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Detection of melamine and cyanuric acid in feed ingredients by near infrared spectroscopy and chemometrics

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This study investigated the detection of contamination of animal feed by melamine and its derivatives by rapid analytical methods. The main goal was to propose an effective tool to detect contaminantion by using multivariate calibration equations built on a large database of non-contaminated feed ingredients. Soybean meal, maize gluten and wheat gluten samples were contaminated by different percentages of melamine and cyanuric acid. The influence of these additives on near infrared (NIR) predicted values of crude protein was studied. The predicted values of protein, in terms of the adulteration percentage, were compared with those obtained by conventional methods (Kjeldahl and Dumas). The addition of the contaminant led to an increase in the protein value when measured by classical methods and to a decrease in the value when predicted by the NIR calibration models. Among the modifications in the spectral profile of affected feed was the intensity of the spectrum at about 2170 nm, characteristic of the absorption of proteins which might explain the reduction in NIR predicted protein values when contaminants were added. An important advantage of the approach is the simultaneous detection of several analytes, making it possible to detect melamine and cyanuric acid at the same time. Contaminated feed was analysed using the near infrared (NIR) general feed ingredient database. Calibration equations were developed and applied to the samples in this study to visualise their distribution with regard to the existing data set that does not contain contaminants. Contaminated samples presented global *H* (*GH)* (Mahalanobis distance) values greater than three and were easily distinguished from the rest. Both the full spectrum and a selected spectral region between 2130 nm and 2230 nm, including wavelengths relevant for discrimination, were used to develop mathematical equations to predict the protein content and to detect contaminated samples.

Keywords: melamine, cyanuric acid, contamination, NIR spectroscopy, chemometrics, *GH*, protein

Introduction

In recent years, public concern about the safety of foods of animal origin has increased. Feed contamination has been the cause of major European crises, such as dioxin-polychlorinated biphenyls (PCB),[1] poultry product contamination by dioxins,[2,3] bovine spongiform encephalopathy (BSE) and mycotoxins.[4] In 2007, a pet food product was recalled in North America by several pet food manufacturers after a number of cats and dogs sickened and died after eating contaminated pet food. The US Food and Drug Administration reported finding melamine in the pet food and in samples of wheat gluten imported from a single source in China. Melamine had been added to give an artificial boost to the nitrogen content of the pet food. There was a similar incident in September 2008, but this time it stemmed from the contamination of Chinese-manufactured milk infant formula with melamine, leading to the hospitalisation of thousands of children, some of whom died.[5]

These problems have drawn the attention of both regulatory authorities and research teams to feeding practices and

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the feed industry. Investigators now believe that the chemical used to make industrial glues, fire retardant and fertilisers was added as supplements to pet food. Purchasers routinely measure the nitrogen content of such supplements to determine their protein content and investigators suspect that suppliers sought to make it appear that their products contained more protein than they actually did by adding melamine. This chemical, with no nutritional value, can be deliberately added to feed supplements to boost apparent protein content. European Parliament and Council Regulation, 1831 (2003), applicable as of 18 October 2004, regulates the use of additives to animal nutrition in order to ensure a high level of protection of human health and welfare, the environment and the interests of users and consumers. It sets out rules for the authorisation, marketing and labelling of feed additives. The European Food Safety Authority (EFSA) is responsible for carrying out scientific assessments of feed additives.[6]

Simultaneously, much research has been done on developing accurate and sensitive analytical techniques for assessing the quality and safety of feeding products. NIR spectroscopy is widely used as a successful quality control tool in the feed industry and animal nutrition. The main quality factors that are routinely assessed in these products are energy value, dry matter, fat, crude fibre and crude protein content. The prediction of crude protein content has been described in several studies that have demonstrated the usefulness of NIR spectroscopy for developing prediction models of protein in wheat, barley, oats and maize, in a mixture of different classes of wheat[10] and in compound feeds.[11] The amount of crude protein has also been measured by NIR reflectance spectroscopy when studying the composition of feed for rabbits,[12] pigs,[13] poultry,[14] and dairy cows.[15] González-Martín *et al*. (2006)[16] used NIR reflectance spectroscopy and a remote reflectance fibre-optic probe to perform an instantaneous determination of crude proteins, fat and fibre in animal feeds.

NIR spectroscopy has proved successful in predicting crude protein content and it has been shown to be able to differentiate protein from non-protein nitrogen sources in feed and food products.[17]

The advances in NIR instrumentation have increased measurement speed and improved sensitivity. In addition, the use of chemometric analysis has helped broaden the use of NIR spectroscopy as an analytical tool. Currently, spectra are easily collected and interpreted. NIR spectroscopy offers a simple and reliable way of conducting routine measurements, but it requires a calibration set that is representative of all future unknown samples in order to ensure the accuracy of the equations developed.[18] National and international networks are being developed in order to offer a selection of pre-calibrated applications for food and feed products in the analysis of moisture, protein, ash, fat, fibre, starch and other physical parameters.

Our objective was to detect, rapidly and efficiently, samples that present anomalies. In the case of the melamine crisis, the contaminant was identified in the urine of dogs and cats that had died after eating pet food. Cyanuric acid, found in the urine and melamine react together to form crystals which could cause severe renal problems leading to death. Feedstuffs should therefore be subject to high and efficient quality assurance systems based on control analysis. Several studies have been undertaken using NIR spectroscopy to detect melamine and/or for determining its content through the development of chemometric methods. The results showed that NIR spectroscopy could provide a rapid way of detecting melamine in infant milk formula. The origin of contamination by melamine could be due to a deliberate external addition to food products or to the adulteration of ingredients in animal feed.[22,23] It is then necessary to check the composition of feed to prevent a possible transmission from feed to the food. Liu *et al*.[24] (2010) worked on of the detection of melamine in fish meal by Fourier transform NIR spectroscopy; they defined the characteristic wave bands of melamine and conducted some trials to quantify the melamine present in concentrations between 0% and 15% by developing partial least-squares (PLS) models.

