Vincent Baeten, Philippe Vermeulen, Juan Antonio Fernández Pierna and Pierre Dardenne
Walloon Agricultural Research Centre (CRA-W), Belgium

From targeted to untargeted detection of contaminants and foreign bodies in food and feed using NIR spectroscopy

For decades, Near InfraRed Spectroscopy (NIRS) has been widely used in the food and feed industry in order to implement rapid, relatively inexpensive and efficient control tools to assure the quality of products. NIRS is a branch of the molecular vibrational spectroscopy that refers to the measurement of radiation intensity (i.e. absorbance) as a function of frequency ranging in the electromagnetic spectrum (usually expressed as a function of wavelength [nm], but with the introduction of Fourier transform based instrument also as a function of wavenumber [cm^{-1}]) in the 780-2500 nm (12820 – 4000 cm^{-1}).

The radiation intensity resulting from the interaction between the infrared radiation and the matter is acquired using a spectrometer (also called spectrophotometer) designed to generate, at different frequencies, the NIR absorbance spectrum of the analysed product, expressed as Log 1/R or Log 1/T for reflection and transmission analysis modes, respectively. As a result, the NIR spectra of food and feed products correspond to absorption bands characteristic of the vibration of O-H, C-H, N-H, S-H and C-C groups. NIR spectra can be affected by

several factors such as the physical state of the product (e.g. solid, liquid or gaseous), the temperature of the sample, the particle size (e.g. powder products), the heterogeneity of the material and the sampling errors, the presence of damaged products and impurities, as well as the environment analytical conditions (e.g. temperature and humidity of the laboratory versus temperature of the line of production).

Figure 1: NIR instrument equipped with a wheel of 30 sample holders (Bruker MPA)

The great success of NIRS is mainly due to its relatively easy implementation procedure; its capacity to simultaneously determine several parameters and the fact that this technique allows implementing rapid analytical solutions. For the food and feed industries, this means mainly a method that can provide an analytical answer in short and real-time; but also a method that provides several analytical answers from a unique analysis. The rapid determination of valuable constituents and parameters is essential for the analysis of raw materials, during the process and of the end-products. The aim is usually to maximise profit by raw product check and effective process control, as well as to avoid financial loss by not delivering products with unwanted characteristics. The existence of international standards and guidelines (e.g. norms ISO 12099:2010 and EN 15948) has reinforced the position of NIRS methods in the global analytical scene[1].

NIRS trends

NIRS instrumentation falls usually into two types: (i) dispersive instruments that consist of a monochromatic system that allows successive portions of a polychromatic light to be sent to the sample, a sample compartment and a detector sensitive in the NIR region; and (ii) Fourier transform (FT) instruments that are similar to the dispersive instruments except that the monochromator is replaced by an interferometer that uses a beam splitter to decompose the light into two beams that are recombined before being sent to the sample compartment. An important aspect of the NIR instrumentation is the sample compartment and even more important the sample accessories allowing the maximum benefit to be achieved from the rapid feature of spectroscopy. The sample compartment of an NIR spectrometer varies from a few centimeters to infinite, depending on how the measurement is designed. Sample presentation techniques are based on different accessories used to present the sample to the instrument and vary depending on the way the spectral information is collected (i.e. transmission, reflection or transflection mode). Over the last decade, companies providing NIR spectrometers have invested a lot of energy in the design of high throughput sampling accessories (based on a fibre optic or an auto-sampler) allowing the analysis of high numbers of samples by unit of time.

Another high active area is the *in situ* analysis using NIR microscope or NIR hyperspectral imaging instruments. The use of hyphenated techniques that combine molecular vibrational spectroscopy devices with specific tools as microscopy or imaging allows adding the spatial dimension to the analysis, which is an interesting solution to address food and feed problems[2-5]. Using such instruments, up to several thousand of spectra per sample can be simultaneously collected, which are gathered in order to generate a hypercube that includes the wavelengths, the absorbance values and the spatial information. NIR microscopes, also known as point scan or staring instruments, allow acquiring spectra at successive single spatial locations using a mapping mode system. The hypercube of a sample can be obtained more quickly using whiskbroom or pushbroom hyperspectral imaging systems. Whiskbroom hyperspectral imaging system (also known as plane-scan

