**Detection of melamine in milk powders using Near-Infrared
Hyperspectral imaging combined with regression coefficient of
partial least square regression model**

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Abstract: Illegal use of nitrogen-rich melamine ($C_3H_6N_6$) to boost perceived protein content of food products such as milk, infant formula, frozen yogurt, pet food, biscuits, and coffee drinks has caused serious food safety problems. Conventional methods to detect melamine in foods, such as Enzyme-linked immunosorbent assay (ELISA), High-performance liquid chromatography (HPLC), and Gas chromatography–mass spectrometry (GC-MS), are sensitive but they are time-consuming, expensive, and labor-intensive. In this research, near-infrared (NIR) hyperspectral imaging technique combined with regression coefficient of partial least squares regression (PLSR) model was used to detect melamine particles in milk powders easily and quickly. NIR hyperspectral reflectance imaging data in the spectral range of 990-1700 nm were acquired from melamine-milk powder mixture samples prepared at various concentrations ranging from 0.02 to 1%. PLSR models were developed to correlate the spectral data (independent variables) with melamine concentration (dependent variables) in melamine-milk powder mixture samples. PLSR models applying various pretreatment methods were used to reconstruct the two-dimensional PLS images. PLS images were converted to the binary images to detect the suspected melamine pixels in milk powder. As the melamine concentration was increased, the numbers of suspected melamine pixels of binary images were also increased. These results suggested that NIR hyperspectral imaging technique and the PLSR model can be regarded as an effective tool to detect melamine particles in milk powders.

Keywords: Hyperspectral imaging, milk powder adulteration, melamine, partial least square regression, regression coefficient

1. Introduction

Melamine (CAS 108-78-1, $C_3H_6N_6$), known as 2,4,6-triamino-1,3,5-triazine, is a nitrogenous chemical substance and is often used in a fertilizer and plastic goods. In particular, melamine resin is widely used in materials for machine parts, glue, adhesives, laminates, coatings, commercial filters, and flame retardants due to the excellent hardness and waterproof properties of melamine resin in combination with formaldehyde [1]. The high nitrogen content of melamine (66% by mass, as shown by its molecular formula) prompted its unlawful use in increasing the perceived protein content of animal feed and human food ingredients [2, 3].

The most representative incident of melamine contamination in food was a melamine-contaminated infant formula scandal that occurred in China in 2008. At that time, some dairy farmers and manufacturers illegally mixed their milk with water in order to increase the milk volume, and melamine was intentionally added to the diluted milk to increase the perceived protein content to meet the specified protein content required for milk (3%), which would be measured by relatively inexpensive nitrogen-content-based methods such as the Kjeldahl or Dumas method [4].

According to the official statistics of the Chinese Ministry of Health, the consumption of milk contaminated with melamine caused 294,000 pediatric patients to suffer from urinary disease, such kidney stones, and six infant deaths [5].

Generally, melamine detection in foods is performed mainly using a variety of chemical assay methods such as Enzyme-linked immunosorbent assay (ELISA), High-performance liquid chromatography–ultraviolet/diode array detection (HPLC-UV/DAD), Gas chromatography–mass spectrometry (GC-MS), Tandem mass spectrometry (MS/MS), GC-MS/MS, and LC-MS/MS [6, 7]. These measurement methods have excellent limits of detection (LOD), but have other disadvantages such

as the need for complicated sample preprocessing procedures, time-consuming determination processes, expensive equipment, and skilled professional operators [8].

Hyperspectral imaging technique has emerged as an effective technology for attaining large amounts of image data with continuous spectrum information, beyond the limits of typical visible/near-infrared spectroscopy methods that acquire spectral information from one point/location at a time and multispectral imaging techniques that acquire spectral images across only a few discrete wavelengths [9, 10]. During the last decade, hyperspectral imaging technology has been widely applied for optical sensing of various physical, chemical, and mechanical characteristics important for the quality and safety of food and agricultural products [11-15].

In particular, optical sensing technologies including hyperspectral imaging and spectroscopy have been recently attempted by many researchers for the detection of melamine in dairy foods [16-24]. Compared to conventional chemical assays, hyperspectral imaging techniques generally need little or no chemical preprocessing of samples and consequently can detect melamine in dairy foods more easily and quickly [16].

Hyperspectral image data are formatted as three-dimensional hypercubes with two spatial dimensions (i.e., images) and one spectral dimension (i.e., wavelength). Because each individual pixel in a hyperspectral image has spectrum information about a specific position in a sample, various multivariate statistical methods such as principal component analysis (PCA), partial least squares (PLS), linear discriminant analysis (LDA), Fishers discriminant analysis (FDA), multi-linear regression (MLR), and artificial neural networks (ANN) can be used to classify normal and abnormal targets [9]. Among them, the most popular PLS regression

(PLSR) in conventional chemometrics has been widely applied to predict the linear relationship between independent variables as hyperspectral spectra and dependent variables as concentration data [23].

