



# Line scan hyperspectral imaging spectroscopy for the early detection of melamine and cyanuric acid in feed

Juan Antonio Fernández Pierna,<sup>a,\*</sup> Damien Vincke,<sup>a</sup> Pierre Dardenne,<sup>a</sup> Zengling Yang,<sup>b</sup> Lujia Han<sup>b</sup> and Vincent Baeten<sup>a</sup>

<sup>a</sup>Walloon Agricultural Research Centre (CRA-W), Valorisation of Agricultural Products Department, Henseval Buiding, 24, Chaussée de Namur, 5030 Gembloux, Belgium. E-mail: [j.fernandez@cra.wallonie.be](mailto:j.fernandez@cra.wallonie.be)

<sup>b</sup>China Agricultural University (East Campus), Qinghua Donglu 17, Haidian District, Beijing 100083, PR China

This study was aimed at exploring the feasibility of detecting and quantifying melamine, and the structural analogue cyanuric acid, contamination in soybean meal, using line-scan near infrared (NIR) hyperspectral imaging spectroscopy (HIS). Soybean meal is one of the main ingredients used in the feed industry because it offers a complete protein profile. Each year, demand increases for soybean products and soya oil, the consumption of which is directly boosted by Chinese consumers' growing wealth, and for soybean meal, which is indirectly affected by the growing demand for meat. Recent cases of deliberate melamine contamination of soybean meal have been reported. This study focuses on the development of a methodology based on NIR–HIS for the acquisition, treatment and interpretation of images and spectra, as well as for the detection and quantification of melamine and cyanuric acid contamination in soybean meal. A total of 40 commercial soybean meal samples were collected, and 17 adulterated samples were prepared by adding different amounts of melamine/cyanuric acid to the samples, with concentrations varying between 0.5% and 5%. The spectral data were collected using line-scan NIR–HIS, and a qualitative model was created based on a principal-component analysis (PCA), whereas partial least-squares discriminant analysis was used to obtain a discrimination model and a semi-quantitative prediction of the content of contaminant. This study has permitted the detection of low levels of melamine and also revealed some limitations for the feasibility of quantifying melamine in soybean meal.

**Keywords:** NIR, line-scan hyperspectral imaging, HIS, feed safety, contaminants, soybean meal, melamine, cyanuric acid

## Introduction

In order to comply with the high quality and safety standards in the food and feed industries, rapid analytical screening methods need to be developed and validated. These methods are preferably based on non-destructive techniques to enable smart control systems to be implemented. Spectroscopic sensors, including those based on near infrared (NIR) spectroscopy technology, have been used for several decades for quality control in many industries. They allow rapid, non-destructive, environmentally friendly and multiparameter

assessments to be made in a variety of products and processes. More recently, new applications of NIRS have emerged due mainly to the development of instruments incorporating technological advances and benefiting from a combination of other techniques. One of the best examples is the combination of NIR with imaging technology to create the NIR hyperspectral imaging spectroscopy (HIS) technique. In the fields of environment, agriculture, life sciences and pharmaceutical research, HIS is no longer an emerging analytical tool, but rather an

approach that has gained in maturity through the publication of hundreds of papers and books, and the organisation of dedicated conferences and scientific networks.<sup>1-3</sup> In addition, the various commercial instruments available in a growing number of fields are taking advantage of the latest developments in analytical techniques and the continued improvement in computer performance and dedicated software. For the research community, HIS constitutes an important revolution that provides the opportunity to collect, spatially, thousands of spectra of one sample or process in a non-destructive way and without interfering directly with the composition or structure of the samples or processes. It allows the repetitive and rapid observation of samples of different types and sizes and the acquisition of information not only about the composition of a product, but also about the spatial distribution of a compound and variability in the field of view (FOV).<sup>4</sup>

NIR-HIS has been used successfully for identifying, mapping and quantifying materials of different origins.<sup>5</sup> The technique has been applied, for example, in the art domain in art-conservation studies and in identifying pigments and other compounds in paintings and palimpsests.<sup>6</sup> It has also been used in the medical sector as a non-invasive method for the detection of melanoma lesions,<sup>7</sup> the early detection of dental caries based on distinct spectral features of healthy and diseased dental tissues,<sup>8</sup> the detection of vascularised tumors in the skin,<sup>9</sup> cancer diagnosis,<sup>10</sup> skin bruises<sup>11</sup> and face recognition,<sup>12</sup> among many other applications. Another important area of application is in the pharmaceutical industry where HIS is currently being used to monitor processes and final products, and to quantify active pharmaceutical ingredients.<sup>13</sup> NIR-HIS is now also being widely used in the agricultural sector.<sup>14,15</sup> It has been applied in the development of rapid methods for accurate feed-component detection,<sup>16,17</sup> species-flora identification,<sup>5,18,19</sup> vegetation-stress detection<sup>20</sup> and quality control and bruise damage detection in fruits and vegetables, including apples,<sup>21</sup> strawberries,<sup>22</sup> tomatoes,<sup>23</sup> mushrooms<sup>24</sup> and cucumbers.<sup>25</sup> In the cereal sector, NIR-HIS has been used, inter alia, for assessing the degree of hardness in maize kernels,<sup>26</sup> studying fungal infestation,<sup>27</sup> tracking the diffusion of conditioning water in single wheat kernels,<sup>28</sup> detecting and quantifying spice adulteration,<sup>29</sup> characterising fonio millet,<sup>4,30</sup> as an alternative technique for genetically modified organisms (GMOs)<sup>31</sup> and for detecting and quantifying contaminants in cereals.<sup>32,33</sup> Several recent works<sup>34-36</sup> have reviewed the application of HIS in agricultural and food science.