In this study, we used NIR spectroscopy to study the influence of melamine on the NIR predicted value of crude protein and to compare the results with those from using conventional methods (Kjeldahl and Dumas). Feed contaminated by melamine and/or cyanuric acid was analysed for comparison with the general feed ingredient database. For that procedure, calibration equations were developed and applied to these new samples to determine their similarity with regard to the existing data set that does not contain contaminated feed ingredients.

Material and method

Samples

A total of 169 synthesised samples of animal feed (65 samples of soybean meal, 65 samples of maize gluten and 39 samples of wheat gluten) was studied. The samples comprised both contaminated and non-contaminated samples; for each type of non adulterated sample (soybean meal, maize gluten and wheat gluten), a percentage of melamine, cyanuric acid or both was added, as described in Table 1. They were synthesised and provided by an international company for feed and food which prepared samples by mixing in a blender. Samples were also homogenised before measurements.

Chemicals

Melamine and cyanuric acid used for adulteration were purchased by the company which provided contaminated samples (described in Table 1), from Acros Organics (www.acros.be). For the identification of the spectral profile of pure melamine, we purchased the product from three sources (VWR, https://be.vwr.com; Sigma–Aldrich, www.sigmaaldrich.com and Fisher Bioblock, www.bioblock.be).

Chemical analysis

Crude protein was analysed using the methods described by Kjeldahl[25] and Dumas.[26] Reagents used in the Kjeldahl method, such as potassium sulphate K_2SO_4 and copper sulphate

Table 1. Description of the samples adulterated by melamine, cyanuric acid and both of them.

		% of melamine added	% of cyanuric acid added	% of mixture added
Soybean meal	SM01	1.00–3.98[4]	0.50–5.55[4]	0.50–6.05[4]
	SM02	1.94–6.00[4]	0.53–5.98[4]	1.06–5.41[4]
	SM03	2.54–5.54[4]	0.94–3.93[4]	2.04–5.93[4]
	SM04	0.53–6.03[4]	1.96–6.04[4]	0.54–4.93[4]
	SM05	0.55–5.03[4]	2.54–5.55[4]	1.50–4.50[4]
Maize gluten	GM01	2.00–6.02[4]	0.54–6.02[4]	0.55–5.34[4]
	GM02	0.52–4.97[4]	1.02–5.54[4]	0.50–6.09[4]
	GM03	1.49–4.58[4]	1.99–6.11[4]	1.10–4.07[4]
	GM04	0.55–5.93[4]	0.55–5.04[4]	2.04–6.17[4]
	GM05	1.04–5.54[4]	1.51–4.56[4]	2.58–5.55[4]
Wheat gluten	GW01	1.07–3.97[4]	0.53–5.55[4]	0.54–5.88[4]
	GW02	2.05–6.03[4]	0.53–6.09[4]	1.06–5.61[4]
	GW03	2.63–5.58[4]	1.00–3.96[4]	2.17–6.15[4]

[4]number of samples adulterated for each matrix and with each contaminant.

$CuSO_4$, were purchased from HUMEAU (,AUTHORS to INSERT URL) and the sulphuric acid H_2SO_4, sodium hydroxide NaOH, hydrochloric acid HCl and boric acid H_3BO_3 were from VWR. A. Gerhardt apparatus was used (type vapodest 20 c www.geminibv.nl). For combustion analysis (the Dumas method), all the reagents were bought from the LECO Corporation and a LECO FP-2000 Protein-nitrogen Analyser (LECO Corporation, St Joseph, MI, USA) was used. The determinations were carried out in duplicate and the results were expressed in g/kg (dry matter). Crude protein content was estimated using a conversion factor based on an estimate of the percentage of nitrogen in the protein. The crude protein was then calculated by multiplying the nitrogen content by a constant based on the average amino acid composition of typical examples of the product; a factor of nitrogen (N) × 6.25 is usually used for feed products.

Spectra acquisition

Spectroscopic analyses were performed in duplicate on feed samples, using a NIRSystems 6500 monochromator (Foss NIRSystems Inc. Laurel, MD 20,723, USA). Spectra were collected between 400 nm and 2498 nm, with an interval of 2 nm and by co-adding 32 scans. Spectra were collected as log $1\,R^{-1}$. As some spectra of the samples in the general feed ingredient database were collected between 1100 nm and 2498 nm, only this region was taken into account and the 400–1098 nm region was excluded.

Data were collected using ISI Windows Near-infrared software WinISI III—version 1.50(e) (Infrasoft International, LLC Foss). The software was also used for data treatment and statistical analyses.

Statistical analyses

A global calibration equation was established using the large database available at CRA-W containing spectra of all the samples of animal feed ingredient database (22,689 samples with known laboratory protein value). The calibration database represents a large variability in chemical composition and sources of animal feed ingredients. Global calibrations were performed using modified partial least squares[27] (MPLS—WinISI, Foss, DK) regression. MPLS is very similar to PLS regression. PLS is modified by normalising the reflectance residuals at every wavelength and before calculating the next factor.[28] MPLS regression was applied with four groups cross validation and a scatter correction by standard normal variate (SNV) and detrend (DT). SNV pre-treatment corrects for the spectral variations associated with particle size.[29] As with SNV, DT is a row-oriented transformation which affects individual spectra. DT removes nonlinear trends from spectroscopic data by fitting a higher-order polynomial to each individual spectrum, then removing the estimated baseline curvature. The spectra were processed using a derivative treatment (1, 5, 5, 1) where the first number indicates the application of the first derivative, the second one is the difference in data points over which the derivative is calculated, the third one is the number of data points used in the first smoothing and the fourth one is the number of data points over which the second smoothing was applied. The best equation was selected on the basis of the lowest standard error of cross-validation (*SECV*). When using one equation to predict a new sample, three control tests were applied; the neighbourhood *H* (*NH*) which should be less than 1.0, the *T* outlier value which should be less than 2.5 (the samples with the highest residual values were eliminated using the $T > 2.5$ criterion) and the *GH* which should be less than 3.0. These values were determined using standard settings in the WinISI software. *GH* can be defined as the standardised *H* distance (Mahalanobis distance) from the average spectrum.[28] Samples with a *GH* greater than three were considered as different from the database.