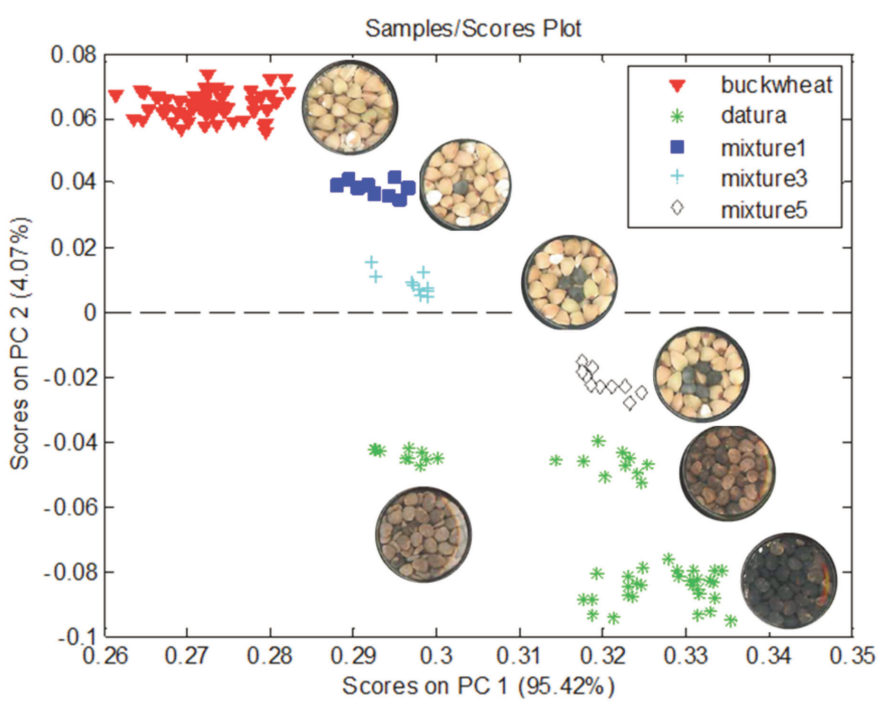

Figure 2: Discrimination of Datura vs. Buckwheat based on PCA analysis

hyperspectral imaging) allows the collection of the absorbance intensities for successive wavelengths. With pushbroom hyperspectral imaging systems (also known as line-scan hyperspectral imaging), the full spectra of the pixels in a line is simultaneously collected. This kind of instrument requires a moving sample stage that can present the successive lines of the scene to be analysed.

The high throughput sampling accessories and the hyperspectral imaging systems are adequate tools to bring the NIRS technology in the field of the detection and quantification of contaminants. Indeed, they allow scanning a sample portion as small as possible in order to lower the limit of the detection of NIRS techniques to the control requirement. This rule is, of course, only valid for contaminants present in the form of particles/bodies. In this paper, three case studies have been selected from projects using NIRS-based techniques to detect and quantify contaminants in agro-food productions:

Case study 1

Detection of plant contaminants in whole grains by NIRS (right sampling procedure allows detecting contaminants at required level)

Recently, we saw a reemergence of the presence of poisonous plants. The intoxication which occurred in France in 2012 was linked to the consumption of bakery products made using buckwheat flour contaminated with *Datura stramonium*, a wild-growing plant found in several crops and well known for its high content in toxic alkaloids. Until now, all the published studies dealing with the detection of Datura during harvest have used visual inspection, optical microscopic or chromatographic methods, which have the drawback of being slow, usually destructive and requiring skilled analysts.

At CRA-W, NIR spectroscopy has been assessed for the detection of the presence of Datura seeds in cereals using an NIR instrument (Bruker MPA) equipped with a wheel for the automatic analysis of 30 vials (**Figure 1**, page 18). With this device a total of 140 spectra were acquired: 60 pure buckwheat spectra, 50 pure Datura spectra and 10 spectra from three different mixtures of buckwheat kernels contaminated with one, three and five Datura seeds respectively placed at the bottom of the vials. **Figure 2** (page 18) shows the score plot of the two first principal components (95.4 per cent and 4.1 per cent of the total variance of the data set respectively) obtained from the PCA analysis of the 140 spectra. According to the score plot, different groups can be observed. Samples of pure buckwheat kernels (red triangle symbol) are located on the top left part of the graph. They are clearly separated from the pure Datura seeds (green stars symbol) that are located to the bottom right part of the graph. The adulterated buckwheat samples with one, three or five Datura seeds (blue square, blue cross and white lozenge symbol respectively) appeared to be situated in the gap between both pure classes and are discriminated according to the second PC with a certain correlation to the degree of contamination. This discrimination is based on the differences in the chemical composition of the seeds analysed. This study demonstrated that the combination of NIR spectroscopy with simple chemometric tools can be used as a fast alternative for the detection of undesirable substances in whole grains.