To date, three instrumentation approaches have been used to acquire hyperspectral images. They have been named according to the orientation of the scanning dimension relative to the 2D spatial sample axes: the point (staring) scan, the push-broom (line) scan and the whiskbroom (plane) scan approaches. With the *point (staring) scan approach*, the reflected light from a single spatial location of scene is collected by means of a Fourier transform or grating spectrometer. Hyperspectral images are then constructed by measuring the spectra successively while the sample is repositioned to a new

point in the  $x$  and  $y$  spatial dimensions. This type of approach is used in spectroscopic microscopy equipped with a high precision  $x$ - $y$  motion stage.<sup>37</sup> With the *push-broom (line) scan approach*, the reflected light of a line of pixels coming from a scene is projected onto a two-dimensional (2D) focal plane array (FPA). This instrument is best suited for remote sensing by satellite, aircraft or online process measurement because the  $y$  spatial axis can be arbitrarily long.<sup>32</sup> With the *whiskbroom (plane) scan approach*, the reflected lights at one frequency of the pixels in a 2D image are collected in one shot. The hyperspectral camera is fixed parallel to the sample surface, allowing the collection of  $x$ - $y$  spatial images, the size of which is determined by the optical design of the instrument and the resolution (i.e. number of pixels/scene) by the number of elements in the 2D detector. Hyperspectral images of a scene are obtained by modulating the radiation reaching the camera via the use of band pass or tunable filters positioned in front of the detector.<sup>38</sup>

For analysts and chemometricians, acquiring and handling data from HIS is a challenge. It includes collecting hundreds or thousands of spectra (including tens or hundreds of variables) for each sample, rather than the typical approach with classical spectroscopic instrumentation where a unique average spectrum is collected.<sup>4,34</sup> Chemometric tools are needed to extract all the relevant information from the hyperspectral images, while simultaneously retaining the spectral and spatial information. HIS provides an opportunity for more sophisticated image analysis that includes using techniques to extract complete information at the pixel scale. For this, new image-processing techniques need to be developed, and multivariate image analysis (MIA) tools become very useful.<sup>39</sup> MIA includes classical statistics and chemometric tools that are indispensable for analysing large amounts of data and for extracting quantitative/qualitative information coming from the images.

The overall objective of this paper is to produce appropriate answers to a particular food and feed problem: the detection and quantification of melamine and cyanuric acid contamination in soybean meal. Soybean meal is one of the main ingredients used in the feed industry because it offers a complete protein profile. Each year, demand increases for soybean products and soya oil, the consumption of which is being directly boosted by Chinese consumers' growing wealth, and for soybean meal, which is indirectly affected by the growing demand for meat. This increased demand for meat is encouraging the consolidation of livestock producers into industrial enterprises that are keener to buy soybean meal than small-time farmers. The deliberate melamine adulteration of soybean meal has recently been reported on two occasions. Melamine (1,3,5-triazine-2,4,6-triamine) and structural analogues (as cyanuric acid—1,3,5-triazine-2,4,6-triol) are small aromatic molecules used to make industrial glues, fire retardants and fertilisers. In 2007, melamine was added during the manufacture of pet feed, and in 2008, it was found in infant formula manufactured from adulterated milk. Manufacturers of organic feed in France, the Netherlands and the UK discov-

**Table 1.** Composition of the artificial mixtures set prepared from pure samples.

Sample	Melamine (%)	Cyanuric acid (%)	Sample	Melamine (%)	Cyanuric acid (%)
Mix 1	0.5	0	Mix 10	0	0.5
Mix 2	1	0	Mix 11	0	1
Mix 3	1.5	0	Mix 12	0	2
Mix 4	2	0	Mix 13	0	2.5
Mix 5	2.5	0	Mix 14	0	3
Mix 6	3	0	Mix 15	0	3.5
Mix 7	3.5	0	Mix 16	0	4
Mix 8	4	0	Mix 17	0	5
Mix 9	5	0			

ered the presence of melamine in organic soybean expellers imported from China in October and November 2008, probably resulting from the fraudulent admixture of melamine.<sup>40</sup>

Melamine can be present at significant levels in certain food/feed products. The maximum levels accepted are 1 mg kg<sup>-1</sup> in infant formula (powdered) and 2.5 mg kg<sup>-1</sup> in other foods and animal feed<sup>41</sup>. In the case of melamine-tainted pet food, it has been shown that a single dose of melamine and cyanuric acid can result in acute renal failure, which eventually causes death in dogs.<sup>42</sup> Having an inexpensive and rapid method to test imported or domestically made products for the presence of melamine would make it easier to assess these products. Most current methods used for the detection of melamine in food, however, are expensive and complicated techniques that need extensive sample preparation.<sup>43,44</sup> Since melamine can be used as a protein substitute for animal feed, which would affect many food sources (e.g. cow milk, fish, pork, cattle), the ideal method would be one that detects melamine early, before its introduction into the food chain. In recent years, vibrational spectroscopy techniques (mainly near and mid-infrared), in combination with chemometric tools, have been used for melamine detection in several analytical studies, most of them focusing on infant formula powder,<sup>45</sup> milk<sup>46,47</sup> and milk powder.<sup>48,49</sup> Some studies have looked at the detection of melamine in feed,<sup>50,51</sup> but only a few of them have applied non-destructive vibrational methods.<sup>52,53</sup> The work described in this paper was conducted within the framework of an EU project, Quality and Safety of Feeds and Food for Europe (QSAFFE: <http://www.qsaffe.eu/>), which aims to deliver better, faster and economically viable means of ensuring the quality and safety of animal feeds in Europe by creating an integrated approach for preventing contamination and fraud, identifying and assessing new risks and providing scientific evidence of the risks of transfer of microbiological and chemical contaminants from feed to food. The specific aim of this work was to develop a methodology based on line-scan NIR–HIS for the acquisition, treatment and interpretation, with a high degree of accuracy, of images and spectra, as well as for the detection and quantification of melamine and cyanuric acid contamination in soybean meal.

## Material and methods

Different sets of ground samples (250 µm) were used. One set consisted of 40 soya samples, including pure soybean meal, soybean hulls (rich in fibre content), full-fat soya (rich in fat content), dehulled soybeans and organic soya. A second set contained four pure melamine and cyanuric acid samples. There was also a set of 17 artificial mixtures (50 g), each created by adding melamine or cyanuric acid, with percentages varying from 0.5% to 5% on a weight/weight basis, to some pure soybean meal, as shown in Table 1. As these artificial mixtures are used for validation, the sets of pure samples used to create them were different from the first two sets.