Results and discussion

Melamine (or 1,3,5-triazine-2,4,6-triamine) is an organic compound rich in nitrogen (66% by mass). Its structure provides a spectral signature rich in peaks. The assignment of main bands of the spectrum (Figure 1) to the appropriate vibrational modes gives detailed information on melamines structure (Table 2).

The peak at 1018 nm is associated with the 2nd overtone of the N–H group, while peaks pointed at 1466 nm, 1490 nm and 1520 nm and 1958 nm can be attributed to the first overtone of N–H. Combination bands appear at 1998 nm and 2058 nm correspond to N–H stretching/N–H deformation combination and N–H stretching H bonded/N–H deformation combination, respectively.[30] Bands at 2160 nm and 2226 nm can be attributed to 1, 3, 5—triazine structural vibrations.

A comparison of the melamine NIR spectrum with those of compounds rich in protein reported in the literature[31] suggests the absence of bands characteristic of protein in the melamine spectrum. The peptide backbone of proteins presents bands mainly at 1600 nm, 2050 nm and 2180 nm, corresponding to the first overtone of amide A and the combination bands of the different types of amide, respectively. In many studies, the band around 2180 nm has been selected to determine the protein content in feed or food products. This band was considered to be an overlapping of absorption bands 2170 nm (peptide) and 2190 nm (amide).[17,32] Yamashita *et al.* (1994)[17] found that the absorption at 2170 nm was the most stable for determining protein content in products where several compounds co-exist.

Melamine added to the studied feed samples led to changes in the shape of the spectra of these compounds (Figure 2). A shoulder at 1466 nm due to the absorbance of the added melamine appeared on the spectrum of contaminated samples. The region between 1956 nm and 2226 nm was also affected, but not in the same way for all types of feed samples. Wheat gluten shows a slight variation when adding melamine; only a rise in the band at 1466 nm appeared when melamine was added. Soybean meal and maize gluten seemed to be more influenced by the presence of melamine and bands 1956 nm, 1992 nm, 2096 nm and 2226 nm in addition to the one at 1466 nm appeared on the spectra of the contaminated samples. The magnitude of the spectral change from melamine seems to be dependent on the feed matrix. In fact, studied matrixes have different particle sizes and shape. Wheat gluten samples are mostly composed of very fine particles while base material particles of maize gluten and soybean meal varies from fine to average or gross depending on the matrix. As melamine powder is electrostatic, good homogenisation is needed, especially with bulk samples as this may influence the "visibility" of melamine in mixtures.

The detection of contaminated samples was based on the modifications in the spectra of the adulterated samples in the 1100–2498 nm region, referred to as the "full spectrum" in this study. In order to accentuate differences, it was decided to look for discriminative spectral regions. The principal wave-

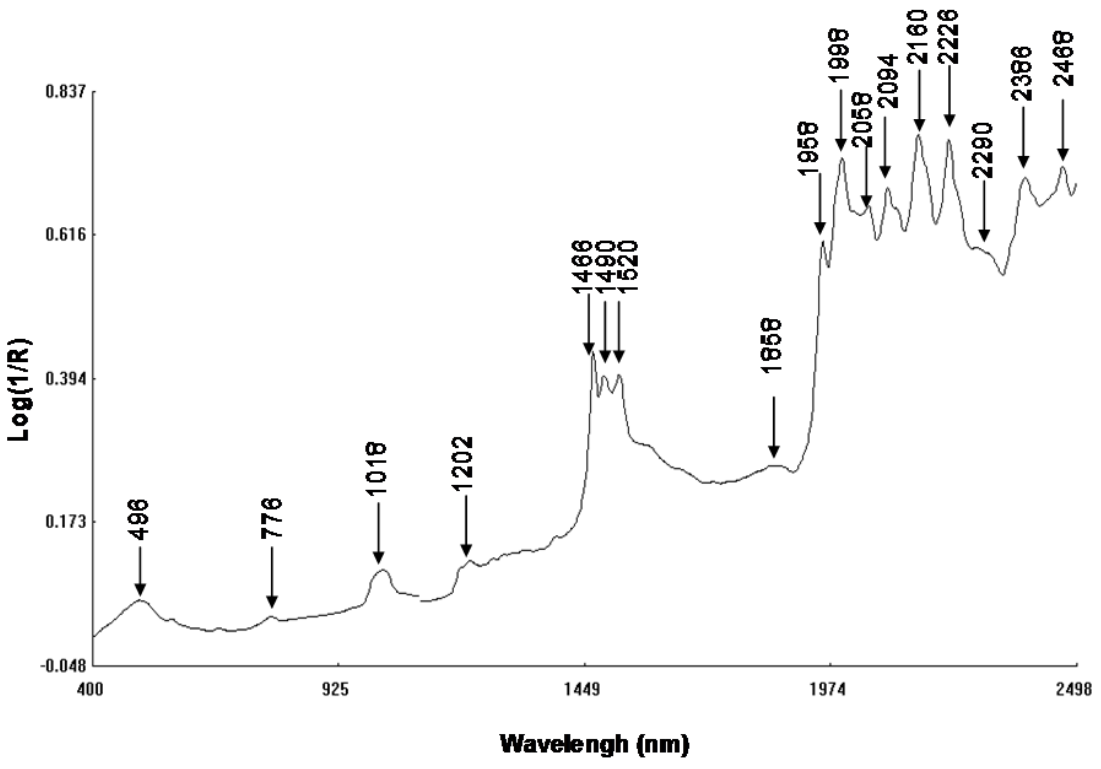

Figure 1. Spectrum of melamine compound recorded between 400 nm and 2498 nm.

