Detection of melamine contamination by near infarared spectroscopy and near infrared microscopy techniques: which perspectives and limits?

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Introduction

Since 2007, CRA-W has conducted several studies in order to assess the potential of NIR techniques in the detection of melamine adulteration. The aim of this presentation was to impart some of the knowledge provided by these different studies.

Material and methods

Powdered infant formula product was purchased from a local Belgian supermarket (Nutrilon Standard, Belgium). The milk powder was first adulterated by a melamine product in order to reach a contamination level of 1%, then the blend was diluted following a step-wise dilution, in order to get levels of contamination of 0.1%, 0.05%, 0.01%, 0.005%, 0.001%, and 0.0001%. Two sets of blank samples were prepared. The first set included non-mixed milk powder samples while the second set included samples mixed with milk powder from the same batch and following the step-wise dilution procedure applied to produce samples spiked with melamine at different levels. NIRS spectra were collected using a FT-NIR spectrometer equipped with a rotating cup and a reflectance integrating sphere accessory (MPA – Bruker Optik GmbH, Germany). Five spectra for each milk powder sample were collected within the region 4000–10000 cm^{-1} with a resolution of 8 cm^{-1} and a total of 64 co-added scans. The OPUS 6.0 software was used for the spectral acquisition while The UNSCRAMBLER software version 9.2 from CAMO (Computer Aided Modelling, Trondheim, Norway) was used for the treatment of the collected spectral data.

The protein contents of a series of soybean meal samples spiked at 0 to 5% with melamine was determined by Kjeldahl, combustion (Leco) and NIR methods. Spectroscopic analyses were performed in duplicate using a NIRSystems 6500 monochromator (NIRSystems, Silver Spring,

MD, USA). Spectra were collected between 400 and 2498 nm with an interval of 2 nm and by co-adding 32 scans. Spectra were collected as log 1/R.

Results and discussion

Learning 1: NIR determination of protein content enables detection of gross melamine contamination

The 5 melamine samples analysed present bands in the same NIR region with relative equivalent absorbance values. When melamine is added to samples, and according to the type of products and the granularity, segregation of the melamine particles from the rest of sample particles was observed and had to be addressed in the NIR analysis.

The protein content of soybean meal samples spiked at 0 to 5% with melamine increased proportionally, as determined by the reference methods (Kjeldahl or Leco). Using NIRS prediction models for proteins constructed using a large soybean meal data base (i.e. INGOT data-base including more than 10,000 spectra with protein reference values; cfr http://www.aunir.co.uk/NIR_product.php?product=INGOT), a decrease in the protein content as determined by NIRS was observed. In the meantime, it was also observed that the difference between the reference and NIR protein values for a dedicated sample increased with the level of melamine contamination, while the maximum standardised Mahalanobis distance (*H* distance) values ranged from 19 to 2566 for the samples spiked respectively at 0.5 to 5% melamine. Clearly, protein content determination by NIR spectroscopy allows bringing to the fore samples spiked with melamine.

Learning 2: NIR limit in the LOD

The process to determine the LOD is well-defined in several international standards. The procedure to follow is different, depending on whether the method is quantitative or qualitative.

Certain inherent limitations of the NIRS technique have to be taken into account in this process. Considering soybean spectra non-adulterated, and adulterated at 1% with melamine in the vicinity of 2166 nm, a mean difference of 0.02 absorbance unit (or 20 000 µlog) is observed. From this figure it can be calculated that the differences that can be observed for samples spiked at 0.1% to 0.0001% should range from to 2 000 µlog to 2 µlog (*cf.* Table 1).

Table 1. Relation between melamine content and absorbance.

AU	µLog	Melamine content %	ppm	ppb
0.02	20,000	1	10,000	10,000,000
0.002	2000	0.1	1000	1,000,000
0.0002	200	0.01	100	100,000
0.00002	20	0.001	10	10,000
0.000002	2	0.0001	1	1000

Taking into account the errors associated with the detector, with the measurement and with the analysis performed in duplicate of respectively 2,200 and 2,000 µlog; the LOD of detection of melamine by NIR spectroscopy should be somewhere between 0.1% and 0.01% (100 ppm to 1000 ppm).

Another limitation is the fact that the NIRS spectra can be influenced by any change in both chemical and physical parameters of the analysed sample. The method of preparation of the samples may affect the spectral features by introducing unexpected sources of variation. An experiment has been done in order to study what could be the impact of the procedure for preparing the spiked samples. Two series (A and B) of spiked samples were prepared, using the same sample of milk powder. Stepwise dilution procedure including several levels of dilution and mixing was used to prepare both series. Series A consisted of the milk powder firstly adulterated with *melamine* at levels of contamination of 1%, then the blend was diluted with milk powder in order to get samples spiked at specified levels. Series B was prepared in exactly the same way as Series A. Milk powder was used as adulterant, so that the series B included milk powder samples spiked with *milk powder* in the 0.0001 to 1% range. This series aimed at to studying the impact of the dilution procedure used. PCA was performed individually on both series of samples (Figure 1b) and on the sample free of melamine [Figure 1(a)].

