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NIR hyperspectral imaging methods for quality and safety control of food and feed products: contributions to four European projects

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Introduction

NIR hyperspectral imaging instruments have significant advantages and are becoming the technique of choice for the analyst seeking to solve specific analytical problems. The addition of the spatial dimension to the frequency and absorbance dimensions opens the door to easy collection of thousands of spectra from one sample; instead of having one spectrum per sample with which to determine a series of parameters, one has thousands of spectra of sub-fractions of the samples on which one can individually determine parameters of interest in order to make some conclusions about the individual sub-fractions or on the status of the entire sample.[1]

Analysts working with NIR hyperspectral imaging techniques have to manage sets of data comprising hundreds or thousands of spectra; the size of any given set depends on the instrument type (whiskbroom versus pushbroom instruments) as well as the number of detector elements in the camera. These spectra comprise responses collected from the analysis of different portions of the same sample. Analysts have also to deal with relatively noisy spectra and poor repeatability/reproducibility of the signal.

This article presents a short summary of the contribution of NIR hyperspectral imaging to the determination of quality parameters, the identification of specific traits as well as the detection of contaminants in food and feed products. These activities have been undertaken (and parts of them are still in progress) since 2004 within European projects financed through the 6th and 7th Framework Programmes. They represent a part of many initiatives of the European Union to develop and validate new tools for the fast and efficient control of the food and feed chains. All of the hyperspectral images discussed were collected using a MatrixNIR Chemical Imaging System (Malvern instruments Ltd).

The FONIO project

The FONIO project ["Upgrading quality and competitiveness of fonio for improved livelihoods in West Africa" (STREP project no. 015403, FP6 INCO programme)] is a three-year project (2006–2009) coordinated by Professor Jean-François Cruz from the Cirad institute (Montpellier, France). The main objective was to improve quality and diversity of Fonio products to widen local and export trading opportunities and thereby increase incomes of the producers/processors (more information can be found at http://inco-fonio-en.cirad.fr). Fonio millet (*Digitaria exilis*) is one of the first cereals cultivated by farmers in Western Africa. Its grains have a diameter ranging from 0.9 mm to 1.8 mm and the weight of 1000 kernels is, on average, 0.5 g. It plays an important role in the food supply on the African continent and its popularity comes mainly from its wide ecological adaptability—Fonio can be found in Conakry Guinea, Mali and Burkina Faso and other countries—as well as the fact that is normally harvested (especially for the short-cycle varieties) at the end of the rainy season when granaries are empty.

One of the sub-objectives of the project was to investigate whether NIR hyperspectral imaging could be used to discriminate samples of Fonio according to their site of production, to determine their physical state (peeled versus unpeeled) and their chemical composition (starch, NDF, dry matter, ADF, ADL and ash contents). It was necessary to perform the discrimination and determinations at single grain level. A total of 174 samples (about 100 kernels per sample) coming from three experimental sites representative of the distribution of Fonio millet in West Africa were recorded and chemometric tools such as principal component analysis