Case study 2

Detection of plant contaminants in whole grains by NIR hyperspectral imaging (use of one analysis for the simultaneous detection of several contaminants)

In recent years, NIR hyperspectral imaging (**Figure 3**, page 20) has proved

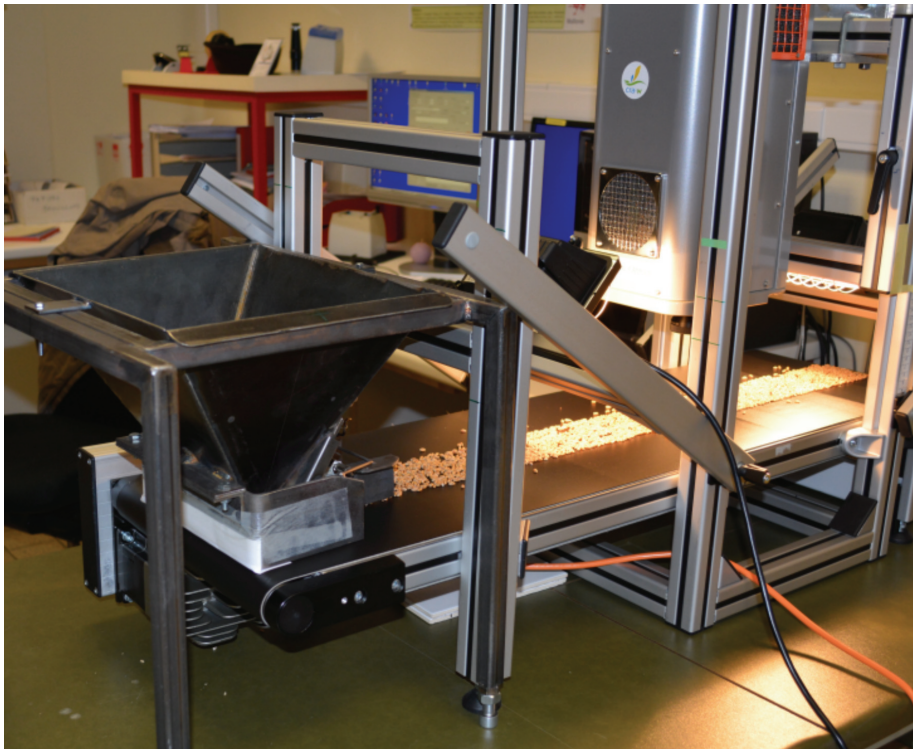

Figure 3: NIR hyperspectral imaging instrument equipped with a grain feed system on a conveyor belt (BurgerMetrics)

its suitability for quality and safety control in the cereal sector by allowing spectroscopic images to be collected at single kernel level[6,7]. Contaminants in cereals include, *inter alia*, impurities such as straw, grains from other crops, insects and other undesirable substances such as ergot (sclerotium of *Claviceps purpurea*). For the cereal sector, the presence of ergot creates an important toxicity risk for animals and humans because of its high level of alkaloid content. The current work, performed in the framework of the EU CONffIDENCE project[12], aims to detect and quantify the presence of ergot bodies in cereals using NIR hyperspectral imaging. In this project, several instrumentation approaches (plane and line scan systems), and chemometrics tools have been tested at laboratory level for the development of a complete procedure for detecting ergot bodies in cereals[8].

A study was sought to transfer and validate the developed procedure using NIR hyperspectral imaging from laboratory to industrial level. All the analyses performed have shown stable and repeatable results with a correlation higher than 0.94 between the predicted values obtained by NIR hyperspectral imaging and those supplied by the stereo microscopic technique, which is the official reference method. The validation of the protocol on blind samples showed that the method could identify and quantify ergot contamination. The transferability of the method has been also demonstrated. This study has been performed on samples with an ergot

concentration of 0.02 per cent, which is lower than the EC limit for cereals destined for humans (0.05 per cent)[9].

The proposed protocol has been extended to the development of procedures for a larger set of contaminants. Figure 4 shows a set of impurities susceptible to be found in a wheat sample (e.g. oilseed rape, Datura seed and ergot body) and its corresponding predicted image obtained after the application of the developed procedure (applying several Partial Least Squares Discriminant Analysis (PLSDA) models). The figure clearly allows discrimination between the different impurities. From laboratory experiments and industrial tests, one can conclude that NIR hyperspectral imaging and chemometrics tools can be used as a control method to develop a protocol to assess the presence and the quantity of impurities and undesirable substances in cereals.

