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NIR fingerprint screening for early control of non-conformity at feed mills

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ABSTRACT

The objective of this work was to devise a complete procedure based on chemometrics and the use NIR spectroscopy at the entrance of a feed mill to provide early evidence of non-conformity and unusual ingredients and thus help to achieve cost-savings. The procedure was validated at laboratory level and was adapted for application at the Cargill Animal Nutrition feed mill. The study focused on the characterisation of pure soybean meal with the aim of creating an early control system for detecting and quantifying any unusual ingredient that might be present in the soybean meal, such as melamine, cyanuric acid or whey powder (milk serum). The study results showed that the use of NIR, combined with some simple chemometric tools based on distances and residuals from regression equations, is appropriate for authenticating important feed products (in this case, soybean meal) and detecting the presence of abnormal samples or impurities in both the laboratory and at the feed mill.

1. Introduction

In recent years, food and feed safety has become an increasing concern for consumers as a result of major crises related directly or indirectly to human health. In March 2007, a pet food recall was initiated in North America by several pet food manufacturers when a number of cats and dogs sickened and died after eating contaminated pet food (http://www.petconnection.com/2007/10/16/recall-insurance/). The US Food and Drug Administration reported finding melamine in the pet food and in samples of wheat gluten imported from China. In November 2008, there was a major food safety incident in China; it involved milk and infant formula adulterated with melamine and affected more than 300,000 people, with six infants dying from kidney stones and other kidney damage (Branigan, 2008). These crises illustrated the need for a sensitive, reliable and rapid procedure for detecting melamine in both food and feed (Chan, Griffiths, & Chan, 2008; Chen, 2009; Dobson et al., 2008; Gossner et al., 2009; Tyan, Yang, Jong, Wang, & Shiea, 2009).

In the feed sector, one of the most important products is soybean meal, with 90% of the soybean seeds produced globally being used as animal feed, which corresponds to an amount exceeding 205 million tons (http://faostat3.fao.org/faostat-gateway/go/to/search/soybeans/E). Soybean seeds are subjected to various types of processing (Berk, 1992, chap. 5) to produce a range of oil-based by-products (cakes, expellers, oilseed meal) used for animal nutrition (Banaszkiewicz, 2011). The soybean meal used in feed is the material remaining after the solvent extraction of oil from soybean flakes; it consists of more than 36% protein and 30% carbohydrate, and is an important source of dietary fibre, vitamins and minerals. Soybean meal also consists of 20% oil, which makes it the most important crop in terms of edible oil production. A by-product from the oil production (soybean cake) is used as high-protein animal feed in many countries. Soy protein products are often used as substitutes for animal products because they have a complete protein profile. They can replace animal-based foods which also have a complete protein profile, but tend to contain more fat, especially saturated fat, without requiring major adjustments elsewhere in the diet (Henkel, 2000). Routinely, feed purchasers measure the nitrogen content of feed products to determine their protein content, normally using the Kjeldahl method, which is based on the decomposition (digestion) of nitrogen in organic samples utilising a concentrated acid solution, followed by a distillation and titration (Jones, 1991). Research has shown that some suppliers try to make it appear that their

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products contain more protein than they actually do by adding melamine, which is an inexpensive and nitrogen-rich compound, but it has no nutritional value and can be quite toxic if animals are constantly exposed to it (Newton & Utley, 1978). That happened in 2008 when almost 300 metric tons of soybean meal destined for organic chickens in western France were withdrawn from the market after the authorities discovered melamine levels 50 times higher than the permitted standard (Adams, 2008). On its own, melamine does not exhibit systemic toxicity, but it is able to mix with other substances such as endogenous uric acid or substances related to melamine to form crystals in the urine, causing kidney damage. Health officials are currently investigating how this chemical, normally used to make industrial glues, fire retardant and fertilizers amongst others got into the food/feed chain (Bann & Miller, 1958; World Health Organization, 2008). Most available procedures, however, deal with the detection of melamine in food and are expensive and time-consuming techniques that need extensive sample preparation (Liu, Todd, Zhang, Shi, & Liu, 2012). The toxicity of melamine can affect directly or indirectly many feed, pet food and food sources, such as cows' milk, fish, pork and cattle. This is forcing the industry to find suitable melamine screening methods. At the request of the European Commission, the European Food Safety Authority (EFSA) provided a scientific opinion on the presence of melamine and the structural analogues in food and feed, including potential melamine sources and associated dietary exposure (EFSA, 2010). The ideal procedure would be one that could ensure the early detection of melamine (i.e., detection before reaching the food/feed chain). Much research has been done on developing accurate and sensitive analytic techniques for assessing feed product quality and safety. Near infrared (NIR) spectroscopy is now widely used as a successful quality control tool in the feed industry and animal nutrition, mainly for the simultaneous determination of crude protein, fat and fibre in animal feed (Barton & Windham, 1988; Murray, 1986, 1993; Norris, Barnes, Moore, & Shenk, 1976; Shenk & Westerhaus, 1995, chap. 10). Cozzolino et al. have shown that NIR spectroscopic methods can be easily implemented directly in the feed mill and could be very useful for initial screening at the early stages in the production chain (Cozzolino, Restaino, La Manna, Fernandez, & Fassio, 2009). Recently, NIR spectroscopy has been used to detect melamine adulteration of soybean meal through the development of multivariate calibration models (Abbas, Lecler, Dardenne, & Baeten, 2013; Haughey, Graham, Cancouet, & Elliott, 2012).

The aim of this work was to devise a procedure based on using NIR spectroscopy and chemometrics to characterise soybean meal and to detect the presence of unusual ingredients (Baeten, Vermeulen, Fernández Pierna, & Dardenne, 2014). This required using statistical tools to interpret the multivariate data obtained from the chemical analyses of soybean meal samples. The data were considered to be fingerprints of the products and the results of the chemometric treatment were used to set specifications (Martens & Naes, 1989). These chemometric and multivariate statistical tools provided pattern recognition techniques that allowed adequate differentiation to be made between authentic and non-authentic soybean meal, as well as regression methods, evaluated according to their ability to handle the available dataset and to predict the status of new samples. Knowledge about soybean meal, combined with pattern recognition techniques and regression models, should lead to the compilation of a set of specifications against which compliance can be checked and decisions made on acceptance or rejection of the meal.

The complete procedure was validated at laboratory level on soybean meal samples contaminated with melamine and whey powder (milk serum). It was then adapted and used in a feed mill where two tests were performed at the reception of the raw material in order to detect anomalies arising from the addition of unusual ingredients or unauthorised additives.

2. Methodology

Three criteria were used to characterise the soybean meal and detect the presence of unusual ingredients: the global H (GH) criterion; and two regression equations evaluated using a spectral residuals ratio.

2.1. GH criteria

The GH criterion is a modification of the Mahalanobis distance (H) of each sample from the average spectrum in which H^2 is divided by the number of dimensions f used to derive H (Guthrie, 2005). This provides information about the distances between each sample and the average calibration sample in the principal components or latent variables space:

$$\mathrm{GH} = \frac{H^2}{f} = (S_i - \bar{S})\left(\frac{(S_i - \bar{S})'(S_i - \bar{S})}{n-1}\right)(S_i - \bar{S})' \quad (1)$$

where S is the $n \times f$ matrix of training samples scores with n the number of samples and f the number of terms.