Table 2. Near infrared (NIR) assignments melamine spectral bands.

Wavelength (nm)	Assignment
1018	–NH str. second overtone
1466, 1490, 1520	N–H str. first overtone
1958	N–H str. first overtone
1998	N–H str./N–H def. Combination
2058	N–H str. H bonded plus N–H def. Combination
2160	Triazine structure vibrations
2226	Triazine structure vibrations

Str: stretching, def: deformation

lengths responsible for the discrimination between spectra were highlighted using the step-up regression method (available on the WinISI software): these were wavelengths between 2130 nm and 2230 nm. Moreover, this region includes the band at 2170 nm responsible for the determination of protein content. It was selected for our study and will be named the "selected region".

Determination of protein

Among the 169 available samples (Table 1), 35 blank and contaminated feed samples (different values of added contaminant) were selected and analysed by both chemical and NIR spectroscopy methods. Samples contaminated by melamine, cyanuric acid or both were selected to cover a range of variation in concentration of additives and matrix type. Soybean meal, maize gluten and wheat gluten were adulterated by one or both of the melamine and/or cyanuric acid contaminants. The calibration equation developed using samples in the general feed ingredient database of feed compounds available at CRA-W (22,689 clean samples with known laboratory protein value). Models were calculated on the "full spectrum" between 1100 nm and 2498 nm and on the "selected region" between 2130 nm and 2230 nm. Measured

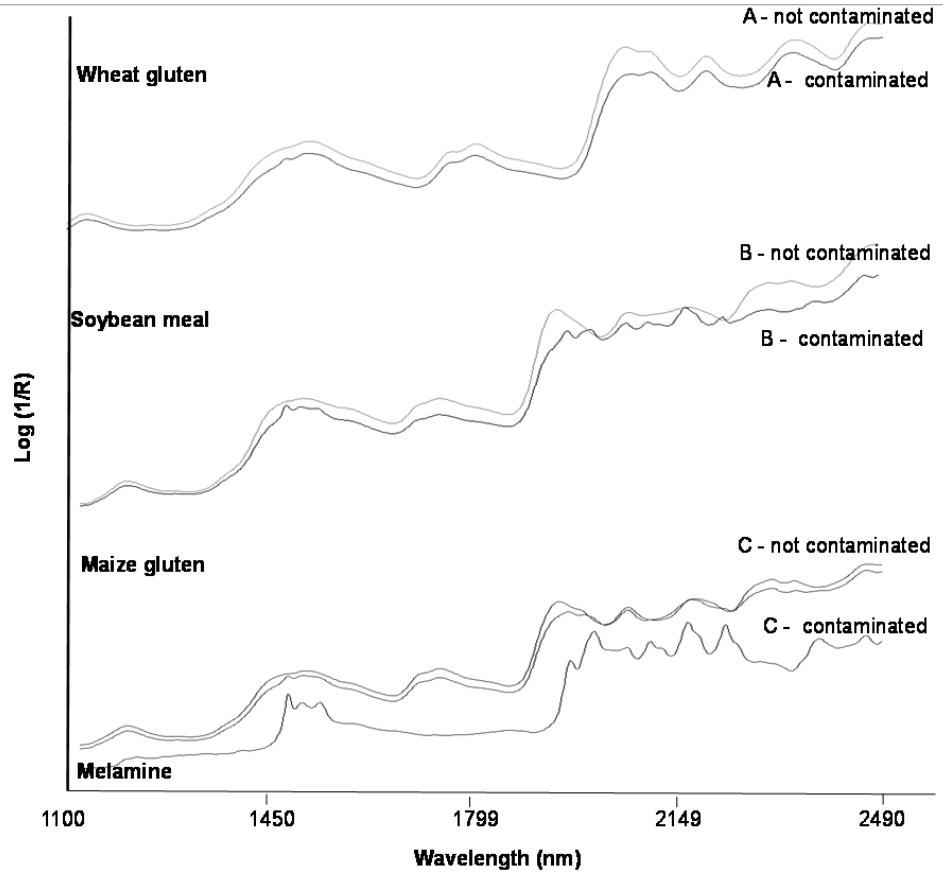

Figure 2. Effect of the contamination by melamine of feed samples spectra (A: 5.57%, B: 3.56%, C: 3.05% of added melamine).

reference values of crude protein obtained by chemical analysis were compared with predicted values produced by NIR spectroscopic calibrations. A plot describing the evolution of the protein values (laboratory values or predicted values) as a function of the percentage of melamine and/or cyanuric acid added was established (Figure 3).