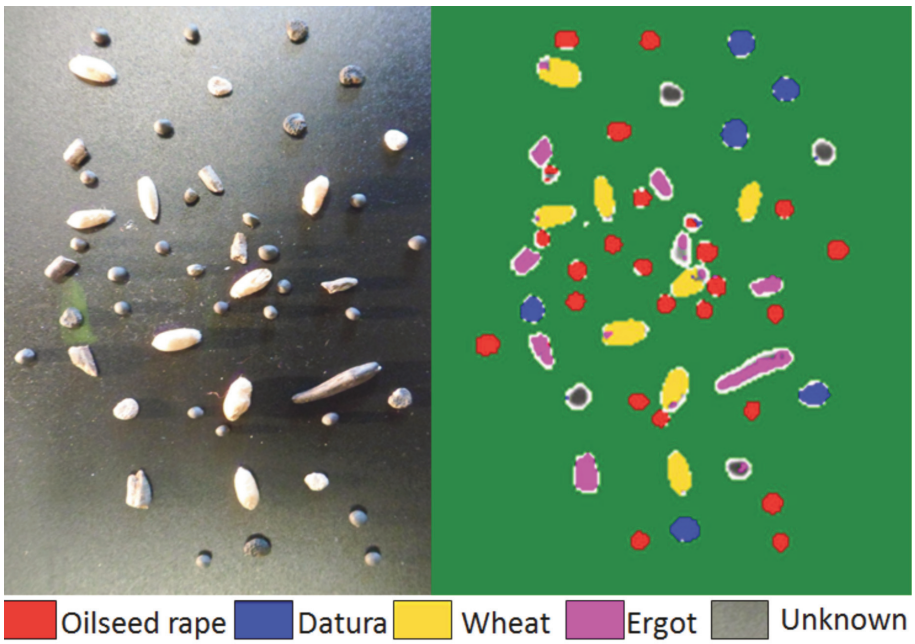

Figure 4: Identification of several known and unknown impurities in wheat based on chemometrics tools

Case-study 3

Untargeted on-line detection of contaminants in feed (look to the unexpected)

In recent years, feed safety has become an increased concern for consumers due to several important crises related directly or indirectly to human health. In 2007, a pet food recall was initiated in North America after a number of cats and dogs became sick and died after eating contaminated pet food with melamine. Again, in 2008, more than 300 tons of soymeal destined for organic chicken in France were withdrawn from the market as authorities discovered melamine levels 50 times higher than the permitted standard. These crises, linked to animal deaths and indirectly human health, put into evidence the