Moreover, and in order to try to assess the limit of quantification of melamine in soybean meal, a comparison was run between two samples, a pure soybean meal sample contaminated with melamine at a concentration of 0.1% and an unadulterated sample from the same soybean meal.

Hyperspectral images were collected using the line-scan NIR hyperspectral or push-broom imaging system from BurgerMetrics SIA (Riga, Latvia), as shown in Figure 1, which

**Figure 1.** Line-scan NIRS-HIS system installed at CRA-W.

was combined with a conveyor belt for better and faster image acquisition. The instrument used for this study was a camera from Xenics (Leuven, Belgium) with a spectrograph from Specim Ltd (Oulu, Finland). The acquisition was carried out using HyperProVB software from BurgerMetrics SIA.

Prior to analysis, the spectral imaging system was calibrated with a dark image (by blocking the lens entrance) and a white image (background) collected from a standard white reference board (empty Teflon plate). The dark image was then automatically acquired every 200 frames and the spectra corrected accordingly. This procedure was performed to compensate for offset owing to the dark current, the light-source temperature drift and the lack of spatial lighting uniformity.<sup>54</sup>

In order to automatically detect the presence of contaminants and therefore to assess the purity of the soybean meal, a complete spectral database was built using the available samples by taking images of pure products (soybean meal, melamine and cyanuric acid) with the line-scan NIR-HIS system. The conveyor belt speed was fixed at  $500 \mu\text{m s}^{-1}$ , and the spatial resolution was  $10 \mu\text{m}$ , using a macro-lens at  $10\times$  magnification. The images were stacked to form a three-sided matrix, where the first two axes defined the image plane (FOV), and the third corresponded to the spectrum at each pixel location. All the images consisted of 200 lines of 320 pixels acquired in less than 20 s at 209 wavelength channels (1118–2424 nm at 6.3 nm intervals; i.e. 64,000 spectra by image). In order to avoid huge datasets and calculation problems, a selection of the most representative spectra was made by, first, creating a mask based on light intensity that allowed background removal and, second, applying a sequential procedure that uniformly selected spectra in the experimental region<sup>55</sup> in order to reduce the dataset to 2400 spectra, including 800 spectra of soybean meal, 800 spectra of melamine and 800 spectra of cyanuric acid.

Computations, chemometric analyses and graphics were performed using HyperSee software (BurgerMetrics SIA) and programs developed in Matlab R2007b (The Mathworks Inc., Natick, MA, USA).

## Results and discussion

Figure 2 shows typical NIR raw and first-derivative spectra from pure soybean meal (Provimi, unknown purity), pure melamine (Sigma Aldrich, 99%) and cyanuric acid (Provimi, unknown purity) obtained using the line scan NIR-HIS system. First-derivative spectra had a greater amplitude than the original spectra, which was especially useful for separating out peaks of overlapping bands. For the soybean meal, absorption bands were observed around 1200 nm, 1480 nm (O–H stretch first overtone, associated with water), 1900 nm (O–H stretch second overtone) and 2300 nm [C–H combination bands, associated with water, amino acids and fatty acids].<sup>56</sup> As shown in the figures, interference between the signals from melamine/cyanuric acid and the soybean meal was minimal around 1450–1600 nm, where both contaminants had distinctive bands

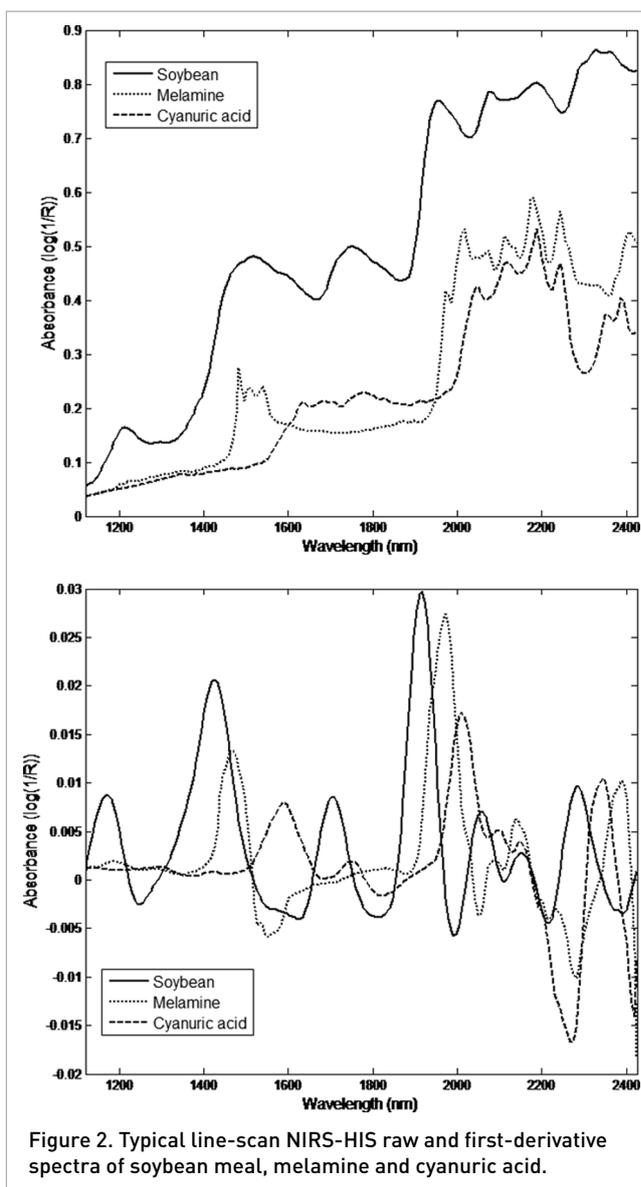


Figure 2. Typical line-scan NIR-HIS raw and first-derivative spectra of soybean meal, melamine and cyanuric acid.

associated with the  $-\text{NH}_2$ , C–N, C=N stretching and deformation vibration, as well as in the 1900–2200 nm region associated with their overtone vibration. All these characteristic bands made it easy to distinguish contaminants from soybean meal, and in this study differentiation was performed based on the spectra by selecting characteristics specific to the different products.<sup>57</sup> These bands were confirmed using the Fisher criterion, which describes the ratio of between-class variance to within-class variance and is helpful in deciding which original variables have an important discriminating ability.<sup>58</sup> Figure 3 shows the Fisher criterion indicating that the 1900–2200 nm region is mainly responsible for the discrimination between the soya and both contaminants.