As recommended by Shenk and Westerhaus, a GH value lower than 3 will guarantee homogeneous spectra, but values greater than 3 will guarantee that the spectra are a result of random chance and hence likely to have outliers (Shenk & Westerhaus, 1991).

2.2. Regression models

In order to characterise the soybean meal, PLS regression models for each property (protein and fat) were determined based on historical (Cargill Animal Nutrition) dataset and then used to predict and characterise new (unknown) samples (Brown, Tauler, & Walczak, 2009; Massart et al., 1988). In all cases, the spectral data were pre-processed using the Standard Normal Variate transform followed by the detrend (Barnes, Dhanoa, & Lister, 1989) and 1st derivative Savitzky–Golay treatment (2nd degree polynomial and a window of 9 points or wavelengths) to remove the scattering effects and smooth the spectra (Gorry, 1990). The optimal equation for each dataset was calculated using Leave-One-Out Cross Validation (LOOCV) over the complete calibration set (Glantz & Slinker, 1990). For the protein content the PLS equation was used in a dataset of 8610 samples (mean of 46.62 and std of 1.93) with a RMSECV of 0.68 and r2 of 0.88. For the fat content the PLS equation was used in a dataset containing 5026 samples (mean of 1.88 and std of 1.59) with a RMSECV of 0.25 and r2 of 0.98.

Once the regression models were constructed, they could be used to predict both properties protein and fat and to extract the respective spectral residuals for unknown samples. When each sample is predicted, a set of scores is found that best fits the model loading vectors to the unknown sample spectrum. By using the calculated scores and the calibration loading vectors, a new model reconstructed spectrum can be calculated (Puryear, 2006). The spectral residual, e, is the difference between the original spectrum, X_{orig}, and this prediction spectrum, X_{pred}, and calculated as follows:

$$e = \sum_{k=1}^{m} (X_{\mathrm{orig}_k} - X_{\mathrm{pred}_k})^2$$

with m being the number of wavelengths in the spectrum.

The spectral F ratio for an unknown sample as defined by Haaland and Thomas has been calculated as follows (Haaland & Thomas, 1988):

$$F_{\mathrm{unk}} = n \frac{\left(\sum_{k=1}^{m} e_{\mathrm{unk},k}^2\right)}{\left(\sum_{i=1}^{m}\sum_{k=1}^{m} e_{\mathrm{cal},k}^2\right)}$$

where n is the number of calibration samples, m the number of wavelengths and e_{cal} and e_{unk} the spectral residuals for the calibration set and unknown sample respectively.

This equation is usually used for detecting samples which are not in compliance with the calibration set in prediction, commonly called prediction outliers (Fernández Pierna, Wahl, de Noord, & Massart, 2002). As the aim of this work is to characterise soybean meal and to detect the presence of unusual ingredients, here the technique is used, not based on prediction values, but to check whether the spectral residuals of the unknown samples fell within the limits defined by the spectral residuals of the calibration set obtained using different PLS models. The fact of using PLS models, built with signal and reference values included in the calibration set has a clear advantage compared to the GH criterion which is only based on signal data. Where the residuals were not in compliance with the calibration set, the samples were considered as suspect and were either rejected or submitted to further investigation. The limits have been defined by the values of the 99.7th percentile calculated in pure soybean meal samples.

3. Materials and methods

This study was conducted in a laboratory and in a feed mill. In both cases, the NIR spectrometer used was an XDS Rapid Content™ Analyzer from FOSS. This instrument is active in the 400–2500 nm VIS-NIR scanning range, with sensitive outlier detection. Its principal advantages are the rapid and non-destructive analysis of samples for routine at line process control and research laboratory application, ease-of-use, rapid answers and flexible sample presentation.

At laboratory level, 65 samples of soybean meal were available. The samples were both contaminated and uncontaminated; for the contaminated samples, a percentage of melamine, cyanuric acid or both was added to pure soybean meal samples (mixtures of various quantities of melamine and cyanuric acid, as indicated in columns 2 and 3 in Table 1).

At feed mill level, a complete strategy and sampling is detailed in Section 4.2 of this paper. Due to security reason at the feed mill plant, melamine contamination simulation could not be performed. However, two tests were carried out where trucks of 25 metric tons of soybean meal were contaminated with whey powder at the feed mill (Cargill Animal Nutrition) entrance. Dehydrated whey (milk serum) is the powder remaining after milk has been curdled and strained. It has been selected as having similar particle size and density as melamine, and being at the same time a non-dangerous important nitrogen source, mainly used in the formulation of baby foods and enteral nutrition (Gonzalez-Tello, Camacho, Jurado, Paez, & Guadex, 1994).

In addition, a series of artificial mixtures were prepared in the laboratory to got indication of the limits of detection by adding different percentages (0.5%, 1%, 2%, 4% and 5%) of whey powder (milk serum) to the soybean meal samples.

Computations, chemometric analyses and graphics were performed using programs developed in Matlab R2014 (The Mathworks Inc., Natick, MA, USA) and the PLS Toolbox (Eigenvector Research, Inc., Wenatchee, USA).

4. Results

4.1. Study in the laboratory

Fig. 1 shows typical spectra (raw and second derivative) of soybean meal, melamine and dehydrated whey powder respectively.

The NIR spectra of soybean meal and dehydrated whey powder are different but they have some common patterns. In all cases, absorption bands in the NIR region were observed around 1215 nm (C–H str. 2nd overtone (CH2)), 1490 nm (O–H str. 1st overtone – intramol. H-bond-related to cellulose), 1720 nm (C–H first overtone, associated with lipids) and between 1900 nm (C═O str. 2nd overtone (–CO2H)), 2058 nm N–H sym. Str. + amide II related to protein) and 2300 nm (C–H combination tones, associated with amino and fatty acids). Bands in the vicinity of 2058 nm and 2174 nm were related to fat and to the peptide absorption of the amide group, and appeared for the soybean meal, but not for the whey powder. Melamine presents very characteristics bands The peak at 1018 nm is associated with N–H symmetric stretching vibration of primary amines (NH_2) second overtone, whilst peaks pointed at 1466 nm and 1520 nm can be specified as N–H asymmetric and symmetric stretching vibration respectively of primary amines (NH_2) first overtone; and 1958 nm is the combination band of N–H stretching and bending in aromatic amines. 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. Bands at 2160 nm and 2226 nm can be attributed to 1,3,5-triazine structural vibrations (Osborne & Fearn, 1986).

