Detecting Melamine Adulteration in Milk Powder

Introduction

Food adulteration is at the top of the list when it comes to food safety concerns, especially following recent incidents, such as the 2008 Chinese powdered milk scandal. That scandal involved milk and infant formula along with other food materials and components being adulterated with melamine. There were an estimated 300,000 victims in total, including six infant deaths, resulting from kidney stones and other kidney damage.

Protein content is a key parameter that measures the quality of food products such as milk powder and infant formula. Protein concentration is traditionally measured by Kjeldahl and Dumas methods through measuring nitrogen content; however, neither method can distinguish non-protein nitrogen from naturally occurring nitrogen in protein. This gap in detectability allowed the protein content in food to be falsified by adding nitrogen-rich chemicals, such as melamine. Melamine adulteration not only causes protein deficiency, but also kidney stones and renal failure when it reacts with cyanuric acid inside the body.

In food products other than infant formula, the FDA concludes that levels of melamine and melamine-related compounds below 2.5 parts per million (ppm) do not raise concerns. They also conclude that melamine or cyanuric acid alone, at or below 1 part per million in infant formula, do not raise public health concerns in babies[1]. To detect melamine at such low concentrations highly sensitive techniques such as LC/MS have been developed; however, it is very time-consuming and does not accommodate rapid screening. Near infrared (NIR) spectroscopy, on the other hand, is a non-destructive, fast screening method used for illicit drug screening, raw material inspection, etc. However, the NIR method is typically not sensitive enough for concentrations less than 1%. To increase the sensitivity of the NIR method, a patent-pending algorithm has been developed for targeted screening at concentrations as low as 0.01%. In this study, the Advanced-ID™ algorithm with an FT-NIR spectrometer will be evaluated for low concentration melamine screening in milk powders.

[1] *"Limits set on melamine levels". People's Daily. 9 October 2008*

Materials and Methods

Materials

Four milk powder samples were purchased from local supermarkets: Market Basket instant nonfat dry milk powder, Carnation instant nonfat dry milk powder, Enfamil infant step 1 powder, and Enfamil newborn powder. Melamine (purity = 99%) was purchased from Aldrich (St. Louis, MO) and added to the milk powder samples in various low concentrations as listed in Table 1.

Table 1: Melamine-Adulterated Sample Concentrations

Sample ID	100% Melamine %
Carnation Mix 1	0.099
Carnation Mix 2	0.191
Carnation Mix 3	0.495
Carnation Mix 4	0.716
Carnation Mix 5	1.020
Carnation Mix 6	1.479
Carnation Mix 7	1.964
Carnation Mix 8	0.355
MB Mix 1	0.139
MB Mix 2	0.267
MB Mix 3	0.407
MB Mix 4	0.600
MB Mix 5	0.919
MB Mix 6	1.457
MB Mix 7	5.384
Enfamil Infant Mix 1	0.197
Enfamil Infant Mix 2	0.077
Enfamil Infant Mix 3	0.436
Enfamil Infant Mix 4	1.333
Enfamil NB Mix 1	0.197
Enfamil NB Mix 2	1.463
Enfamil NB Mix 3	0.231
Enfamil NB Mix 4	0.782

Sample Measurement

NIR spectra were collected using a QuasIR™ 3000 (Galaxy Scientific, Nashua, NH, USA) equipped with a sample spinner accessory. Samples were placed into a 98 mm cup with low OH quartz window and then loaded onto the sample spinner, which is mounted off-center on the 23 mm sample window of the integrating sphere. This increases the quantity of sample measured and is suitable for inhomogeneous samples. Each sample was measured five times with 4 cm^{-1} resolution and 50 scans. Samples were reloaded between measurements.

Data Processing

Spectral Sage™ software was used for data collection and the CLS-based Advanced-ID™ algorithm and software were used for analysis.

Result and Discussion

For a sample comprising *n* components, its spectrum *S* can be modeled as the sum of the spectra of the *n* components $K_1...K_n$, assuming the Beer-Lambert law is obeyed.

$$S = \sum_{i=1}^{n} (Ci * Ki) + R$$

Where *K* is the matrix of reference spectra of the sample components, $c_1...c_n$ are unknown coefficients and *R* is a residual, or error. The least squares solution to this equation for the coefficients can be found by standard matrix algebra, and is otherwise known as Classical Least Squares (CLS), or *K*-matrix regression.

