical Components, In *Proceeding of the 2nd International NIRS Conference*(Eds M. Iwamoto and S. Kawano), Tsukuba Japan, 1989. pp. 393-404.

Thenkabail, P.S., Smith, R.B. and De Pauw, E. (2000) Hyperspectral Vegetation Indices for Determining Agricultural Crop Characteristics, *Remote Sensing of environment*, 158-182.

Tran, C.D. and Grishko, V.I. (2004) Determination of water contents in leaves by a near-infrared multispectral imaging technique, *Microchemical Journal*, **76**, 91-94.

Tsuta, M., Sugiyama, J. and Sagara, Y. (2002) Near-infrared imaging spectroscopy based on sugarabsorption band for melons, *Journal of Agricultural and Food Chemistry,* **50** (1), 48-52.

Vane, G. and Goetz, A.F.H. (1993) Terrestrial Imaging Spectrometry: Current status, Future Trends, *Remote Sending of Environment,* **44**, 117-126.

Wessman, C.A., Bateson, C.A. and Benning T.L. (1997) Detecting fire and grazing patterns in a tallgrass prairie using spectral mixture analysis, *Ecological Applications*, **7**, 493-511.

Wetzel, D.L., Eilert, A.J., Pietrzak, L.N., Miller, S.S. and Sweat, J.A. (1998). Ultraspatially-Resolved Synchrotron Infrared Microspectroscopy of Plant Tissue in situ, *Cell. Mol. Biol.*, 44, 145.

Yang, C., Bradford, J.M. and Wiegand, C.L. (2001). Airbone multispectral imagery for mapping variable growing conditions and yields of cotton, grain sorghum, and corn. *Transactions of the ASAE*, 44 (6), 1983-1994.

Yang, C., Everitt, J.H. and Bradford, J.M. (2004). Airbone hyperspectral imagery and yield monitor data for estimating grain sorghum yield variability. *Transactions of the ASAE*, 47 (3), 915-924.

Yao, H., Tian, L. and Noguchi, N. (2001) Hyperspectral Imaging System Optimization and Image Processing, *ASAE meeting paper no: 01-1105* Sacramento, California, USA, July 30-August 1, 2001.