4.1.1. Melamine contamination

The procedure created using GH and the spectral F ratio based on regression models was applied to the samples in the laboratory in order to validate it (Abbas et al., 2013). Table 1 shows the results of the 65 contaminated and uncontaminated soybean meal samples. The first columns indicate the sample number and the percentage of melamine and/or cyanuric acid included. One sample every 13 is a blank sample, i.e. pure soybean meal without contamination. For each criterion, the predicted value (GH and F ratio) is indicated and a conclusion is drawn based on those values whether they are below or above the limit defined by the 99.7th percentile calculated in pure soybean meal samples for the protein (8.42) and fat (8.54) criteria and by a GH larger than 3 (C: contaminated; NC: not contaminated). The last column indicates whether the conclusion is correct (✓) or not (X) compared to the true answer. Similar results are obtained in all criteria. With both spectral F ratio criteria, all the pure soybean samples were correctly detected as such, i.e., there were no false positive results (i.e. a result that indicates that an unusual ingredient is present when it is not) .and only three contaminated samples were not detected (false negative results, i.e. results that appears negative when it should not), all of them being samples with a low quantity of cyanuric acid – between 0.13% and 0.53% –, and in all the cases with results close to the limits defined by the pure soybean meal samples, giving an indication of the limit of detection of the techniques. The GH criterion had a false positive result by considering the blank sample 40 as contaminated, however with a value of 3.16 near the limit.

4.1.2. Whey powder contamination

The procedure was applied to synthetic samples where soybean meal had been contaminated with 0.5%, 1%, 2%, 4% and 5% of dehydrated whey powder, in addition to one pure soybean sample and one whey powder sample. Table 2 shows the results when applying the procedure. Both the spectral F ratio using the fat equation and the GH criterion allowed a sensitivity specificity of 100% (i.e., all the samples, pure and mixed, were correctly detected, indicating that both criteria can detect mixtures contaminated with 0.5% of whey powder). The spectral F ratio based on protein failed in detecting the pure soybean meal but with a value close to the limit defined by the pure soybean meal samples.

4.2. Study in the feed mill

Once the procedure had been validated in the laboratory, tests were carried out at a feed mill. Two tests were developed focusing

Table 1
Predicted values (spectral *F* ratios and GH) for the 65 contaminated and non-contaminated soybean meal samples.

Sample	Melamine (%)	Cyan acid (%)	Total (%)	*F* ratio protein	Conclusion prot (*C* if >8.42)		*F* ratio fat	Conclusion fat (*C* if >8.54)		GH	Conclusion GH (*C* if >3)	
1	0	0	0	1.00	NC	✓	0.87	NC	✓	0.97	NC	✓
2	1.94	0	1.94	60.46	C	✓	48.30	C	✓	13.42	C	✓
3	2.95	0	2.95	42.48	C	✓	34.15	C	✓	9.73	C	✓
4	5.05	0	5.05	313.00	C	✓	248.59	C	✓	63.85	C	✓
5	6	0	6	503.22	C	✓	399.55	C	✓	101.12	C	✓
6	0	0.53	0.53	1.67	NC	X	1.37	NC	X	1.52	NC	X
7	0	1.95	1.95	13.15	C	✓	10.14	C	✓	11.19	C	✓
8	0	4.54	4.54	57.48	C	✓	44.02	C	✓	47.36	C	✓
9	0	5.98	5.98	133.12	C	✓	102.18	C	✓	110.78	C	✓
10	0.53	0.53	1.06	5.52	NC	X	4.69	NC	X	2.59	NC	X
11	1.31	1.31	2.62	54.22	C	✓	43.83	C	✓	19.43	C	✓
12	2.58	0.91	3.49	120.89	C	✓	96.95	C	✓	31.14	C	✓
13	2.7	2.7	5.4	130.09	C	✓	104.80	C	✓	44.78	C	✓
14	0	0	0	0.83	NC	✓	0.69	NC	✓	0.90	NC	✓
15	2.54	0	2.54	81.56	C	✓	65.18	C	✓	17.18	C	✓
16	3.04	0	3.04	178.70	C	✓	142.36	C	✓	36.43	C	✓
17	4.95	0	4.95	338.65	C	✓	269.22	C	✓	68.62	C	✓
18	5.54	0	5.54	1142.28	C	✓	899.77	C	✓	244.81	C	✓
19	0	0.94	0.94	47.21	C	✓	36.70	C	✓	41.89	C	✓
20	0	1.48	1.48	66.44	C	✓	51.62	C	✓	60.03	C	✓
21	0	3.56	3.56	398.36	C	✓	310.03	C	✓	361.15	C	✓
22	0	3.93	3.93	489.03	C	✓	380.30	C	✓	448.12	C	✓
23	1.49	0.55	2.04	213.42	C	✓	169.49	C	✓	53.51	C	✓
24	0.82	2.28	3.1	169.95	C	✓	135.65	C	✓	100.39	C	✓
25	2	2.01	4.01	322.73	C	✓	257.55	C	✓	115.67	C	✓
26	1.48	4.46	5.94	339.15	C	✓	270.13	C	✓	216.16	C	✓
27	0	0	0	1.60	NC	✓	1.47	NC	✓	1.96	NC	✓
28	0.53	0	0.53	68.65	C	✓	55.45	C	✓	18.06	C	✓
29	1.97	0	1.97	2251.73	C	✓	1773.88	C	✓	497.58	C	✓
30	4.48	0	4.48	3618.86	C	✓	2853.95	C	✓	805.04	C	✓
31	6.03	0	6.03	7068.65	C	✓	5555.57	C	✓	1575.18	C	✓
32	0	1.96	1.96	583.72	C	✓	457.02	C	✓	512.59	C	✓
33	0	2.98	2.98	466.13	C	✓	368.11	C	✓	400.75	C	✓
34	0	4.96	4.96	742.44	C	✓	583.42	C	✓	648.05	C	✓
35	0	6.04	6.04	1793.01	C	✓	1404.16	C	✓	1624.22	C	✓
36	0.4	0.14	0.54	18.80	C	✓	15.88	C	✓	9.41	C	✓
37	0.55	0.53	1.08	37.27	C	✓	31.26	C	✓	19.59	C	✓
38	2.61	0.93	3.54	1481.06	C	✓	1180.04	C	✓	432.00	C	✓
39	3.73	1.2	4.93	2226.34	C	✓	1769.05	C	✓	575.96	C	✓
40	0	0	0	2.24	NC	✓	2.29	NC	✓	3.16	C	X
41	0.55	0	0.55	81.51	C	✓	64.57	C	✓	21.16	C	✓
42	0.94	0	0.94	300.01	C	✓	237.63	C	✓	69.82	C	✓
43	3.56	0	3.56	1112.48	C	✓	881.08	C	✓	244.33	C	✓
44	5.53	0	5.53	2207.28	C	✓	1745.78	C	✓	488.96	C	✓
45	0	2.54	2.54	120.56	C	✓	96.57	C	✓	105.95	C	✓
46	0	3.04	3.04	863.00	C	✓	677.97	C	✓	745.38	C	✓
47	0	4.93	4.93	1592.99	C	✓	1246.44	C	✓	1394.09	C	✓
48	0	5.55	5.55	1337.01	C	✓	1051.49	C	✓	1169.07	C	✓
49	0.41	1.09	1.5	62.33	C	✓	50.69	C	✓	39.90	C	✓
50	0.82	2.26	3.08	136.28	C	✓	110.88	C	✓	91.71	C	✓
51	2	2.01	4.01	707.29	C	✓	570.81	C	✓	375.27	C	✓
52	1.08	3.42	4.5	2415.73	C	✓	1921.24	C	✓	1784.39	C	✓
53	0	0	0	0.99	NC	✓	0.75	NC	✓	1.04	NC	✓
54	1	0	1	56.06	C	✓	44.62	C	✓	12.45	C	✓
55	1.55	0	1.55	175.90	C	✓	139.70	C	✓	37.66	C	✓
56	3.51	0	3.51	852.98	C	✓	674.66	C	✓	180.83	C	✓
57	3.98	0	3.98	917.80	C	✓	727.20	C	✓	191.04	C	✓
58	0	0.5	0.5	11.07	C	✓	8.49	NC	X	9.44	C	✓
59	0	0.98	0.98	29.42	C	✓	22.76	C	✓	24.83	C	✓
60	0	3.56	3.56	262.68	C	✓	203.71	C	✓	222.45	C	✓
61	0	5.55	5.55	543.70	C	✓	421.85	C	✓	460.37	C	✓
62	0.37	0.13	0.5	13.76	C	✓	11.15	C	✓	4.20	C	✓
63	0.37	1.11	1.48	30.22	C	✓	24.18	C	✓	19.35	C	✓
64	3.72	1.27	4.99	772.89	C	✓	616.19	C	✓	194.17	C	✓
65	1.53	4.53	6.06	426.35	C	✓	339.97	C	✓	271.17	C	✓