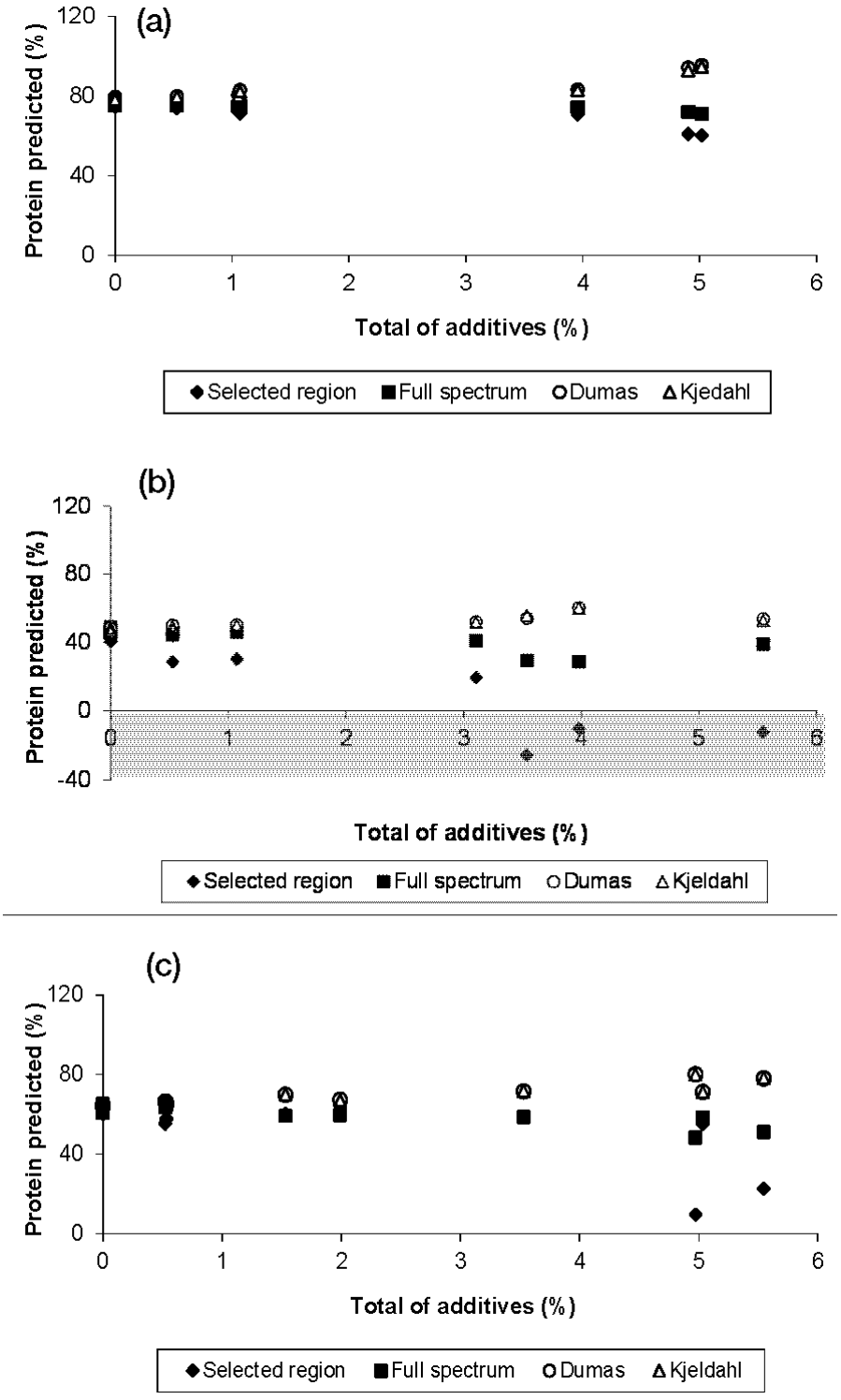

Figure 3. Comparison between predicted values of nitrogen obtained by spectroscopic analysis (selected region between 2130 nm and 2230 nm, full spectrum between 1100 nm and 2498 nm) and reference values obtained by chemical analyses [Dumas and Kjeldahl) of studied samples (a): wheat gluten, (b) soybean meal, (c) maize gluten].

The results in Figure 3 show that the Dumas and Kjeldahl values were very similar, demonstrating the precision of both wet chemistry methods. The means of difference between the Dumas values and the Kjeldahl values were 0.71 for soybean samples, 0.5 for wheat gluten samples and 0.17 for maize samples. It should also be noted that the predicted value of crude protein was inversely correlated to the reference values. When adding the contaminant, the value of protein obtained by classical Kjeldahl and Dumas methods increased, but it decreased when predicted by the calibration models based on NIR spectra. In some cases, values predicted were even negatives.

From graphs presented in Figure 3, the influence of adulteration on feed samples depends on their type. Values of difference between measured and predicted values of protein were not similar for the three types of feed compounds. The difference was more significant for soybean meal than for maize gluten which, in turn, showed a bigger gap than for wheat gluten. Moreover, this difference is also affected by the type and relative concentration of added melamine or cyanuric acid.

In order to better illustrate this phenomenon, the case of maize gluten will be presented in detail, as an example. The values of protein in those samples measured by the Kjeldahl reference method and the predicted values obtained by equations developed on the selected region and the full spectrum are presented on Table 3. If we consider as an example the case of the matrix coded GM02, the values resulting from chemical methods increased from about 64.51 to 79.91% when adulteration with melamine was increased from 0% to 4.97%, but there was a decrease in the values predicted either by the equation developed on the full spectrum (from about 65.18 to 48.35%) or by the one developed on the selected region (from 60.65% to 9.62%) when melamine varied from 0% to 4.97%. It should be noted that two samples, GM03 and GM04 (before contamination), exhibit slightly elevated GH values which may explain the difference observed in the predicted protein values (equation developed on full NIR spectrum) comparing to the other non-contaminated maize gluten samples.

The table also shows that the difference between the measured value and the predicted one increased markedly with an increase in contamination. The effect of contamination was very marked when an adulterant was added at a high percentage.

The Kjeldahl and Dumas methods gave high nitrogen values because the nitrogen from contaminants was measured as protein. In contrast, NIR spectroscopy clearly differentiates between nitrogen from protein and nitrogen from the other sources used in this study. Prediction of chemical values or properties was based on the correlation between specific bands of the spectrum and reference chemical values. The absorption at 2170 nm is usually used to determine protein content.[17] The intensity of this band did not increase with melamine contamination nor did the predicted value of protein content. Rather, in some cases there were large decreases. The spectral profile of feed ingredient samples was modified by the presence of melamine and/or cyanuric acid. This may explain why the crude protein value dropped. This reduction was more evident when working on the selected region of the NIR spectrum (2130 nm–2230 nm).