If each spectrum contains *m* data points, then we can write this in matrix notation as:

$$S = K * c + R$$

where *S* and *R* are *m* x 1 matrices, *K* is a *m* x *n* matrix of reference spectra, and *c* is an *n* x 1 matrix of coefficients. Often, all of the components represented in *K* are known to be present and the objective of the regression is to find the coefficients *c* that can then be used to calculate their relative concentrations. In certain cases, however, one of the components may be an unknown that needs to be identified, or a suspected component whose presence in the mixture needs to be confirmed. If we designate this component as a target component and the spectrum of this component as *T* (the target spectrum), then for convenience we can rewrite the equation as:

$$S = T * c_0 + K' * c' + R$$

where *S*, *T*, and *R* are *m* x 1 matrices, *K'* is an *m* x (*n*-1) matrix of reference spectra of known components that does not contain the spectrum in *T*, *c'* is an (*n*-1) x 1 matrix of coefficients, and c_0 is a scalar coefficient.

Various methods can be used to judge the quality of the model, which includes the common practice of examining

the size of the residuals, R. However, if the contribution of the target component to the spectrum *S* is very small, then the residual is a very poor indicator of the presence of the target component. This is because the regression of only the spectra of the known components *K'* will result in a very good fit to the sample spectrum *S*, resulting in a very small residual (close to zero).

The patent-pending Advanced-ID™ algorithm finds a new approach to resolve this issue. It first calculates an approximation to the target spectrum by performing a regression that includes the target and known spectra ($S = T * c_0 + K'*c' + R$), and then calculates a residual with the coefficient for the target spectrum, c_0, set to 0, thus defining the extracted spectrum *E*:

$$E = S - K'*c'$$

This can be compared with the expression for the residual *R*:

$$R = S - Tc_0 - K'*c'$$

The residual *R* will be small if either the target component is not present and $K'*c'$ is a good approximation to *S*, or if the target component is present and $T*c_0 + K'c'$ is a good approximation to *S*. As noted above, this is therefore not a good indicator of the presence of the target component. The extracted spectrum *E* will also be small and will resemble *R* if the target component is not present and $K'*c'$ is a good approximation to *S*. However, if the target component is present in the sample at any significant concentration and $T*c_0 + K'c'$ is a good approximation to *S*, then the extracted spectrum will resemble the spectrum of the target component. Additionally, if the target component is not present and $K'*c'$ is not a good approximation to *S* because another component is present that was not included in the regression, then the extracted spectrum will not resemble either *R* or the target spectrum.

Comparison of the extracted and target spectrum, typically scaled by the regression coefficient c_0, can therefore be a reliable indicator of the target's presence. The comparison could be mathematical or visual by overlaying the two spectra on the computer screen.

As long as the spectra of all components present are included in the regression, the method described above will also work if the sample contains more than one suspected component that needs to be confirmed. In this case, one of the target spectra is *T*, all the other target spectra are included in *K'* and the extracted spectrum is calculated and compared with the target spectrum. This is then repeated for each of the other target spectra. The method described above may also work with more than one unknown component, especially if the principal spectral features of the unknowns are in different spectral regions. In this fashion, individual components in a mixture may be identified.

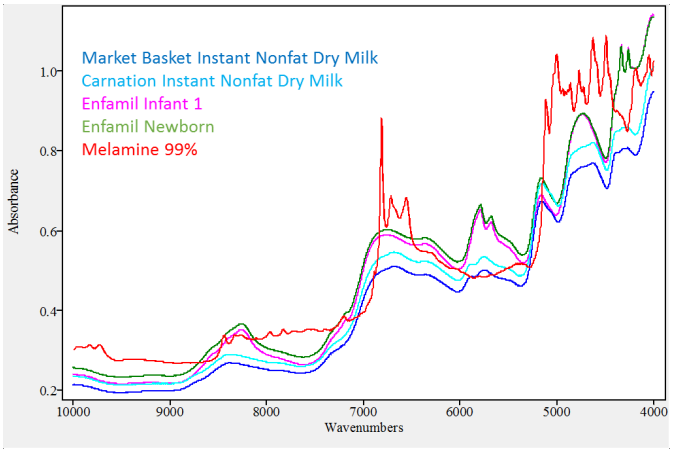
In this study, the target spectrum is the 99% melamine sample. The extracted spectrum is expressed as

$$E = S_{AMP} - K_{MK}'*c'$$

where S_{AMP} is the spectrum of adulterated milk powder, K_{MK} are the spectra of milk powder components. The Advanced-ID package was used to solve the unknown coefficients and calculate the correlation coefficient between the extracted spectrum and melamine reference spectrum.