C: contaminated.
NC: not contaminated.

on the actual contamination of 25 metric tons of soybean meal with whey powder at the feed mill (Cargill Animal Nutrition) entrance. The soybean meal arrived at the mill by truck and was unloaded into an empty concrete pit at the mill entrance. The pit was equipped with a redler conveyor (horizontal transporter), which allows continuous movement at the bottom of the pit. The

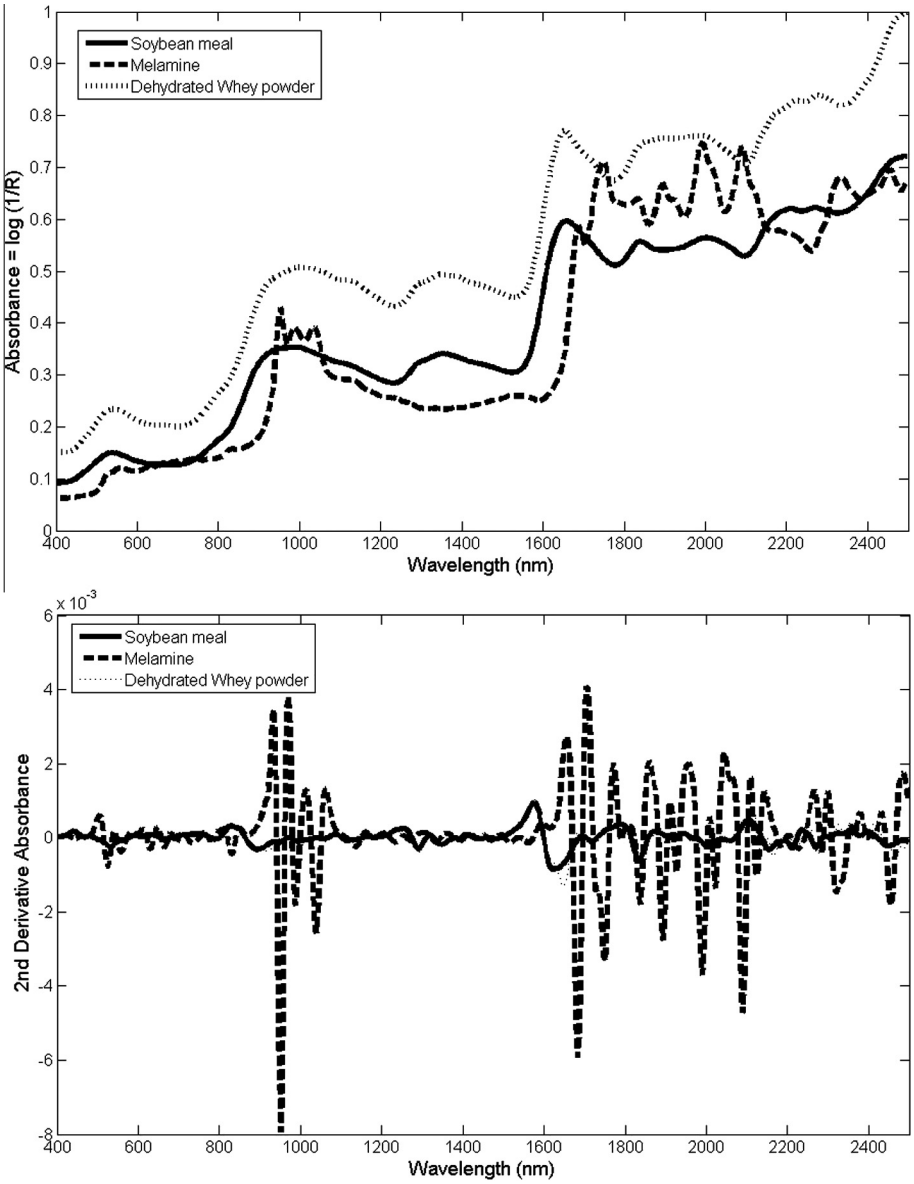

Fig. 1. Typical NIR spectra of soybean meal, melamine and whey powder; a) raw data b) second derivative data.

Table 2
Predicted values (spectral *F* ratios and GH) for the soybean samples contaminated with dehydrated whey powder at different concentrations (0.5%, 1%, 2%, 4% and 5%) using PLS regression models.

Sample	Whey powder (%)	*F* ratio protein	Conclusion prot (*C* if >8.42)		*F* ratio fat	Conclusion fat (*C* if >8.54)		GH	Conclusion GH (*C* if >3)	
1	0	8.61	C	X	7.57	NC	✓	1.29	NC	✓
2	0.5	19.91	C	✓	16.21	C	✓	3.50	C	✓
3	1	34.41	C	✓	27.15	C	✓	5.83	C	✓
4	2	144.41	C	✓	109.25	C	✓	21.20	C	✓
5	4	233.96	C	✓	177.74	C	✓	33.01	C	✓
6	5	299.43	C	✓	226.59	C	✓	41.08	C	✓
7	100	2007.49	C	✓	1481.39	C	✓	224.81	C	✓

C: contaminated.
NC: not contaminated.

redler conveyor ensures that products unloaded into the pit move in the same direction and allows the pit to be emptied quickly. An automatic sampling device was placed at the end of the redler conveyor in order to extract enough samples for analysis. With this system, which uses air compressed for the movement of the internal drawer, samples of 1 kg can be captured at one go and in less than a second. The only limiting factor in obtaining a high number of samples is manpower.

Table 3
Predicted values (spectral *F* ratios and GH) for test 1.