Figure 1(b) presents the scores plot PC1 vs PC2 for the samples of series A (labelled Mela) and series B (labelled Milk). It is observed that samples spiked with milk powder (series B) clustered with the samples spiked with melamine (series A). In our experiments, the stepwise dilution

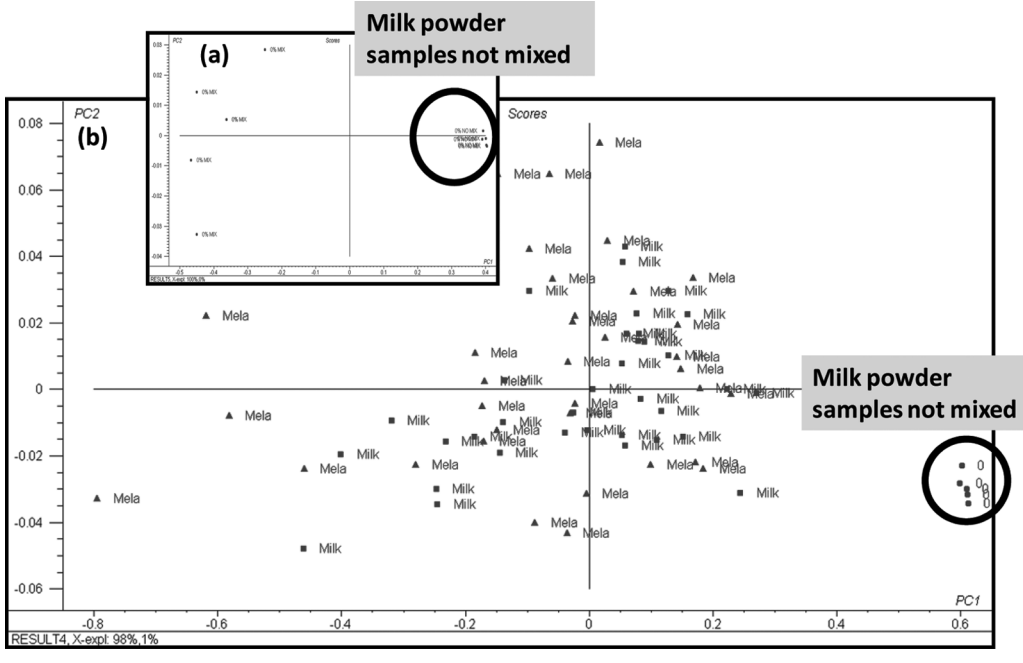

Figure 1. Score plot of the (a) samples of milk powder not mixed and mixed according to the stepwise dilution procedure and (b) samples of milk powder adulterated with melamine (Mela) and milk powder (Milk).

procedure introduced spectral variation that partially overlapped with the one introduced by addition of low content of melamine. The impact of the stepwise procedure on the spectral response was also observed on the PCA performed only on the unmixed milk powder and mixed milk powder samples (both free of melamine) [Figure 1(a)]. The variation introduced by the preparation of the samples could be explained by specific hygroscopic and repacking phenomena affecting the milk powders during the spiking procedure. This underlines the importance to test the LOD on real samples and not only on "artificial" samples.

Learning 3: How to reach low LOD with NIRS

In the detection of undesirable substances, the proposed methods should be able to detect such substances at the ppm or ppb levels. It is feasible to reach low levels of melamine detection by NIR spectroscopy, but it is necessary to put aside one of NIR spectroscopy's basic rules for quantitative analysis of major constituents (e.g. protein determination): *"To scan a sample portion as large as possible in order to get a representative spectrum of the sample"*. For the detection of contaminants it is imperative that the spectral pattern of the contaminant affects the spectrum of the product in a way that it is detectable by NIR spectroscopy.

It is difficult to detect a contaminant at a level below 1% on the basis of a single sample spectrum representative of it. But if 100 spectra of very small fractions of the sample are collected, most of the spectra will not conform to the spectral pattern of the contaminants, while a few spectra will occur where the spectral pattern of the contaminants will represent a significant fraction of the sample portion analysed. Important factors are the particle sizes of the contaminants and the food/feed products as well as the sample portion analysed, as well as the efficiency of the blending. In the melamine samples tested at CRA-W about 60% and 40% of the particles had respectively a diameter lower and higher than 125 µm.

NIR microscopy and NIR imaging are suitable NIR techniques to collect spectra of small portions of food or feed materials.[1,2] In our trials, NIR microscopy and NIR imaging instruments respectively allow obtaining spectra from a sample portion as small as 10 µm × 70 µm. It means that, if enough NIR microscopy or NIR imaging spectra from a powder contaminated with melamine at ppm level are acquired, some of them should have the distinguishable features of melamine particles. A new basic rule of NIR spectroscopy, as applied to the detection of contaminants at low level could be: *"To scan a sample portion as small as possible in order to lower the LOD"* or *"To collect as many spectra as possible in order to lower the LOD"*.

Conclusion

NIR spectroscopy has a role in the methods used to ensure optimum food and feed safety. However the basic rules of the proper application of NIR have to be adapted to the detection of small amounts of contaminants.

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References

1. V. Baeten, C. von Holst, A. Garrido, J. Vancutsem, A. Michotte Renier and P. Dardenne, *Anal. Bioanal. Chem.* **382**, 149 (2005).
2. 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. John Wiley & Sons, Ltd, Chichester, UK, p. 289 (2007).