The overlap between the signals was minimal on several bands in the NIR spectra, making the detection of melamine or cyanuric acid as contaminants in soybean meal fairly straightforward.

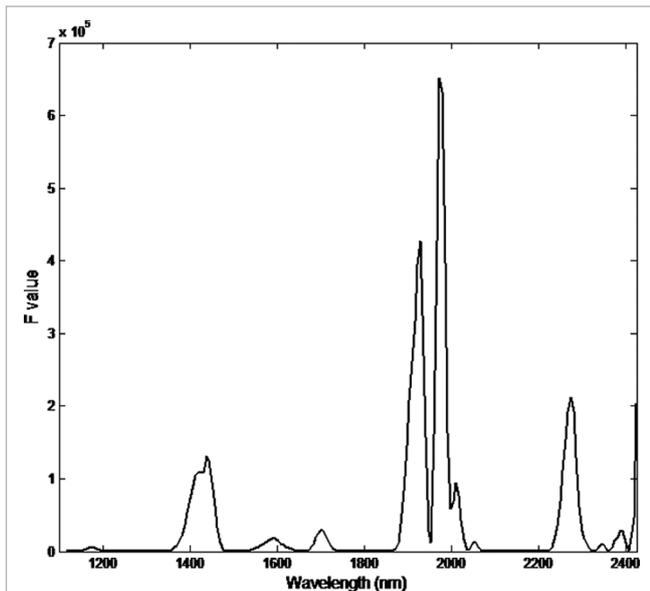


Figure 3. Fisher criterion for soya vs. contaminant (melamine and cyanuric acid).

The exploratory PCA analysis was applied to the spectral library of pure samples in order to extract important information from the data. PCA is an established statistical technique for extracting the major axes of variation from a high-dimensional dataset. Figure 4 shows a projection with principal components (PC1 and PC2) after first-derivative Savitzky-Golay (15:2:1).<sup>59</sup> PC1 explained 72.97% of the variation in the entire dataset, and PC2 explained 21.66% of the remaining variation. From Figure 4, it is clear that there are three classes, indicating that soybean meal can be easily characterised, and both contaminants can be detected.

The supervised clustering method, partial least-squares discriminant analysis (PLS-DA), was implemented to

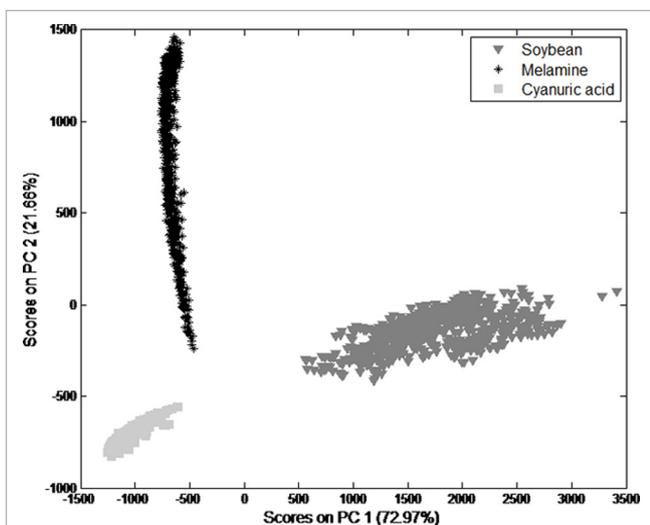


Figure 4. Principal components (PC1 and PC2) after first-derivative Savitzky-Golay (15:2:1) differentiating soybean meal from both contaminants.

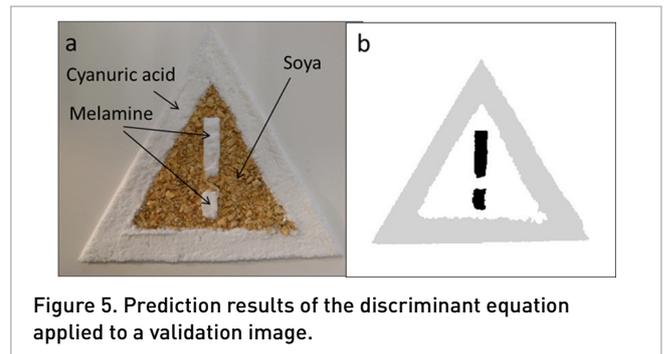


Figure 5. Prediction results of the discriminant equation applied to a validation image.

enhance the good perspectives of separation obtained with the PCA model by automatically building discriminant equations between soybean meal, melamine and cyanuric acid. The model was built using four latent variables and showed a sensitivity (a measure of how often the model correctly identified a positive sample as positive) and a specificity (a measure of how accurate the model was against false positives) of 100% for the three classes (soybean meal, melamine and cyanuric acid). The same results were obtained when applying PLS-DA models only on wavelength ranges selected using the Fisher criterion, confirming the relatively easy detection of melamine or cyanuric acid as contaminants in soybean meal. In order to validate the equation, a validation image dataset was created in the same way as the calibration samples, using the same imaging instrument; it consisted of an image of 298 lines, which corresponded to 95,360 spectra. These data consisted of pure melamine, pure cyanuric acid and pure soya particles (not used at the calibration step) arranged in a special shape, as indicated in Figure 5(a). The constructed classification model was applied to all the pixels in this dataset image, and the results are shown in Figure 5(b). In this figure, the different symbols indicate different classes as detected by the discrimination model.

Once the PLS-DA model had been built and validated, it could be used for the prediction of the 17 artificial mixtures. Each sample was measured online using the line-scan NIR-HIS system with the same parameters as previously specified. For each sample, this led to 10 images of 200 lines each. As in the previous case, the constructed classification model was applied to all the pixels in the field of view (FOV) of each dataset image (2200 lines  $\times$  320 pixels  $\times$  209 wavelengths). This made it possible to quantify the possible contamination of the dataset.