Sample	Sample id	*F* ratio protein	Conclusion prot (*C* if >8.42)	*F* ratio fat	Conclusion fat (*C* if >8.54)	GH	Conclusion GH (*C* if >3)
1	1/A – SOYBEAN MEAL	36.19	C	31.61	C	27.61	C
2	1/A – SOYBEAN MEAL	10.98	C	8.17	NC	2.97	C
3	1/A – SOYBEAN MEAL	9.09	C	6.97	NC	1.68	NC
4	1/A – SOYBEAN MEAL	8.18	NC	6.41	NC	1.45	NC
5	1/A – SOYBEAN MEAL	8.08	NC	6.43	NC	1.41	NC
6	1/A – SOYBEAN MEAL	8.14	NC	6.35	NC	1.34	NC
7	1/A – SOYBEAN MEAL	7.64	NC	5.99	NC	1.32	NC
8	1/A – SOYBEAN MEAL	8.19	NC	6.33	NC	1.38	NC
9	1/A – SOYBEAN MEAL	8.40	NC	6.80	NC	1.45	NC
10	1/A – SOYBEAN MEAL	7.73	NC	6.09	NC	1.30	NC
11	1/A – SOYBEAN MEAL	8.10	NC	6.41	NC	1.32	NC
12	1/A – SOYBEAN MEAL	8.17	NC	6.36	NC	1.36	NC
13	1/A – SOYBEAN MEAL	7.68	NC	6.04	NC	1.29	NC
14	1/A – SOYBEAN MEAL	7.73	NC	5.99	NC	1.35	NC
15	1/A – SOYBEAN MEAL	8.38	NC	6.44	NC	1.50	NC
16	1/B – SOYBEAN MEAL + WHEY	8.79	C	6.80	NC	1.54	NC
17	1/B – SOYBEAN MEAL + WHEY	295.61	C	226.17	C	242.06	C
18	1/B – SOYBEAN MEAL + WHEY	204.49	C	158.97	C	178.52	C
19	1/B – SOYBEAN MEAL + WHEY	238.56	C	184.05	C	203.14	C
20	1/B – SOYBEAN MEAL + WHEY	203.71	C	158.77	C	179.41	C
21	1/B – SOYBEAN MEAL + WHEY	192.81	C	150.70	C	170.72	C
22	1/B – SOYBEAN MEAL + WHEY	298.22	C	228.13	C	244.78	C
23	1/B – SOYBEAN MEAL + WHEY	167.20	C	130.62	C	148.64	C
24	1/B – SOYBEAN MEAL + WHEY	149.44	C	117.89	C	135.89	C
25	1/B – SOYBEAN MEAL + WHEY	203.40	C	157.72	C	176.59	C
26	1/B – SOYBEAN MEAL + WHEY	286.38	C	220.45	C	240.32	C
27	1/B – SOYBEAN MEAL + WHEY	288.98	C	222.01	C	239.98	C
28	1/B – SOYBEAN MEAL + WHEY	185.36	C	140.36	C	141.89	C
29	1/B – SOYBEAN MEAL + WHEY	257.95	C	197.23	C	209.23	C
30	1/B – SOYBEAN MEAL + WHEY	271.30	C	208.83	C	227.91	C
31	1/B – SOYBEAN MEAL + WHEY	228.06	C	175.80	C	192.11	C
32	1/B – SOYBEAN MEAL + WHEY	236.58	C	179.56	C	186.41	C
33	1/B – SOYBEAN MEAL + WHEY	258.36	C	197.11	C	209.33	C
34	1/B – SOYBEAN MEAL + WHEY	133.62	C	100.11	C	96.60	C
35	1/B – SOYBEAN MEAL + WHEY	32.50	C	24.26	C	15.25	C
36	1/B – SOYBEAN MEAL + WHEY	25.01	C	18.62	C	10.28	C
37	1/B – SOYBEAN MEAL + WHEY	48.23	C	35.73	C	27.44	C
38	1/B – SOYBEAN MEAL + WHEY	14.55	C	10.98	C	3.70	C
39	1/B – SOYBEAN MEAL + WHEY	14.47	C	10.92	C	3.73	C
40	1/C – SOYBEAN MEAL	17.62	C	13.33	C	14.80	C
41	1/C – SOYBEAN MEAL	2.27	NC	2.04	NC	2.05	NC
42	1/C – SOYBEAN MEAL	1.23	NC	1.21	NC	1.27	NC
43	1/C – SOYBEAN MEAL	1.08	NC	1.01	NC	1.21	NC
44	1/C – SOYBEAN MEAL	1.23	NC	1.16	NC	1.31	NC
45	1/C – SOYBEAN MEAL	1.10	NC	1.09	NC	1.27	NC
46	1/C – SOYBEAN MEAL	1.06	NC	0.94	NC	1.16	NC
47	1/C – SOYBEAN MEAL	1.06	NC	1.04	NC	1.19	NC
48	1/C – SOYBEAN MEAL	1.07	NC	1.06	NC	1.17	NC
49	1/C – SOYBEAN MEAL	1.24	NC	1.02	NC	1.32	NC
50	1/C – SOYBEAN MEAL	1.04	NC	0.98	NC	1.17	NC
51	1/C – SOYBEAN MEAL	1.12	NC	0.99	NC	1.30	NC
52	1/C – SOYBEAN MEAL	1.11	NC	0.99	NC	1.27	NC
53	1/C – SOYBEAN MEAL	1.08	NC	0.99	NC	1.20	NC
54	1/C – SOYBEAN MEAL	1.21	NC	1.18	NC	1.31	NC
55	1/C – SOYBEAN MEAL	1.09	NC	0.97	NC	1.24	NC
56	1/C – SOYBEAN MEAL	1.18	NC	1.07	NC	1.32	NC
57	1/C – SOYBEAN MEAL	1.04	NC	0.91	NC	1.19	NC
58	1/C – SOYBEAN MEAL	1.24	NC	0.97	NC	1.42	NC
59	1/C – SOYBEAN MEAL	1.59	NC	1.15	NC	1.76	NC
60	1/C – SOYBEAN MEAL	1.52	NC	1.16	NC	1.71	NC
61	1/C – SOYBEAN MEAL	1.35	NC	1.05	NC	1.59	NC
62	1/C – SOYBEAN MEAL	1.46	NC	1.15	NC	1.65	NC
63	1/C – SOYBEAN MEAL	1.47	NC	1.10	NC	1.73	NC
64	1/C – SOYBEAN MEAL	1.32	NC	1.07	NC	1.49	NC
65	1/C – SOYBEAN MEAL	1.40	NC	1.09	NC	1.65	NC
66	1/C – SOYBEAN MEAL	1.28	NC	1.02	NC	1.47	NC
67	1/C – SOYBEAN MEAL	1.06	NC	1.00	NC	1.25	NC
68	1/C – SOYBEAN MEAL	2.70	NC	2.19	NC	2.37	NC
69	1/D – SOYBEAN MEAL + WHEY	1.19	NC	1.01	NC	1.37	NC
70	1/D – SOYBEAN MEAL + WHEY	1.79	NC	1.37	NC	1.93	NC
71	1/D – SOYBEAN MEAL + WHEY	48.26	C	37.41	C	43.33	C
72	1/D – SOYBEAN MEAL + WHEY	21.30	C	16.36	C	18.60	C
73	1/D – SOYBEAN MEAL + WHEY	5.67	NC	4.39	NC	4.90	C

(*continued on next page*)

Table 3 (*continued*)