The average NIR spectra of pure milk powder and infant formula used in this study and the spectrum of 99% melamine are shown in Figure 1. In contrast to milk powders, melamine has very sharp peaks in the region of 6500-7000 cm^{-1}; this difference was used to develop the Advanced-ID method.

Figure 1: Spectra of pure components



For Enfamil Infant step 1 formula, melamine was added in four concentrations: 0.077%, 0.197%, 0.436%, and 1.333%. By visually checking the NIR spectra of adulterated samples an elevated peak can be noticed around 6800 cm^{-1} (Figure 2) only when melamine concentration is higher than about 0.5%.

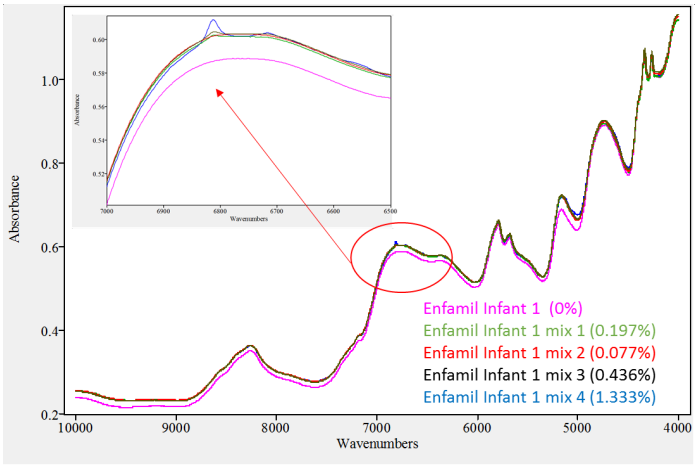


Figure 2: Original spectra of Enfamil Infant 1 Mixture

Figure 3 presents the extracted spectra of the mixtures using the Advanced-ID™ method. As can be seen, extracted spectra of the four mixtures have apparent absorbance peaks around 6800 cm^{-1} where the Enfamil Infant step 1 reference sample shows none.

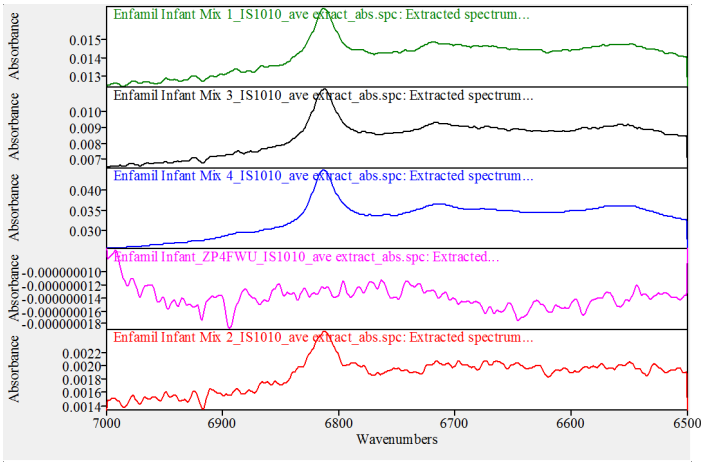


Figure 3: Extract spectra of Enfamil Infant 1 Mixture

If we overlay the extract spectrum of Enfamil Infant step 1 mixture 2, which had the lowest melamine concentration, with its original spectra and that for pure melamine, no melamine is detected in the original spectra, whereas in the extracted spectrum we see the absorbance matching the melamine sample in the designated region (Figure 4).