The results were divided into two tables: results for the mixtures contaminated with melamine (Table 2) and results for the mixtures contaminated with cyanuric acid (Table 3). For each table, the real percentage of added contaminant was included, as was the detected number of pixels for each product. Figures 6 and 7 show the regression lines between the real percentages of added contaminant and the pixels detected as melamine or cyanuric acid, respectively, by applying the PLS-DA discrimination model.

Table 2. Contaminant detection results obtained using PLS-DA and NIR-HIS analysis of the mixtures of soybean meal and melamine.

Sample <sup>a</sup>	Theoretical melamine (%)	Detected melamine (number of pixels)	Detected cyanuric acid (number of pixels)	Detected soya(number of pixels)
Mix 1	0.5	834	48	462,043
Mix 2	1	831	0	634,460
Mix 3	1.5	1975	0	568,091
Mix 4	2	2174	1	636,503
Mix 5	2.5	2388	0	604,950
Mix 6	3	3043	0	644,489
Mix 7	3.5	5134	0	554,416
Mix 8	4	7700	0	603,682
Mix 9	5	7771	1	512,598

<sup>a</sup>Each image contains 2200 lines.

Table 3. Contaminant detection results obtained using PLS-DA and NIR-HIS analysis of the mixtures of soybean meal and cyanuric acid.

Sample <sup>a</sup>	Theoretical cyanuric acid (%)	Detected melamine (number of pixels)	Detected cyanuric acid (number of pixels)	Detected soya (number of pixels)
Mix 10	0.5	4	223	677,264
Mix 11	1	8	556	546,939
Mix 12	2	0	1183	626,962
Mix 13	2.5	15	1476	639,837
Mix 14	3	3	1727	464,566
Mix 15	3.5	4	3006	222,171
Mix 16	4.5	2	4264	567,847
Mix 17	5	13	5045	421,928

<sup>a</sup>Each image contains 2200 lines.

Both regression lines between the real percentages and the pixels detected as melamine or cyanuric acid by the PLS-DA discrimination model showed a high  $r^2$ , indicating that both contaminants could be easily detected, even at levels of 0.5%. In order to try to assess the limit of quantification of melamine in soybean meal, a comparison was run between a pure soybean meal sample contaminated with melamine at 0.1% and an unadulterated sample from the same soybean meal. The total mass of both samples was 1000 mg. As in the

previous samples, images were acquired using the line scan NIR-HIS system, using a macro-lens with an FOV of  $28 \times 1$  cm. To avoid particle superimposition, the scanning procedure for powders involved spreading the samples as a mono-layer on the conveyor belt. In addition, to meet the constraints of spreading and a fixed FOV, each sample was subdivided into smaller parts (series). Each series comprised 28–30 images representing 34–38 mg of the sample. Prior to image acquisition, the sample measurement repetition was set at 10 repli-

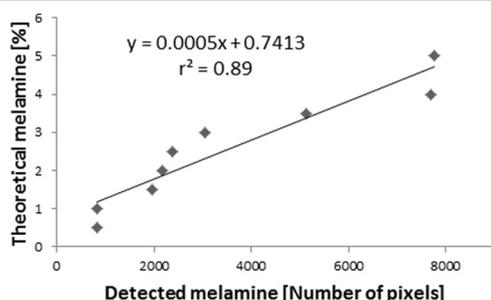


Figure 6. Regression line between the laboratory percentage of added melamine and the number of pixels detected as melamine using PLS-DA.

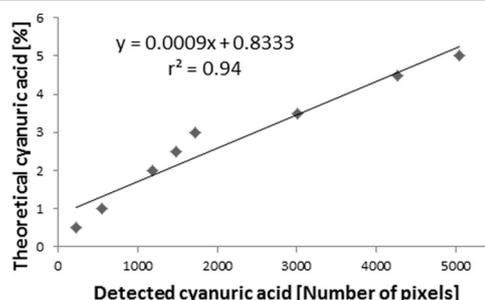


Figure 7. Regression line between the laboratory percentage of added cyanuric acid and the number of pixels detected as cyanuric acid using PLS-DA.

cates for each sample. The spreading led to a high loss (up to 20% in some cases) in the sample mass, which meant that only two repetitions could be run for both samples.

The PLS-DA model was applied to each image for each repetition. For each repetition, the results of each series were gathered in order to obtain a total prediction from the sample repetition. In general, the obtained results (not shown) indicated that samples contaminated at 0.1% could still be detected; the quantification predictions, however, were well under the actual 0.1% value. The quantitative predictions did not seem to be reliable for a melamine mixture of 0.1% concentration, but could be more reliable with higher concentrations of melamine. These weak predictions might have been linked to the significant loss of sample mass between repetitions owing to the spreading. The loss of mass increased with the number of series per repetition, as well as the number of potential manipulation errors. Lighting heterogeneity might also have reduced prediction quality. Despite the weak results obtained for quantification, it is important to emphasize that melamine was still detectable at low concentrations.

## Conclusion

Soya is one of the major ingredients of cattle feed. Soybean production and use are likely to show steady growth in the foreseeable future because of the strong and growing demand for protein meals and vegetable oils. There is therefore a need for standardised methodologies for setting specifications that characterise soybean meal and for detecting the presence of contaminants such as melamine. The results presented in this study show the feasibility of line-scan NIR-HIS combined with unsupervised (PCA) and supervised (PLS-DA) data-mining methods, applied at the laboratory level with adequate preprocessing of the data and variable selection (Fisher criterion), for accurately characterising soybean meal destined for food or feed and for detecting the presence of melamine and analogues. The main limitations of HIS are cost and complexity. Fast computers, sensitive detectors and large data-storage capacities are needed for the data analysis, all of which greatly increase the cost of acquiring and processing hyperspectral data. The main advantages are the rapid image acquisition of data and the ability to acquire an entire spectrum at each point, avoiding the need for prior knowledge of a sample. Using online measurements, the procedure could be easily applied in the processing industry, where accurate and fast inspection is needed. The method allows a low level of melamine in soybean meal to be detected and could be extended to other types of contaminants. The results also indicate that quantitative prediction is not reliable for a melamine mixture of 0.1% concentration, but predictions could be more reliable with higher concentrations of melamine. These poor predictions might be linked to the significant loss of sample mass during the repetitions. The results could also be explained by the fact that a poor spectral profile was obtained even when the integration time was set to maximum, probably because

of less-than-optimal light distribution. More work is therefore needed on using this method for quantitative purposes.