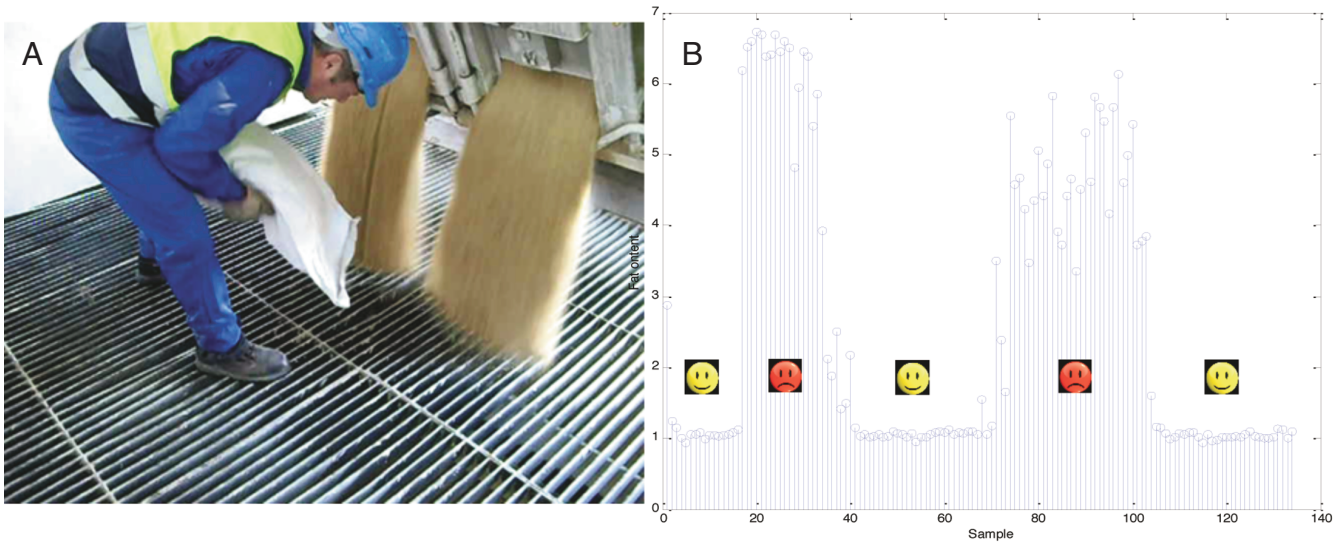

Figure 5: (a) Soybean meal contamination when loading the truck at the entrance of the feed factory; (b) Spectral similarity criterion used to characterise soybean meal and detect the presence of possible contaminants

need of a sensitive, reliable and rapid procedure for the determination of melamine and other contaminants in feed[10,11].

In the framework of the EU project QSAFFE[13] CRA-W proposed a procedure based on NIR spectroscopy and chemometrics in order to characterise soybean meal and to detect the presence of any possible known or unknown contaminant before reaching the feed chain. Using statistical tools to interpret multivariate data obtained from the NIR analysis of soybean meal samples, has led to the creation of some decision rules. They allow checking compliance against specifications in order to decide whether to reject or accept compliance. The procedure was validated at laboratory level and adapted to be applied at the feed mills in order to detect anomalies due to an eventual addition of contaminant or not authorised additives. At the feed mill, a complete experimental plan was designed where trucks containing soybean meal

were contaminated during the unloading with certain types of contaminants (**Figure 5a**, page 22). The application of the decision rules allowed to detect the presence of the contaminants during the unloading of the truck are indicated in **Figure 5b** (page 22) where two clear contaminations can be detected using a simple criterion of NIR spectral similarity (Mahalanobis distance).

Conclusion

The results obtained in these different case studies showed that NIR, combined with some simple chemometrics tools, fits the purpose of authenticating products and detecting targeted and untargeted contaminants at the laboratory and factory levels.

About the Authors

Dr. Ir. Vincent Baeten is head of the Food and Feed Quality Unit of the Valorisation of Agricultural Products Department of the Walloon Agricultural Research Centre (CRA-W, Gembloux – Belgium). The Food and Feed Quality Unit is involved in the development of methods based on spectroscopy (NIR, NIR imaging, MIR, Raman, fluorescence), optical microscopy and chemometrics. The Food and Feed Quality Unit is accredited (ISO 1705) for 3 analytical methods. Dr. Ir. Vincent Baeten has about 20 years of experience on European projects dealing with the development of spectroscopic methods. In the last 10 years he has participated to several projects dealing with quality and safety of food and feed including aspects of traceability and authentication (STRATFEED, TYPIC, MEDEO, CO-EXTRA, TRACE, SAFEED-PAP, CONFFIDENCE, QSAFFE). Recently, he has been awarded of the 2011-Q-Interline Sampling Awards for the outstanding contribution in sampling applied to spectroscopy methods.

Pierre Dardenne is an Agronomy Engineer from Gembloux Agricultural University. In 1980, he was employed by the Walloon Agricultural Research Centre (CRA-W) to lead researches in NIRS, a role he still holds today. In 1991, he got his PhD in Agronomical Sciences at Gembloux Agricultural University in the field of spectroscopy and chemometrics. He has 30 years of expertise in the development of agronomical and agro-industrial applications in NIRS and is involved in several European programmes. Since 2000, Pierre has led the Valorisation of Agricultural Products department at CRA-W. He is leading other groups of scientists working on biomass, feed and food chemical composition, contaminants (heavy metals, antibiotics), milk microbiology and GMO detection. Authenticity, anti-fraud and food safety are keywords in many research programs of his department. The department has 90 employees with 35 scientists.

Ir. Philippe Vermeulen is a Research Engineer at the Valorisation of Agricultural Products Department of CRA-W. He received his Engineering degree in Agricultural Sciences from the Catholic University of Louvain in 1988. Philippe worked for 10 years in the area of breeding on a European Research Program in hybrid wheat for a private company called Hybritech-Monsanto. Since 2001, he has worked at CRA-W inside the Food and Feed Quality Unit, and is involved in the development of NIR methods for the control on-line of agro-food products and feed.