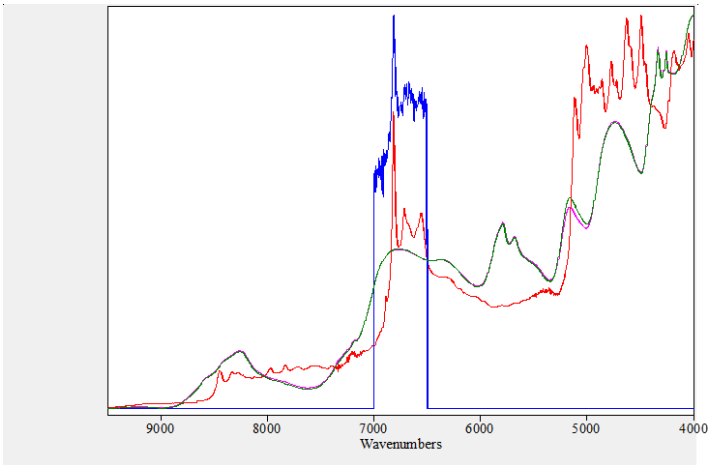


Figure 4: Overlay of melamine spectrum, original and extract spectra of Enfamil infant mixture 2

Table 2 lists the correlation coefficients of the extracted spectra to the melamine reference spectrum at different concentrations. The extracted spectrum of pure Enfamil infant step 1 powder shows no correlation with the melamine spectrum, whereas extracted spectra of adulterated samples, with melamine levels as low as about 0.1%, have a high correlation with the melamine spectrum, with a correlation coefficient higher than 0.97. The reported NIR melamine detection limit is about 0.05%, showing the excellent sensitivity of the Advanced-ID™ algorithm.

Table 2: Advanced-ID result of Enfamil infant mixture

Sample ID	Melamine (%)	Ave. Corr. Cofficient
Enfamil Infant Mix 1	0.197	0.9961
Enfamil Infant Mix 2	0.077	0.9726
Enfamil Infant Mix 3	0.436	0.9968
Enfamil Infant Mix 4	1.333	0.9979
Enfamil Infant Pure	0.000	-0.1392

Similar results were obtained with the rest of the mixtures as shown in Table 3. All extracted spectra of melamine-adulterated samples (down to 0.1%) had a higher than 0.97 correlation coefficient with the pure melamine reference spectrum, with the exception of Market Basket instant nonfat dry milk powder mixture 1, which had a melamine concentration of 0.139% and correlation coefficient value of 0.881. This could be due to the fact that the milk powder is freeze-dried, which leaves a porous structure and various particle sizes that are

more inhomogeneous compared to the rest. Grinding the sample before measurement could possibly increase the accuracy of the result.

Table 3: Advanced-ID™ result of milk powder and Enfamil New Born mixtures

Sample ID	Melamine (%)	Ave. Corr. Cofficient
MB Mix 1	0.139	0.881
MB Mix 2	0.267	0.9698
MB Mix 3	0.407	0.9883
MB Mix 4	0.600	0.9932
MB Mix 5	0.919	0.9952
MB Mix 6	1.457	0.9969
MB Mix 7	5.384	0.9983
MB powder pure	0.000	-0.1454
Melamine Mix 1	0.099	0.9755
Melamine Mix 2	0.191	0.9834
Melamine Mix 3	0.495	0.9976
Melamine Mix 4	0.716	0.9978
Melamine Mix 5	1.020	0.9987
Melamine Mix 6	1.479	0.9988
Melamine Mix 7	1.964	0.9981
Melamine Mix 8	0.355	0.9964
Carnation Pure	0.000	-0.1448
Enfamil NB Mix 1	0.197	0.9909
Enfamil NB Mix 2	1.463	0.9977
Enfamil NB Mix 3	0.231	0.9824
Enfamil NB Mix 4	0.782	0.997
Enfamil NB Pure	0.000	-0.1512

The spectra of other possible chemicals that could be added to milk powder to increase the nitrogen reading, could also be measured and used them as target spectra, allowing their identification in a similar way. It is also possible to compare the extracted spectrum to the standard residual spectrum from the Advanced-ID™ algorithm, which may indicate the presence of an unknown adulterant, even at a low concentration.

Conclusion

Using our CLS-based Advanced-ID™ algorithm, we can detect melamine adulteration at concentrations down to 0.1% or lower. Even though this is not at the FDA mandated 2.5 ppm level, there is no economic motivation to adulterate milk powder with such low concentrations because 0.25% melamine added to milk powder only increases protein content by 1%. Therefore, with a detection limit of 0.1% melamine, NIR can be used as a fast screening tool for milk powder adulteration.

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