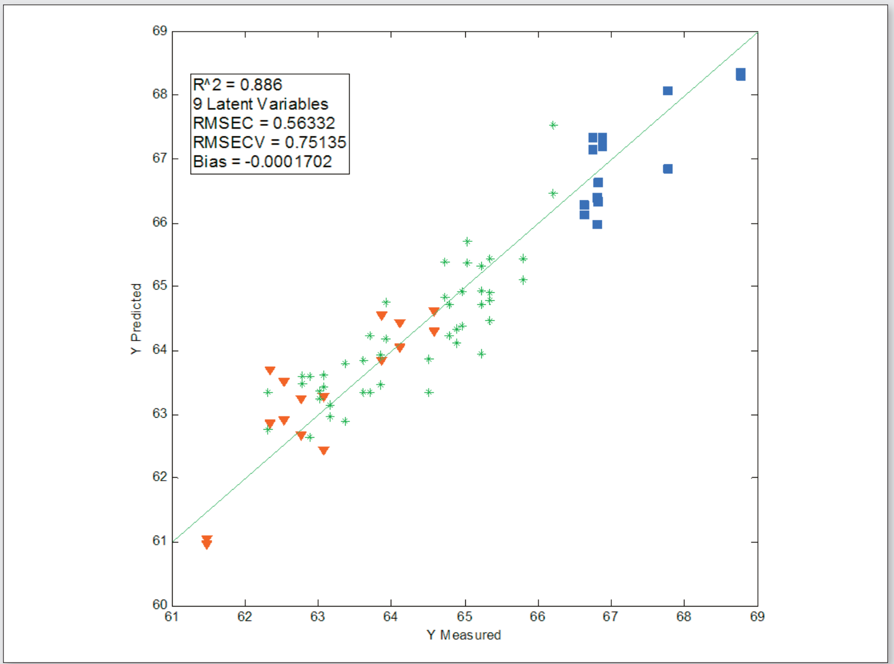

Figure 1. Regression line between NIR hyperspectral predicted values and reference starch content for Fonio samples.

(PCA), partial least squares (PLS) and PLS-discriminant analysis (PLSDA) were used in order to deal with the huge amount of spectral data and to display results. In the case of the discrimination of Fonio on the basis of production origin, results showed that all the sites of production were easily discriminated (at the single grain level) using PLS-DA with an average of 94.6% of correct classification. NIR hyperspectral imaging was also calibrated to determine some chemical parameters (i.e. starch, NDF, dry matter, ADF, ADL and ash) at both the sample and individual seed levels. Models were constructed using PLS regression and promising results were obtained (*RMSECV* values of 0.75, 0.65, 0.28, 0.38, 0.38 and 0.17 were obtained for starch, NDF, dry matter, ADF, ADL and ash contents, respectively). Figure 1 shows the graph of measured versus predicted starch values produced using the leave-one-out cross-validation method on a pixel basis. This work was presented at the 14th ICNIRS conference.

The CO-EXTRA project

CO-EXTRA ["GM and non-GM supply chains: their CO-Existence and TRAceability" (Integrated project no. 007158, Project—FP6 Food Quality and Safety programme)] was a four-year project (2005–2009) coordinated by Professor Yves Bertheau from INRA (Versailles, France). The main objectives were to develop comprehensive tools and methodologies and integrate them along with existing ones into embedded decision-support systems aimed at enabling co-existence between GM and non-GM (conventional and organic) crops as well as tracing genetically-modified organic materials and derived products along the food and feed chains (more information can be found at http://www.coextra.eu).

In CO-EXTRA, NIR hyperspectral imaging was proposed for the development of cost-effective screening (at the single kernel level) methods. This proposal was made on the basis of earlier reports indicating the potential of classical NIR instrument in discriminating the geographical source of soybean samples.[2]

Roundup Ready™ soybean kernels were recorded and statistical procedures including PCA, PLS, support vector machines (SVM) and artificial neural networks (ANNs) were applied to develop calibrations from NIR hyperspectral data from single kernels. Chemometric methods were also used in order to build models to discriminate between transgenic and non-transgenic samples; in this case, the best results were obtained using ANNs (88.9% correct classification). In parallel, another study examined the potential of NIR imaging analysis to discriminate the geographical origin of barley samples collected in Belgium and the United Kingdom; these samples comprised different varieties, some of them from transgenic production. Results showed clear differences between kernels from different varieties; when comparing a transgenic line with its isogenic equivalent, it could be discriminated on the basis of differences in spectral characteristics. However, when taking into account the diversity included from several barley varieties, it was no longer possible to distinguish the GM varieties from the others on the basis of the spectral data. Figure 2 shows an image of ten barley kernels at 1325 nm and a mask performed on such an image; these results were presented at the NIR-2007 conference in Umea, Sweden.[3,4]

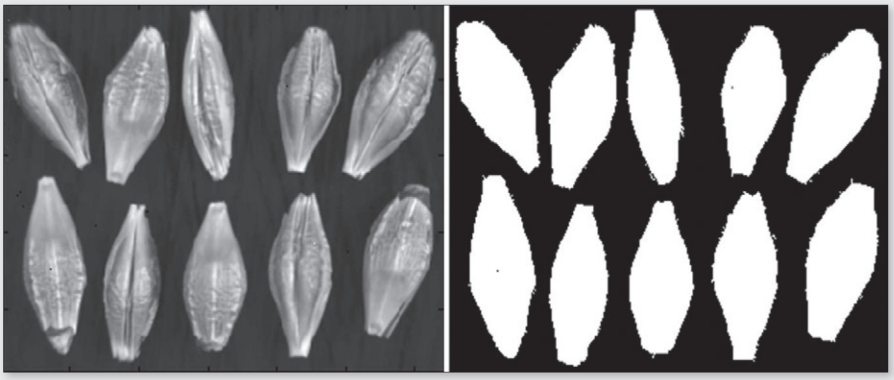

Figure 2. Original and masked images obtained with the NIR hyperspectral camera.