Table 3. Values of protein measured by chemical methods and predicted by spectroscopic techniques for maize gluten samples.

Sample Identification	Melamine Added (%)	Cyanuric acid added (%)	Total of Additives (%)	Kjedahl Method (%)	Equation on Full NIR spectrum (%)	Equation on Selected NIR region (%)
GM01	0.00	0.00	0.00	65.02	65.18	60.16
GM01-C	0.54	0.00	0.54	65.40	64.94	57.63
GM02	0.00	0.00	0.00	64.51	65.18	60.65
GM02-M	0.52	0.00	0.52	66.14	63.32	55.25
GM02-M	4.97	0.00	4.97	79.91	48.35	9.62
GM02-X	0.13	0.39	0.53	65.46	63.96	57.75
GM03*	0.00	0.00	0.00	64.49	60.85	64.84
GM03-C	0.00	1.99	1.99	67.07	59.53	59.43
GM03-X	0.94	2.59	3.53	71.66	58.65	58.26
GM04*	0.00	0.00	0.00	64.16	60.61	64.15
GM04-C	0.00	5.04	5.04	71.09	58.43	54.93
GM04-M	1.53	0.00	1.53	69.93	59.17	60.13
GM05	0.00	0.00	0.00	64.02	63.20	60.04
GM05-X	4.10	1.44	5.55	78.25	51.11	22.48

M: Melamine added, C: Cyanuric acid added, X: Mixture of melamine and cyanuric acid added,
*Samples having slightly elevated *GH* values; NIR: near infrared

It seems that melamine has more effect on the feed matrix than cyanuric acid. In fact, the comparison of reference and predicted values obtained when mixtures of cyanuric acid and melamine were added to GM03 and GM05 showed that the increase in the reference value or the decrease in the predicted value was more marked when the mixture added was mainly composed of melamine (the case of GM05-X).

Detection of outliers

The NIR spectra of contaminated samples were visibly different from those not contaminated. In order to investigate this distinction, mathematical models have been developed to statistically compare the NIR spectra of contaminated compounds to those of the database. Two methods were attempted; first, a calibration equation was developed on the basis of "the general feed ingredient database" (the same as ones used for the prediction of protein), then second models based on the different "specific feed ingredient databases" (soybean meal, maize gluten and wheat gluten) (Figure 4).

MPLS calibrations were based on the spectra of the general feed ingredient database available at CRA-W. Both the 1100–2498 nm "full spectrum" and the 2130–2230 nm "selected region" were studied. The objective was to obtain an equation that could be applied to the samples of soybean meal, maize gluten and wheat gluten whether contaminated or not.

The application of those models showed that the equation developed using the 2130–2230 nm selected region allowed the contaminated samples to be better distinguished by giving higher *GH* values compared with those obtained by using the 1100–2498 nm full spectrum. However, because the objective was to determine whether it was possible to discriminate or detect contaminated samples on the basis of their spectra without *a priori* knowledge, we chose to present the work done on the full spectrum.

Table 4. Calibration performance of developed equations on the basis of the general feed ingredient database and the specifics ones (soybean meal, maize gluten and wheat gluten). Full NIR spectra were used.

	SECV	1-*VR*
General feed ingredient	1.13	0.99
Soybean meal	0.72	0.86
Maize gluten	0.74	0.93
Wheat gluten	0.67	0.90

MPLS models developed on the full NIR spectra were selected on the basis of statistical parameters in order to precisely predict the *H* distance (*GH*). The resulting values for *SECV* and the coefficient of determination in cross-validation (1-*VR*) are listed in Table 4.

The use of a large database for calibration is advantageous because it increases the robustness of the calibration, but it decreases the accuracy of prediction (*SECV* = 1.13) compared to that for the specific feed ingredient databases (soybean meal, maize gluten or wheat gluten), where *SECV* was equal to 0.72, 0.74 and 0.67 for soybean meal, maize gluten and wheat gluten, respectively. The general database includes a great variety of samples which may influence the performance of the model for one class or one group of ingredients. Specific equations were developed on only one database of the appropriate samples leading to more accurate results.

Detection of outliers on the basis of the general feed ingredient database

One example is presented; that of soybean meal. Histograms of *GH* values as a function of the percentage of (a) melamine, (b) cyanuric acid and (c) a mixture of additives added, respec-