Sample	Sample id	*F* ratio protein	Conclusion prot (*C* if >8.42)	*F* ratio fat	Conclusion fat (*C* if >8.54)	GH	Conclusion GH (*C* if >3)
74	1/D – SOYBEAN MEAL + WHEY	152.97	C	120.15	C	145.45	C
75	1/D – SOYBEAN MEAL + WHEY	105.83	C	82.43	C	96.64	C
76	1/D – SOYBEAN MEAL + WHEY	112.92	C	87.99	C	103.93	C
77	1/D – SOYBEAN MEAL + WHEY	98.52	C	76.51	C	89.63	C
78	1/D – SOYBEAN MEAL + WHEY	67.63	C	52.00	C	60.68	C
79	1/D – SOYBEAN MEAL + WHEY	104.34	C	80.86	C	96.07	C
80	1/D – SOYBEAN MEAL + WHEY	147.20	C	114.19	C	135.60	C
81	1/D – SOYBEAN MEAL + WHEY	111.99	C	86.70	C	103.23	C
82	1/D – SOYBEAN MEAL + WHEY	130.30	C	101.54	C	120.96	C
83	1/D – SOYBEAN MEAL + WHEY	177.56	C	139.33	C	170.28	C
84	1/D – SOYBEAN MEAL + WHEY	91.91	C	70.70	C	84.11	C
85	1/D – SOYBEAN MEAL + WHEY	80.79	C	62.00	C	73.42	C
86	1/D – SOYBEAN MEAL + WHEY	107.51	C	83.52	C	99.46	C
87	1/D – SOYBEAN MEAL + WHEY	122.76	C	95.24	C	112.83	C
88	1/D – SOYBEAN MEAL + WHEY	59.48	C	45.90	C	53.96	C
89	1/D – SOYBEAN MEAL + WHEY	114.83	C	88.66	C	105.81	C
90	1/D – SOYBEAN MEAL + WHEY	148.65	C	116.24	C	139.19	C
91	1/D – SOYBEAN MEAL + WHEY	120.02	C	93.05	C	110.35	C
92	1/D – SOYBEAN MEAL + WHEY	160.43	C	126.55	C	154.75	C
93	1/D – SOYBEAN MEAL + WHEY	160.87	C	126.38	C	153.93	C
94	1/D – SOYBEAN MEAL + WHEY	145.59	C	114.59	C	139.75	C
95	1/D – SOYBEAN MEAL + WHEY	97.01	C	74.99	C	88.53	C
96	1/D – SOYBEAN MEAL + WHEY	165.03	C	129.35	C	156.26	C
97	1/D – SOYBEAN MEAL + WHEY	176.78	C	139.84	C	170.60	C
98	1/D – SOYBEAN MEAL + WHEY	118.65	C	92.05	C	109.41	C
99	1/D – SOYBEAN MEAL + WHEY	121.42	C	95.17	C	114.20	C
100	1/D – SOYBEAN MEAL + WHEY	144.18	C	113.20	C	135.99	C
101	1/D – SOYBEAN MEAL + WHEY	73.32	C	56.75	C	66.66	C
102	1/D – SOYBEAN MEAL + WHEY	74.20	C	57.39	C	67.19	C
103	1/D – SOYBEAN MEAL + WHEY	74.21	C	57.59	C	67.33	C
104	1/E – SOYBEAN MEAL	5.96	NC	4.69	NC	5.25	C
105	1/E – SOYBEAN MEAL	1.48	NC	1.30	NC	1.38	NC
106	1/E – SOYBEAN MEAL	1.28	NC	1.16	NC	1.30	NC
107	1/E – SOYBEAN MEAL	1.24	NC	1.23	NC	1.23	NC
108	1/E – SOYBEAN MEAL	1.30	NC	1.33	NC	1.37	NC
109	1/E – SOYBEAN MEAL	1.22	NC	1.17	NC	1.21	NC
110	1/E – SOYBEAN MEAL	1.03	NC	0.97	NC	1.21	NC
111	1/E – SOYBEAN MEAL	1.09	NC	1.00	NC	1.20	NC
112	1/E – SOYBEAN MEAL	1.02	NC	1.00	NC	1.18	NC
113	1/E – SOYBEAN MEAL	1.09	NC	0.96	NC	1.27	NC
114	1/E – SOYBEAN MEAL	1.03	NC	1.12	NC	1.21	NC
115	1/E – SOYBEAN MEAL	1.44	NC	1.60	NC	1.66	NC
116	1/E – SOYBEAN MEAL	1.02	NC	1.02	NC	1.16	NC
117	1/E – SOYBEAN MEAL	1.23	NC	1.29	NC	1.33	NC
118	1/E – SOYBEAN MEAL	1.17	NC	1.26	NC	1.22	NC
119	1/E – SOYBEAN MEAL	1.11	NC	1.16	NC	1.26	NC
120	1/E – SOYBEAN MEAL	0.99	NC	1.01	NC	1.22	NC
121	1/E – SOYBEAN MEAL	1.04	NC	1.15	NC	1.27	NC
122	1/E – SOYBEAN MEAL	1.08	NC	1.02	NC	1.19	NC
123	1/E – SOYBEAN MEAL	1.21	NC	1.21	NC	1.26	NC
124	1/E – SOYBEAN MEAL	1.13	NC	1.07	NC	1.19	NC
125	1/E – SOYBEAN MEAL	1.05	NC	0.98	NC	1.25	NC
126	1/E – SOYBEAN MEAL	1.15	NC	1.11	NC	1.24	NC
127	1/E – SOYBEAN MEAL	1.12	NC	1.16	NC	1.24	NC
128	1/E – SOYBEAN MEAL	1.32	NC	1.30	NC	1.28	NC
129	1/E – SOYBEAN MEAL	1.34	NC	1.29	NC	1.41	NC
130	1/E – SOYBEAN MEAL	1.19	NC	1.10	NC	1.47	NC
131	1/E – SOYBEAN MEAL	1.33	NC	1.04	NC	1.52	NC
132	1/E – SOYBEAN MEAL	1.17	NC	1.03	NC	1.30	NC
133	1/E – SOYBEAN MEAL	1.16	NC	1.11	NC	1.31	NC
134	1/E – SOYBEAN MEAL	1.36	NC	1.04	NC	1.55	NC
135	PURE WHEY	144.09	C	124.13	C	146.36	C

C: contaminated.
NC: not contaminated.

4.2.1. Experimental plan

The actual contamination of the soybean meal with whey powder was performed when the truck unloaded the soybean meal into the concrete pit.

The experimental procedure was as follows:

Step A – 5 tons of soybean meal unloaded directly from the truck

Step B – 5 tons of soybean meal unloaded from the truck simulating a spot (local) contamination (all the contaminated samples of whey powder unloaded at the same time).

Step C –5 tons of soybean unloaded directly from the truck

Step D – 5 tons of soybean meal unloaded from the truck and contaminated by mixing simultaneously during the unloading with whey powder.