## Acknowledgements

The authors are very grateful for the soybean sample sets used in the work that were kindly donated by Provimi, Crévin, France and John Thompson & Sons Ltd, Belfast, UK. The work was funded by the European Commission 7th Framework Project: QSAFFE-265702: Collaborative Project under the Seventh Framework Programme, KBBE-2010-2-4-03: Quality and Safety Aspects of Feed, as well as by the Bruxelles-Wallonie International cooperation project between the Walloon Agricultural Research Centre (CRA-W) in Gembloux, Belgium and the China Agricultural University (CAU) in Beijing, China. We also gratefully acknowledge the technical assistance provided by Sandrine Mauro and Nicaise Kayoka for the line-scan NIR-HIS measurements.

## References

1. M. Moroni, E. Lupo, E. Marra and A. Cenedese, "Hyperspectral image analysis in environmental monitoring: setup of a new tunable filter platform", *Proc. Environ. Sci.* **19**, 885 (2013). doi: <http://dx.doi.org/10.1016/j.proenv.2013.06.098>
2. A.Y. Fong and E. Wachman, "Hyperspectral imaging for the life sciences", *Biophoton. Int.* **15**, 38 (2008).
3. S.J. Hamilton and R.A. Lodder, "Hyperspectral imaging technology for pharmaceutical analysis", *SPIE Proc. BiOS* **2002**, 1 (2002).
4. E.N. Lewis, L.H. Kidder and E. Lee "NIR chemical imaging – near infrared spectroscopy on steroids", *NIRS News* **16(6)**, 3 (2005). doi: <http://dx.doi.org/10.1255/nirn.841>
5. R.N. Clark and G.A. Swayze, "Mapping minerals, amorphous materials, environmental materials, vegetation, water, ice, and snow, and other materials: The USGS Tricorder Algorithm", in *Fifth Annual JPL Airborne Earth Science Workshop 95-1*, 39 (1995).
6. K. Rapantzikos and C. Balas, "Hyperspectral imaging: potential in non-destructive analysis of palimpsests", in *IEEE International Conference on Image Processing, II-618* (2005).
7. D.T. Dicker, N. Kahn, K.T. Flaherty, J. Lerner and W.S. El-Deiry, "Hyperspectral imaging: a non-invasive method of imaging melanoma lesions in a patient with stage IV melanoma, being treated with a RAF inhibitor", *Cancer Biol. Ther.* **12**, 326 (2011). doi: <http://dx.doi.org/10.4161/cbt.12.4.17142>
8. P. Usenik, M. Bürmen, A. Fidler, F. Pernuš and B. Likar, "Automated classification and visualization of healthy and diseased hard dental tissues by near-infrared hyperspectral imaging", *Appl. Spectrosc.* **66**, 1067 (2012). doi: <http://dx.doi.org/10.1366/11-06460>