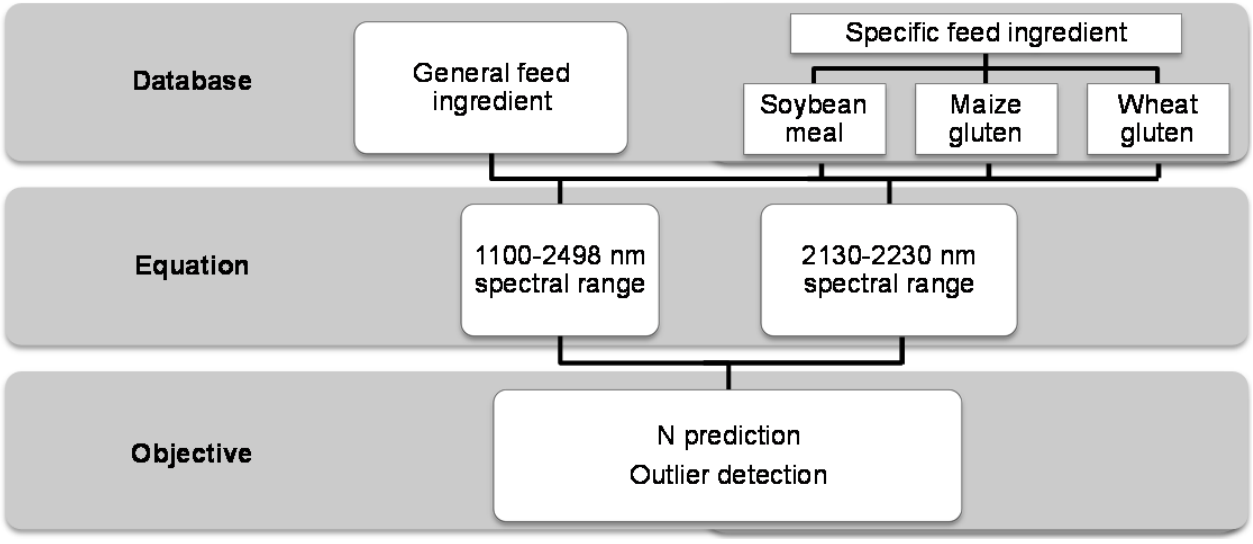

Figure 4. Scheme of the procedure used to calculate global *H* (*GH*) and to predict N.

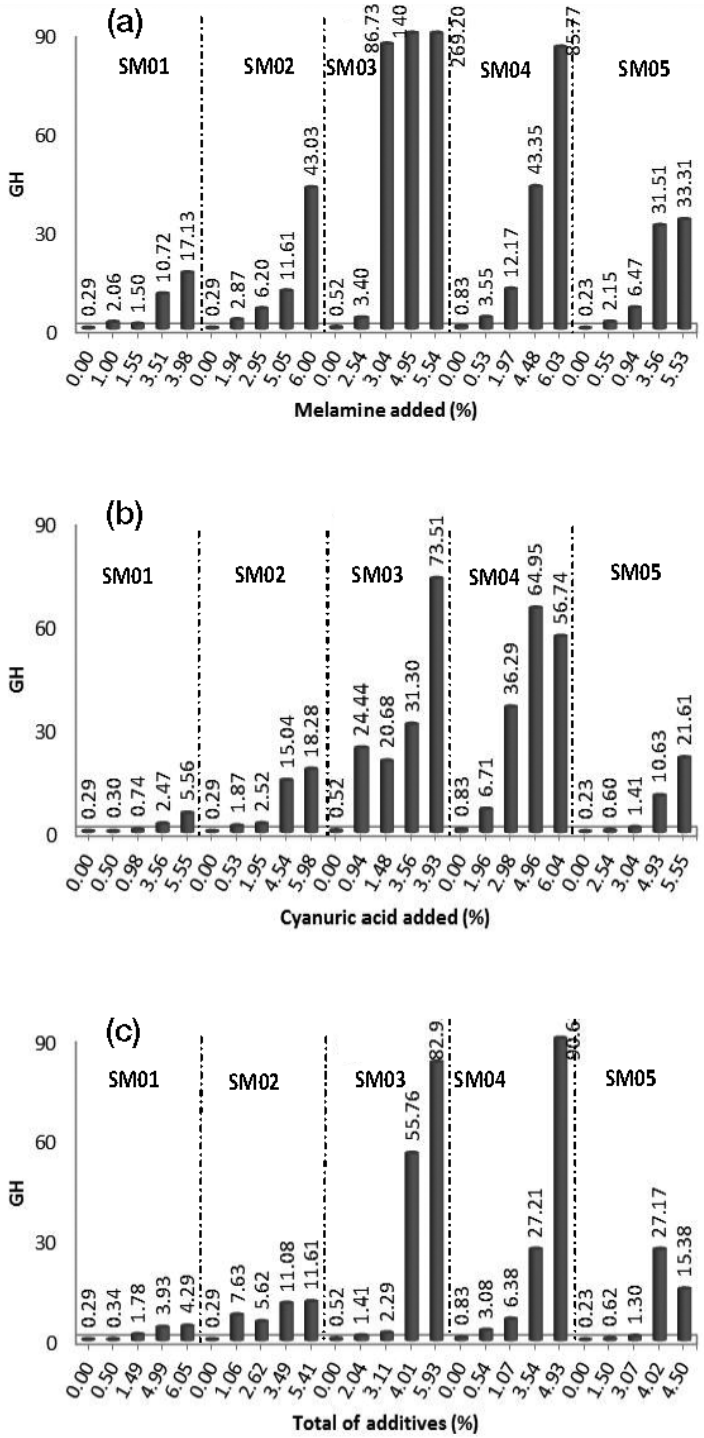

Figure 5. Evolution of *GH* in function of the percentage of adulteration of soybean meal when using the general feed ingredient equation [(a): melamine, (b): cyanuric acid, (c): total of additives].

tively are shown in Figure 5. Each part of the histogram corresponds to one sample adulterated at various percentages.

Figure 5. Soybean meal samples showed *GH* values varying between 0.23 and 0.83 in the absence of any contaminants. When melamine was added to matrix SM04 at between 0.53% and 6.03%, the *GH* values increased and varied between 3.55 and 85.77 [Figure 5(a)]. The addition of cyanuric acid at percentage less than 3.5% did not influence the *GH* values of SM01, SM02, SM05 matrixes. At this level of contamination, cyanuric acid had less influence than melamine [Figure 5(b)]. Both melamine and cyanuric acid were then added to soybean meal samples in different percentages [Figure 5(c)] leading to

GH values 7.63 and 11.61 (SM02) for contaminations between 1.06% and 5.41%. The composition of samples (SM03 and SM05) was not affected when the contaminant was added at a level of around 3%; mixtures added were mainly composed of cyanuric acid. In addition, the comparison of Graphs a, b and c of Figure 5 shows that, in most cases, the *GH* values corresponding to the addition of melamine were higher than those due to the addition of cyanuric acid.

Detection of outliers on the basis of the specific soybean meal database

The soybean meal database is composed of 8630 samples for which the reference values of protein were already measured by wet chemistry.