Juan Antonio Fernandez Pierna received his degree in Physical Chemistry at the University of Zaragoza, Spain in 1997. In 2003 he received his PhD in Pharmaceutical Sciences (Chemometrics) at the Analytical Chemistry department of the Vrije Universiteit Brussels under Professor D. L. Massart, with a thesis entitled 'Improvements in the multivariate calibration processes'. Since 2003 he has worked as a research assistant at the CRA-W in Belworks as research assistant at the CRA-W where he has been working for the statistical treatment of the data, the application of chemometrics and the validation of methods. From end 2009, he is also responsible of the Hyperspectral Imaging laboratory installed at the Food and Feed Quality Unit. He is the author or co-author of numerous chapters and around 65 scientific papers mainly related to the statistical treatment of spectroscopic data (including homogeneity detection and uncertainty estimation), food and feed authentication and imaging techniques. He is a member of the Belgian Chemometric Society and is still involved in various EU projects.

References

1. Baeten, V., Fernández Pierna, J.A., Dehareng, F., Sinnaeve, G. & Dardenne, P. (2010a). Regulatory considerations in applying vibrational spectroscopic methods for quality control In Applications of Vibrational Spectroscopy in Food Science, John Wiley & Sons, Ltd, 2, 595-607.
2. Baeten V., von Holst C., Garrido A., VanCutsem J., Michotte Renier A. & Dardenne P. (2005). Detection of banned Meat and Bone Meal in Feedingstuffs by Near-infrared Microscopy Analysis of the Sediment Fraction. Anal. Bioanal. Chem., 382, 149-157.
3. Fernández Pierna, J.A., Baeten, V., Michotte Renier, A., Cogdill, R.P. & Dardenne, P. (2005). Combination of SVM and NIR imaging spectroscopy for the detection of MBM in compound feeds. Journal of Chemometrics, 18(7-8), 341-349.
4. Baeten V., Fernández Pierna, J.A. & Dardenne P. (2007). Hyperspectral Imaging Techniques: An attractive Solution for the Analysis of Biological and Agricultural Materials. In Techniques and applications of hyperspectral image analysis. (Eds Hans F. Grahn and Paul Geladi). Wiley, 2007, Chapter 12, 289-312.
5. Fernández Pierna, J.A., Baeten V., Dubois, J., Burger, J., Lewis, E.N. & Dardenne, P. (2009). NIR Imaging – Theory and applications. In Comprehensive Chemometrics, volume 4, pp. 173-196 Oxford, Elsevier (Steve Brown, Romà Tauler and Beata Walczak eds.).
6. Baeten V., Fernández Pierna, J.A., Vermeulen, Ph., & Dardenne, P. (2010b). NIR hyperspectral imaging methods for quality and safety control of food and feed products: contributions to 4 European projects. Nirsnews, 21(6), 10-13.
7. Fernández Pierna J.A., Vermeulen P., Amand O., Tossens A., Dardenne P. & Baeten V. (2012) NIR hyperspectral imaging spectroscopy and chemometrics for the detection of undesirable substances in food and feed. Chemometrics and Intelligent Laboratory Systems, 117, 233-239.
8. Vermeulen P., Fernández Pierna J.A., Van Egmond H., Dardenne P. & Baeten V. (2012). On-line detection and quantification of ergot bodies in cereals using near infrared hyperspectral imaging. Food Additives & Contaminants, 29 (2), 232-240.
9. Vermeulen P., Fernández Pierna J.A., Van Egmond H., Zegers J., Dardenne P. & Baeten V. (2013). Validation and transferability study of a method based on near infrared hyperspectral imaging method for the detection and quantification of egot bodies in cereals. Analytical and Bioanalytical Chemistry, CONffIDENCE Special issue, 405 (24), 7765-7772.
10. Haughey S.A.., Graham S.F., Cancouet E. & Elliott C.T. (2013), 'The application of Near-Infrared Reflectance Spectroscopy (NIRS) to detect melamine adulteration of soya bean meal'. Food Chemistry, 136 (3–4), 1557–1561.
11. Abbas O., Lecler B., Dardenne P. & Baeten V. (2013), 'Detection of melamine and cyanuric acid in feed ingredients by near infrared spectroscopy and chemometrics', Journal of Near Infrared Spectroscopy, vol. 21, issue 3, p. 183.
12. http://www.conffidence.eu
13. http://www.qsaffe.eu