The SAFEED-PAP project

SAFEED-PAP ["Species-specific detection of processed animal proteins in animal feed" (STREP project no. 036221—FP6 Food Quality and Safety programme)] is a three-year project (2006–2009) coordinated by Dr Vincent Baeten from CRA-W (Gembloux, Belgium). The main objective was to solve the problem of species-specific detection of animal proteins and animal feeds in compound feeds by the development and validation of suitable methods as well as the quantification of animal protein in compound feed. Strict EU legislation has been developed and amended year after year since the earliest case of the bovine spongiform encephalopathy (BSE) epidemic. Nowadays, the European Commission has come to the stage that amendments of certain measures could be envisaged, without endangering the health or the policy of eradicating BSE, provided that the positive trend continues and necessary scientific conditions are in place. The achievement of the objectives of this project should help to allow the amendment of the extended total ban (more information can be found at http://safeedpap.feedsafety.org).

In SAFEED-PAP, one of the operational objectives was to propose two different microscopic methods (NIR microscopy and NIR hyperspectral imaging) as viable alternative techniques to the EU reference method (optical microscopy). The University of Córdoba (UCO), the Joint Research Centre—Institute for Reference Materials and Measurements (JRC-IRMM) and CRA-W have contributed to these tasks.

A NIR microscopy protocol[5] was successfully transferred to the NIR hyperspectral instrument. This transfer was performed by appropriate decision rules based on absorbances at specific wavelengths as well as the transfer of discriminant equations.[6] Moreover, the method and the corresponding decision rules were also successfully transferred from the laboratory which constructed the decision rules (CRA-W) to the JRC-IRMM laboratory, which was not involved in the calibration process of the method. Studies aimed at determining the performance characteristics and robustness of the protocol were performed in order to present a framework for developing and validating a NIR hyperspectral imaging method as a standard protocol for detecting processed animal proteins in feedstuffs which would be accepted by regulatory authorities. These studies included the use of criteria and tests to assess the limit of detection, the repeatability, the absence of cross-contamination and the full method

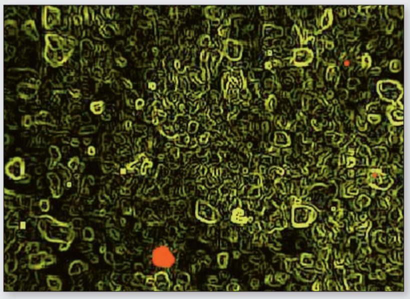

Figure 3. Example of a result obtained by hyperspectral imaging using a SVM discrimination model. In yellow: contour of the different particles included in the image. In red: pixels detected as containing processed animal protein.

validation according to international standards. Figure 3 shows an example of a result obtained by the procedure with the results generated after applying a SVM discrimination model. In red: pixels detected as containing processed animal protein by the SVM model.

The CONFFIDENCE project

CONFFIDENCE ["Contaminants in Food and Feed: Inexpensive detection for control of exposure" (Collaborative project no. 211326—FP7 Food, Agriculture and Fisheries, and Biotechnology)] is a four-year project (2008–2012) coordinated by Dr Jacob de Jong from The Institute of Food Safety (RIKILT) (Wageningen, The Netherlands). The objective of the CONFFIDENCE project is to develop new, simplified, inexpensive detection methods for chemical contaminants from farm to fork in order to assure chemical safety and quality in the European food supply (more information can be found at http://www.conffidence.eu). Contamination of cereals with ergot, formed by the fungus *Claviceps purpurea*, is well known. A survey on the presence of undesirable botanic substances in feed, carried out in 2006 by official control labs from all member states of the European Union, showed a resurgence of ergot in cereal samples.[7] For the farmer, damage caused by ergot is a yield reduction but for the feed/food sector, the presence of ergot in feeding-stuffs and agro-food products represents an unacceptable risk of high toxicity for animals and humans. This toxicity is related to the alkaloids in ergot bodies. In the CONFFIDENCE project, NIR hyperspectral imaging was investigated in order to develop a fast method for the on-line detection and quantification of ergot bodies in cereals.