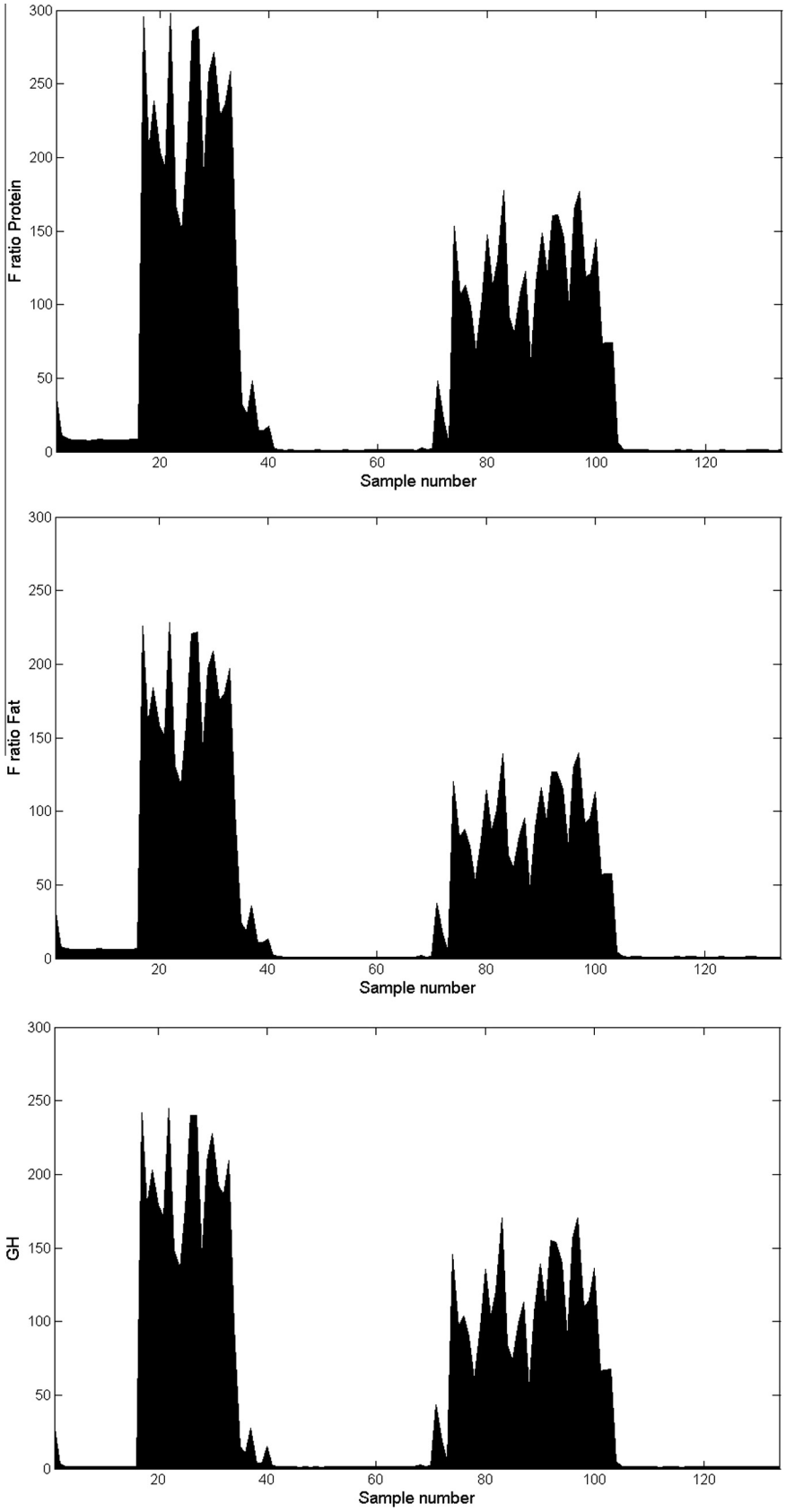

Fig. 2. Spectral F ratio for the predicted protein content, fat content and GH, respectively, for test 1.

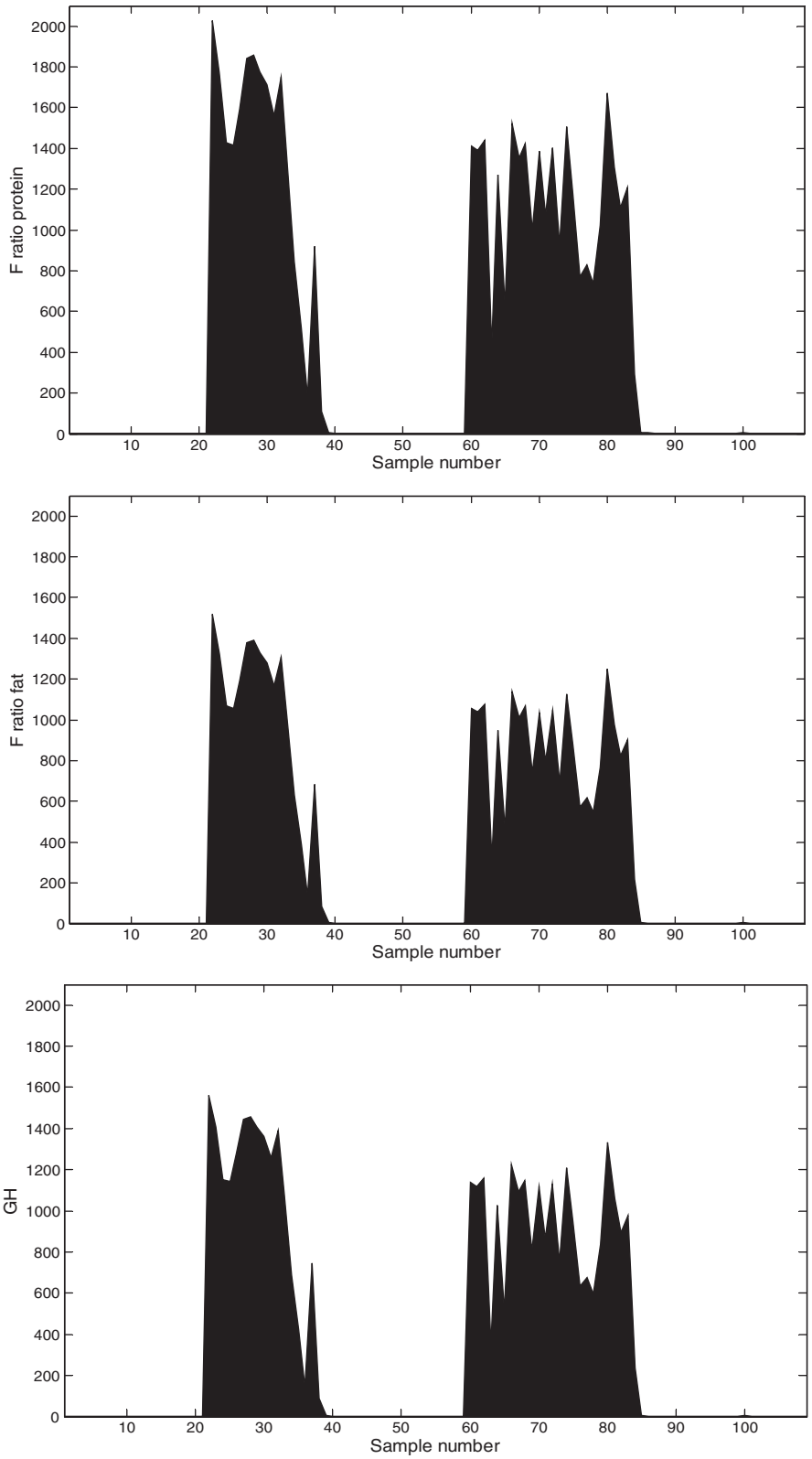

Fig. 3. Spectral F ratio for the predicted protein content, fat content and GH, respectively, for test 2.