9. D. Hattery, M. Hassan, S. Demos and A. Gandjbakhche, "Hyperspectral imaging of Kaposi's sarcoma for disease assessment and treatment monitoring", in *AIPRIEEE Computer Society*, 124 (2002).
10. R.S. Gad, N. Vetrekar, I. Nazareth, J.S. Parab and G.M. Naik, "Human tissue diagnostics for physiological information using Hyperspectral Image model", *Proceedings of the International Conference on Biomedical and Health Informatics (BHI), IEEE-EMBS*, 2-7 January 2012, Hong Kong and Shenzhen, China, pp. 741 (2012).
11. L.L. Randeberg and J. Hernandez Palacios, "Hyperspectral imaging of bruises in the SWIR spectral region", in *Proceedings of the SPIE Conference, Photonic Therapeutics and Diagnostics VIII*, 3 February 2012, 82070N (2012).
12. Z. Pan, G. Healey, M. Prasad and B. Tromberg, "Face recognition in hyperspectral images", *IEEE Trans. Pattern Anal. Mach. Intell.* **25(12)**, 1552 (2003). doi: <http://dx.doi.org/10.1258/rsmmsl.47.3.225>
13. C. Gendrin, Y. Roggo and C. Collet, "Content uniformity of pharmaceutical solid dosage forms by near infrared hyperspectral imaging: A feasibility study", *Talanta* **73**, 733 (2007). doi: <http://dx.doi.org/10.1016/j.talanta.2007.04.054>
14. V. Baeten, J.A. Fernández Pierna, P. Vermeulen and P. Dardenne, "NIR hyperspectral imaging methods for quality and safety control of food and feed products: contributions to 4 European projects", *NIRS News* **21(6)**, 10 (2010). doi: <http://dx.doi.org/10.1255/nirn.1200>
15. J.A. Fernández Pierna, P. Vermeulen, O. Amand, A. Tossens, P. Dardenne and V. Baeten, "NIR hyperspectral imaging spectroscopy and chemometrics for the detection of undesirable substances in food and feed", *Chemom. Intell. Lab. Syst.* **117**, 233 (2012). doi: <http://dx.doi.org/10.1016/j.chemolab.2012.02.004>
16. J.A. Fernández Pierna, V. Baeten, A. Michotte Renier, R.P. Cogdill and P. Dardenne, "Combination of support vector machines (SVM) and near infrared (NIR) imaging spectroscopy for the detection of meat and bone meat (MBM) in compound feeds", *J. Chemom.* **18**, 341 (2004). doi: <http://dx.doi.org/10.1002/cem.877>
17. J.A. Fernández Pierna, V. Baeten and P. Dardenne P., "Screening of compound feeds using NIR hyperspectral data", *Chemom. Intell. Lab. Syst.* **84**, 114 (2006). doi: <http://dx.doi.org/10.1016/j.chemolab.2006.03.012>
18. L.M. Dale, J.A. Fernández Pierna, P. Vermeulen, B. Lecler, A.D. Bogdan, F.S. Păcurar, I. Rotar, A. Thewis and V. Baeten, "Research on crude protein and digestibility of *Arnica montana* L. using conventional NIR spectrometry and hyperspectral imaging NIR", *J. Food Agric. Environ.* **10**, 391 (2012).
19. L.M. Dale, A. Thewis, C. Boudry, I. Rotar, F.S. Păcurar, O. Abbas, P. Dardenne, V. Baeten, J. Pfister and J.A. Fernández Pierna, "Discrimination of grassland species and their classification in botanical families by laboratory scale NIR hyperspectral imaging: preliminary results", *Talanta* **116**, 149 (2013). doi: <http://dx.doi.org/10.1016/j.talanta.2013.05.006>
20. R.N. Merton, *Multi-Temporal Analysis of Community Scale Vegetation Stress with Imaging Spectroscopy*. PhD thesis, University of Auckland (1999).
21. R. Lu, "Detection of bruises on apples using near-infrared hyperspectral imaging", *Trans. ASAE* **46**, 1 (2003).
22. M. Nagada, J.G. Tallada and T. Kobayashi, "Bruise detection using NIR hyperspectral imaging for strawberry", *Environ. Control Biol.* **44**, 133 (2006). doi: <http://dx.doi.org/10.2525/ecb.44.133>
23. G. Polder, G.W.A.M. Heijden and I.T. Young, "Tomato sorting using independent component analysis on spectral images", *Real-Time Imaging* **9**, 253 (2003). doi: <http://dx.doi.org/10.1016/j.rti.2003.09.008>
24. A.A. Gowen, C.P. O'Donnell, M. Taghizadeh, P.J. Cullen, J.M. Frias and G. Downey, "Hyperspectral imaging combines with principal component analysis for bruise damage detection on white mushrooms (*Agaricus bisporus*)", *J. Chemometrics* **22**, 259 (2008). doi: <http://dx.doi.org/10.1002/cem.1127>
25. D. Ariana and R. Lu, "Hyperspectral imaging for defect detection of pickling cucumbers" in *Hyperspectral Imaging for Food Quality Analysis and Control*, Ed by D.W. Sun. Academic Press, San Diego, CA, pp. 431-447 (2010).
26. P. Williams, P. Geladi, G. Fox and M. Manley, "Maize kernel hardness classification by near infrared (NIR) hyperspectral imaging and multivariate data analysis", *Anal. Chim. Acta* **653**, 121 (2009). doi: <http://dx.doi.org/10.1016/j.aca.2009.09.005>
27. P. Williams, P. Geladi, T.J. Britz and M. Manley, "Investigation of fungal development in maize kernels using NIR hyperspectral imaging and multivariate data analysis", *J. Cereal Sci.* **55**, 272 (2012). doi: <http://dx.doi.org/10.1016/j.jcs.2011.12.003>
28. M. Manley, G. du Toit and P. Geladi, "Tracking diffusion of conditioning water in single wheat kernels of different hardnesses by near infrared hyperspectral imaging", *Anal. Chim. Acta* **686**, 64 (2011). doi: <http://dx.doi.org/10.1016/j.aca.2010.11.042>
29. D.J.F. September, *Detection of Spice Adulteration by Near Infrared Hyperspectral Imaging*. PhD thesis, Stellenbosch University (2010).
30. J.A. Fernández Pierna, P. Vermeulen, D. Stilmant, B. Dupuis, P. Dardenne and V. Baeten, "Characterisation of fonio millet by near infrared hyperspectral imaging", in *Proceedings of the 14th International Conference on NIR Spectroscopy*, Bangkok, Thailand, Ed by S. Saranwong, S. Kasemsumran, W. Thanapase and P. Williams (2010).
31. M. Pla, A. Nadal, V. Baeten, C. Bahrtdt, G. Berben, Y. Bertheau, A. Coll, J.P. van Dijk, D. Dobnik, J.A. Fernández Pierna, K. Gruden, S. Hamels, A. Holck, A. Holst-Jensen, E. Janssen, E.J. Kok, J.L. La Paz, V. Laval, S. Leimanis, A. Malcevski, N. Marmioli, D. Morisset, T.W. Prins, J. Remacle, G. Ujhelyi and D. Wulff, "New multiplexing tools for reliable GMO