Regarding the detection issue, NIR hyperspectral imaging was used with success to discriminate ergot bodies from wheat kernels; this discrimination was based on a specific spectral region characteristic of ergot. For quantification, three spectral libraries (ergot, wheat and background were built and used for the construction of two discriminant equations (i.e. equation 1: "background versus wheat + ergot", equation 2: "ergot versus wheat") using PLSDA as classification method. These equations were successively applied to all pixels in images acquired from mixtures of adulterated and unadulterated samples. This approach also allowed calculation of the number of pixels detected as ergot in a given quantity of cereals. Figure 4 shows an example of the results obtained; a similar study is ongoing using a line scan NIR imaging instrument (BurgerMetrics SIA). With this camera, acquisition time (expressed as the number of grains analysed per unit of time) is reduced and the wavelength range is extended to 2400 nm allowing the inclusion of other spectral information to discriminate ergot in food and feed. This work was presented at the 14th ICNIRS conference.

Conclusions

Experience acquired through participation in these different European projects allows us to highlight the main requirements for NIR hyperspectral imaging to be applied in the control of food and feed products:

Need 1—Basic research to study the fraction of samples really taken into account when performing the analysis;

Need 2—Design and validation of a robust system for adequate sample presentation and to focus on specific regions of interest (ROI);

Need 3—Development of robust software to acquire and store rapidly the data collected, to process instantly the data and present the qualitative and quantitative results in an intuitive way for the analyst;

Need 4—Basic research in order to propose fast chemometric algorithms to support the vast amount of data generated by these imaging instruments;

Need 5—Setting up new and adapted calibration strategies that take into account the classical rules for spectrometer calibration but also the specificity of NIR hyperspectral imaging technology (mainly the high S/N ratio and poor reproducibility of the measurement).

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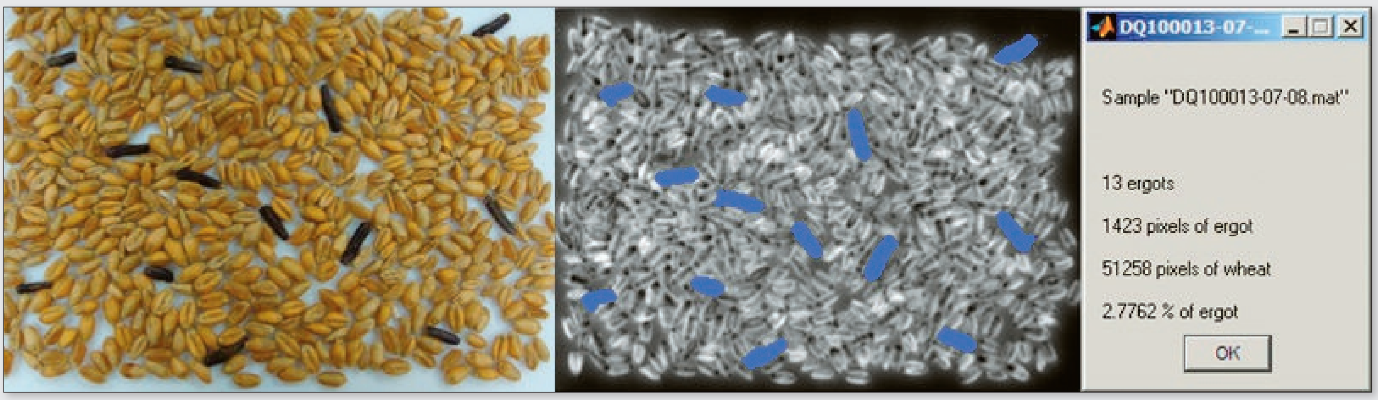

Figure 4. Results of the detection of ergot bodies by NIR hyperspectral imaging in a cereal sample. Detected ergot bodies are in blue. The number of bodies, the number of pixels classified as being ergot as well as their relative percentage in the image are also given.

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