Step E – Truck emptied. No deliberate contamination carried out.

This procedure was performed twice on different days (test 1 and test 2) and using different soybean meal batches. For each step, samples were collected using the specified sampling system. All the samples were then analysed in duplicate using the NIR spectrometer described in Section 3 and the spectra were submitted to the complete methodology in order to characterise the soybean meal and detect the presence of unusual ingredients.

Before performing this work, the volume flow rate (i.e., the volume of soybean meal samples being unloaded into the concrete pit from the truck per unit time) had been calculated. In order to make this calculation, a truck containing soybean samples similar to those to be used in the final study was unloaded, trying to keep the flow as constant as possible.

Five loads of about 5 tons each were necessary to ensure that the flow rate from the truck could be managed, as opposed to the flow rate of the redler conveyor, which was much easier to determine and control. Determining the flow rate was also necessary to prevent the systematic weighing step of the truck between each load. Several trials were done with several trucks unloading according to the various steps. Each truck driver had his own way of proceeding and it was necessary to determine the best way to obtain the most constant (stable) flow rate. In these trials, on average more than 25 tons per truck were unloaded; often, it was closer to 29–30 tons. It was found that the 'calculated' time needed to unload 5 tons varied considerably (between 4′30″ and 3′30″). This was mainly because soybean meal with different densities was delivered, and more time was needed (4′30″) to unload the one with the lowest density (0.597). Because only one out of five loads had such a low density, it was decided to set the unloading time at 3′30″ per group (four groups at about 3′30″ and the fifth and last group as long as necessary until the truck was empty).

In total 134 and 109 samples were collected for test 1 and test 2 respectively following the steps previously explained.

Step A – Only soybean meal was unloaded. Simultaneously, the horizontal transporter was running in order to carry the soybean meal, and the samples were captured with the automatic sampler. In total, 15 and 20 samples were obtained for tests 1 and 2, respectively.
Step B – About 5 tons of soybean meal were unloaded. To effect local contamination, 500 kg of whey powder (i.e. 10% of contamination) were then poured from a large bag onto the top of the soybean meal. The transporter was then moved to empty the concrete pit, with the soybean meal and whey moving down together. During this time, samples were collected. In total, 24 and 18 samples were collected for tests 1 and 2, respectively. At the end of the operation, the transporter was turned off.
Step C – About 5 tons of soybean meal were unloaded. No deliberate contamination was carried out. In total, 29 and 20 samples were collected for tests 1 and 2, respectively.
Step D – About 5 tons of soybean meal were unloaded. To simulate a more global contamination, 500 kg of the same whey powder used earlier were poured manually onto the top of the soybean meal. A time of 3′30″ were not enough to empty all 20 whey powder bags of 25 kg as scheduled ($20 \times 25 = 500$ kg). Only 14 and 17 bags were poured onto the soybean meal (350 kg and 425 kg which corresponds to 7% and 8.5% of contamination respectively) for tests 1 and 2, respectively. In total, 35 and 25 samples were collected under the pit for tests 1 and 2, respectively, using the automatic sampler.
Step E – No deliberate contamination was carried out. A total of 31 and 26 samples were collected for tests 1 and 2, respectively.

4.2.2. Results

As in the previous examples, Table 3 shows the results for the first set of samples using the experimental procedure.

For test 1, a total of 135 samples were analysed, including one sample of pure whey. As before, for each criterion, a conclusion is drawn based on whether the predicted values are inside the limits defined by the 99.7th percentile calculated in pure soybean meal samples for the protein (8.42) and fat (8.54) criteria and by a GH larger than 3 (C: contaminated; NC: not contaminated). Because it was a continuous process, the indications given in column 2 (sample ID) of Table 3 are intended simply to be informative; they were recorded at the moment the contaminant was loaded. The first 15 first samples related to Step A are supposed to be only soybean meal going into the pit. However, as the table shows, the first samples were detected as contaminated, probably because the pit still contained the remains of a previous loading that was not soybean meal. Soon, however, the presence of pure soybean meal was detected. With the arrival of contaminated samples, Step B started, lasting from sample 16 to sample 39. As previously explained, due to the continuous process, the sampling has been performed at the same time as the loading of the truck, which can explain the fact that the first sample of step B (sample 16) is still considered as not contaminated by two criteria as the contaminant did not reach the sampling device. Step C started from sample 40, which was still being detected as contaminated because of the accumulation of whey at the end of the redler conveyor in the concrete pit; soon, however, the methodology showed normal results and the presence of pure soybean meal. Step D started from sample 69 and Step E lasted from sample 104 until the end of the process, with one sample of pure whey added as reference. In general, similar results were obtained with all the criteria. The spectral F ratio based on the fat equation presented 2 false positive results and 4 false negatives whereas the spectral F ratio based on protein content and the GH presented each 4 false positive and 3 false negative results. Fig. 2 shows, for each numerical criterion, the results following the loading of the contaminant.

For test 2, a total of 109 samples were analysed, including one sample of pure whey. As in test 1, all criteria enabled an easy characterisation of the soybean meal and detection of most of the contaminants. Fig. 3 shows, for each numerical criterion, the results following the loading of the contaminant.

In both tests, even if some false positive and negatives results have been found, the methodology proposed allowed the detection of irregular samples and then it should permit the development of an automatic alert to stop the loading of the truck.

5. Conclusions

Feed industries strive to produce quality products at the lowest cost and in the shortest time. The introduction of fast and non-destructive analytical methods to measure not only the final product but also the raw materials at the start of the production chain can offer technical and cost-saving advantages over conventional techniques for authenticating feed products and therefore for manufacturing the final feed compound. NIR spectroscopy has become one of the most important techniques in feed companies, especially for the quality control of the final product, due to its easy implementation and use. Applying NIR at the start of the chain, however, is less common, although it could help to achieve important cost-savings by detecting non-conformity. In most cases, only a portion of the samples loaded on trucks bound for feed mills is collected and measured, with the result used as an indication of the quality of the whole load and therefore introducing significant sampling uncertainty. In this study we proposed a complete procedure that involved installing a sampling device at the entrance of a feed mill and measuring the samples using NIR. The study results showed that the use of NIR, combined with some simple chemometric tools based on distances and regression equations, is appropriate for authenticating important feed products (in this case, soybean meal) and detecting the presence of abnormal samples or unusual ingredients in both the laboratory and at the feed mill. The cases shown in this study, melamine and whey, are just two examples of products contaminating soybean meal. Another example of bulk contamination at the entrance of the feed mill

was also performed using DDGS (Dried Distillers Grains with solubles), a residual product from ethanol production, with similar results and conclusions (data not shown). This study is limited by the nature of the method of analysis (NIR spectroscopy), which one of the main perceived disadvantages has been stated to be the low sensitivity to some minor constituents, though this can also depend on the chemical species being detected and the complexity of the feed matrix under analysis (Ellis et al., 2012). The encouraging results of this work have led to the installation of an online NIR system opposite the sampling device, allowing for the measurement of a larger portion of the samples being loaded into the feed mill.

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