- detection”, in *Genetically Modified and Non-Genetically Modified Food Supply Chains: Co-Existence and Traceability*, Ed by Y. Bertheau, John Wiley & Sons, Chichester, UK, pp. 333–336 (2012).
32. P. Vermeulen, J.A. Fernández Pierna, H.P. van Egmond, P. Dardenne and V. Baeten, “Online detection and quantification of ergot bodies in cereals using near infrared hyperspectral imaging”, *Food Addit. Contam.* **29**, 232 (2012). doi: <http://dx.doi.org/10.1080/19440049.2011.627573>
33. P. Vermeulen, J.A. Fernández Pierna, H.P. van Egmond, J. Zegers, P. Dardenne and V. Baeten, “Validation and transferability study of a method based on near-infrared hyperspectral imaging for the detection and quantification of ergot bodies in cereals”, *Anal. Bioanal. Chem.*, **405**, 7765 (2013). doi: <http://dx.doi.org/10.1007/s00216-013-6775-7>
34. V. Baeten and P. Dardenne, “Application of near-infrared imaging for monitoring agricultural food and feed products”, in *Spectrochemical Analysis using Infrared Multichannel Detectors*, Ed by R. Bhargava and I. Levin. Wiley-Blackwell, Oxford, pp. 283–302 (2005).
35. V. Baeten, J.A. Fernández Pierna and P. Dardenne, in *Techniques and Applications of Hyperspectral Image Analysis*, Ed by H.F. Grahn and P. Geladi. Wiley, New York (2007).
36. J.A. Fernández Pierna, V. Baeten, J. Dubois, J. Burger, E.N. Lewis and P. Dardenne, in *Comprehensive Chemometrics*, Ed by S. Brown, R. Tauler and B. Walczak. Elsevier, Amsterdam, pp 173–196 (2009).
37. Z. Yang, L. Han, J.A. Fernández Pierna, P. Dardenne and V. Baeten, “Review: The potential of near infrared microscopy to detect, identify and quantify processed animal by-products”, *J. Near Infrared Spectrosc.* **19**, 211 (2011). doi: <http://dx.doi.org/10.1255/jnirs.935>
38. J.A. Fernández Pierna, P. Dardenne and V. Baeten, “In-house validation of a near infrared hyperspectral imaging method for detecting processed animal proteins (PAP) in compound feed”, *J. Near Infrared Spectrosc.* **18**, 121 (2010). doi: <http://dx.doi.org/10.1255/jnirs.872>
39. G. El Masry and D.W. Sun, “Principles of hyperspectral imaging technology”, in *Hyperspectral Imaging for Food Quality and Control*, Ed by D.W. Sun. Academic Press, San Diego, CA, pp. 3 (2010).
40. FEFAC, “FEFAC welcomes Commission decision to extend testing requirements on melamine to soybean products from China” (press review) (2008).
41. EFSA (2010) “Scientific opinion on melamine in food and feed”, *EFSA J.* **8**, 1573.
42. Y. Tian, M. Yang, S. Jong, C. Wang and J. Shiea, “Melamine contamination”, *Anal. Bioanal. Chem.* **395**, 729 (2009). doi: <http://dx.doi.org/10.1007/s00216-009-3009-0>
43. K. Ai, Y. Liu and L. Lu, “Hydrogen-bonding recognition-induced color change of gold nanoparticles for visual detection of melamine in raw milk and infant formula”, *J. Am. Chem. Soc.* **131**, 9496 (2009). doi: <http://dx.doi.org/10.1021/ja9037017>
44. N. Yan, L. Zhou, Z. Zhu and X. Chen, “Determination of melamine in dairy products, fish feed, and fish by capillary zone electrophoresis with diode array detection”, *J. Agric. Food Chem.* **57**, 807 (2009). doi: <http://dx.doi.org/10.1021/jf803429e>
45. L.J. Mauer, A.A. Chernyshova, A. Hiatt, A. Deering and R. Davis, “Melamine detection in infant formula powder using near- and mid-infrared spectroscopy”, *J. Agric. Food Chem.* **57**, 3974 (2009). doi: <http://dx.doi.org/10.1021/jf900587m>
46. Y.W. Dong, Z.H. Tu, D.Z. Zhu, Y.W. Liu, Y.N. Wang, J.L. Huang, B.L. Sun and Z.N. Fan, “Feasibility of using NIR spectroscopy to detect melamine in milk”, *Guang Pu Xue Yu Guang Pu Fen Xi* **29**, 2934 (2009).
47. S.V. Smirnov, “Neural network based method for melamine analysis in liquid milk”, in *International Conference on Intelligent Computation Technology and Automation (ICICTA)*, Volume 2, pp. 999 (2011).
48. C. Lu, B. Xiang, G. Hao, J. Xu, Z. Wang and C. Chen, “Rapid detection of melamine in milk powder by near infrared spectroscopy”, *J. Near Infrared Spectrosc.* **17**, 59 (2009). doi: <http://dx.doi.org/10.1255/jnirs.829>
49. V. Baeten, O. Abbas, B. Lecler and P. Dardenne, “Detection of melamine contamination by NIRS and NIR microscopy techniques: which perspectives and limits?”, in *Proceedings of the 14th International Conference on Near Infrared Spectroscopy (ICNIRS): Breaking the Dawn*, 7 November 2009, Bangkok, Thailand, pp. 427 (2011).
50. M. Lin, L. He, J. Awika, L. Yang, D.R. Ledoux, H. Li and A. Mustapha, “Detection of melamine in gluten, chicken feed and processed foods using surface enhanced Raman spectroscopy and HPLC”, *J. Food Sci.* **73**, 129 (2008). doi: <http://dx.doi.org/10.1111/j.1750-3841.2008.00901.x>
51. M. Lin, “A review of traditional and novel detection techniques for melamine and its analogues in foods and animal feed”, *Front. Chem. Eng. China* **3**, 427 (2009). doi: <http://dx.doi.org/10.1007/s11705-009-0244-z>
52. S.A. Haughey, S.F. Graham, E. Cancouët and C.T. Elliott, “The application of near-infrared reflectance spectroscopy (NIRS) to detect melamine adulteration of soya bean meal”, *Food Chem.* **136**, 1557 (2013). doi: <http://dx.doi.org/10.1016/j.foodchem.2012.01.068>
53. O. Abbas, B. Lecler, P. Dardenne and V. Baeten, “Detection of melamine and cyanuric acid in feed ingredients by near infrared spectroscopy and chemometrics”, *J. Near Infrared Spectrosc.* **21**, 183 (2013). doi: <http://dx.doi.org/10.1255/jnirs.1047>
54. S. Piqueras, J. Burger, R. Tauler and A. de Juan, “Relevant aspects of quantification and sample heterogeneity in hyperspectral image resolution”, *Chemom. Intell. Lab. Syst.* **117**, 169 (2012). doi: <http://dx.doi.org/10.1016/j.chemolab.2011.12.004>

55. R.W. Kennard and L.A. Stone, "Computer aided design of experiments", *Technometrics* **11**, 137 (1969). doi: <http://dx.doi.org/10.1080/00401706.1969.10490666>
56. I. Murray, "The NIR spectra of homologous series of organic compounds", in *Proceedings of the International NIR/NIT Conference*, Akademiai Kiado, Budapest, Hungary, Ed by J. Hollo, K.J. Kaftka and J.L. Gonczy, pp. 13 (1986).
57. X. Liu, G. Jia, C. Wu, K. Wang and X. Wu, "Determination of characteristic wave bands and detection of melamine in fishmeal by Fourier transform near infrared spectroscopy", *J. Near Infrared Spectrosc.* **18**, 113 (2010). doi: <http://dx.doi.org/10.1255/jnirs.871>
58. R.O. Duda, P.E. Hart and D.G. Stork, *Pattern Recognition*, 2nd Edn, Wiley, New York (2000).
59. A. Savitzky and M.J.E. Golay, "Smoothing and differentiation of data by simplified least squares procedures", *Anal. Chem.* **8**, 1627 (1964). doi: <http://dx.doi.org/10.1021/ac60214a047>