Figure 6 shows that: *GH* values for uncontaminated samples were around 3 or less, but were higher when melamine, cyanuric acid or both were added.

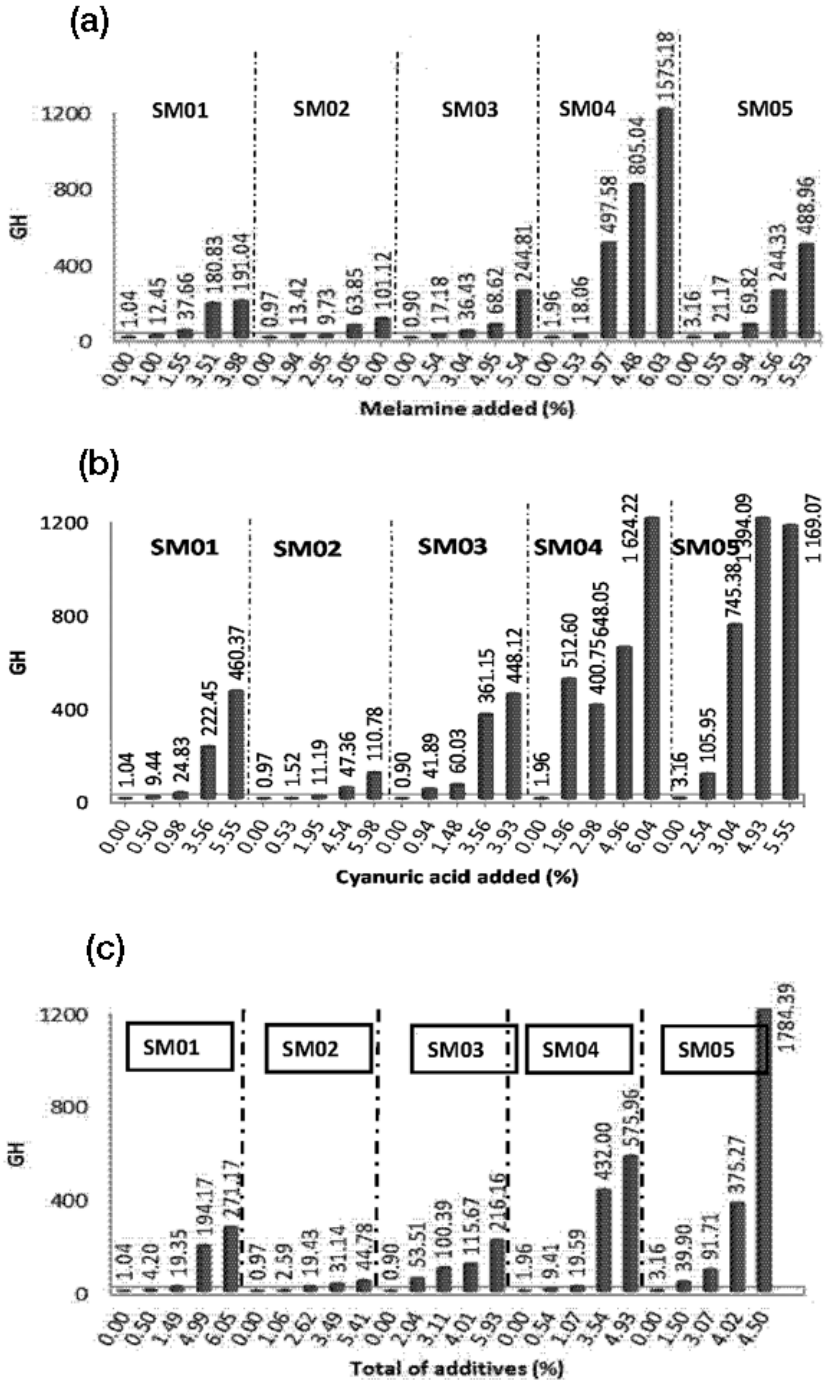

Figure 6. Evolution of *GH* in function of the percentage of adulteration of soybean meal when using the soybean specific feed ingredient equation [(a): melamine, (b): cyanuric acid, (c) total of additives).

The addition of melamine to soybean meal in percentages varying between 0.53 and 6.03 caused an increase in GH to between 18.06 and 1575.18 (SM04). The addition of cyanuric acid in percentages from 0.94 to 3.93 (SM03) lead to *GH* values of between 41.89 and 448.12. The contamination by the mixture of the additives in percentages varying between 0.50 and 6.05 (SM01) resulted in an increase of *GH* values from 4.20 to 271.17.

From graphs 6(a), 6(b) and 6(c), it can be noticed that the influence of one or several contaminants is closely dependent on the type of soybean meal matrix. This was also observed when applying the general feed ingredient database, but *GH* values were greater when the specific soybean meal database was used. In the case of the example of a contamination by melamine (0%–5%), *GH* values ranged from 0 to 269.20 when the general database was used and from 0 to 1575.18 when the soybean meal database was used. This can be explained by the fact that, when using the equation built using exclusively spectra from the soybean meal database, higher *GH* are observed than those obtained using the equation built on spectra from the feed ingredient database. This is quite logical and it can be easily explained by the fact that the variability included in the feed ingredient database is larger due to the database composition. This database contains not only soybean meal samples, but also other kinds of ingredient that can, eventually, mask some of the effects of the contaminant.

Conclusion

The results of this study illustrate the ability of NIR spectroscopy to detect contamination by melamine and its derivatives like cyanuric acid in feed samples such as soybean meal, maize gluten and wheat gluten. The calibration equations developed enabled the prediction of the protein content that decreased instead of increasing, as shown by chemical analysis. In addition, the use of a large robustly gathered feed database allowed identification of the adulterated samples with *GH* values higher than 3.

The use of a specific feed ingredient database was found to accentuate the detection of anomalies existing in one sample type.

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