In this paper, NIR hyperspectral imaging technique combined with regression coefficient calculated based on PLSR model and applications of individual pixel-based global threshold values (no false positives in pure milk powder) was used to detect and quantify the melamine particles contained in milk powder. To achieve this objective, the main goals and process were as follows;

(1) A newly created reflectance spectrum (independent variables) multiplying a weighting factor by melamine concentration (dependent variables) using average reflectance spectrum of hyperspectral imaging acquired pure milk powder and pure melamine was used for PLSR model development.

(2) Performance of the developed PLSR models using various pretreatment methods was evaluated by comparison of R_c^2 , SEC, R_v^2 , SEP, and F values.

(3) Calculated regression coefficient (Beta coefficient, β) from each PLSR model developed in accordance with pretreatment methods was applied to hyperspectral imaging. The global threshold value with no false-positive melamine spots in a reconstructed PLS image of pure milk powder was used to detect melamine particles in every other hyperspectral imaging of milk powder-melamine mixture samples.

(4) Original reflectance spectra of suspected melamine pixels were compared with spectrum of pure melamine for verification of pixel identification.

2. Materials and methods

2.1 Hyperspectral imaging system

Schematics of the NIR hyperspectral imaging system and its core components used for this research are shown in Figure 1. The imaging hardware includes an InGaAs focal-plane-array (FPA) camera (Xenics, Model Xeva-1.7-320, Leuven, Belgium), a spectrograph (SWIR Hyperspec, Headwall Photonics, MA, USA), and a 25 mm focal length lens (Optec, Model OB-SWIR25/2, Parabiago, Italy). The InGaAs FPA camera has 320 spatial and 256 spectral pixels on a 30 μm pitch. It is thermoelectrically cooled to -20 °C via a double-stage Peltier device and image data are digitized at 14 bit A/D resolutions. The imaging spectrograph covers from approximately 950 nm to 1700 nm and has a spectral resolution of 8 nm full width at half maximum. The imaging system was used to collect images from approximately 990 nm to 1700 nm wavelength range (144 wavelengths).

Two 150 W (DC 21 V) quartz tungsten halogen (QTH) lamps (Dolan Jenner, Model DC-950, MA, USA) with DC power regulation were used to illuminate samples for SWIR reflectance image measurements. The QTH light transfers through a flexible fiber optic (0.8 m length, 15.9 mm diameter, Dolan Jenner, DC-950, MA, USA) by the opposite side equipped with a thin line light of fibers 254 mm length. The programmable uniaxial transfer stage moved the mixture samples incrementally across the linear field of view of the line-scan imaging system.

2.2 Sample preparation

The dry milk powder used in this study was nonfat dry milk (Organic Valley Family of Farm, La Farge, Wisconsin, USA) purchased from a local grocery store and was used for sample preparation and imaging without any chemical or physical preprocessing. Melamine of stated purity 99% (Sigma-Aldrich, St Louis, MO, USA) was used to prepare 10-g mixture samples with concentrations levels of melamine in

milk powder at 0.02%, 0.04%, 0.06%, 0.08%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1% by weight (g/g). In addition, 10 g samples of pure milk powder and pure melamine were also prepared. Three such sets (I, II, and III) each containing 10 mixture samples and 2 pure samples were prepared, for a total of 36 samples. All samples were weighed by an electronic balance (Mettler-Toledo International, Model AL204, Shanghai, China) with a measuring sensitivity of 0.0001 g. The 10 g quantified samples were poured into 50 mL polypropylene centrifuge tubes marked for 50 mL and blended using a Vortex mixer (Scientific Industries, Inc., Model K-500-J, Greens Village, NY, USA) to mix homogenize the powder mixtures. After mixing for more than one min, the powder mixtures were transferred to plastic petri dishes (47 mm inside diameter, 7.0 mm depth, Fisher Scientific Inc., Hampton, NH, USA). Each petri dish was completely filled with powder and the powder surface was leveled flush with the rim of the petri dish, without any pressure for packing down the powder. Imaging of all 36 samples was replicated three times for hyperspectral imaging measurement.

2.3 Hyperspectral imaging acquisition

The set-up of the hyperspectral imaging system for line-scan imaging of the powder samples is shown in Figure 2.A. The distance (a) of powder samples from the lens is 380 mm, and the line illumination is fixed at 30 mm in height (b) from the surface of the sample. Distance (c) and angle (d) of the two line lights are 32 mm and 16 degrees, respectively. The 12 powder samples were prepared in 3 set (I, II, III) and each set was arranged on a rectangular black plate (230 mm X 170 mm) in a 3 x 4 array and scanned in the direction as shown in Figure 2.B.

Line-scan images of the samples were acquired using a 7 ms exposure time with the programmable translation stage advancing the samples in incremental steps

of 0.25 mm, for a total of 900 line-scan images to fully capture each 3 X 4 array of samples arranged within a 225 mm X 170 mm area. The resolution of the resulting 320 X 900 pixels had a resolution of approximately 0.132 mm²/pixel. Hyperspectral images were collected from 992.0 nm to 1681.2 nm in 144 spectral bands with about 4.82 nm increment. As shown in Figure 2.C, each hyperspectral image thus obtained created a three-dimensional hyper-cube with spatial information (320 X 900 pixels) and spectral information (144 wavelengths). For each set of powder samples (Figure 2.B), the samples were imaged, remixed and prepared in Petri dishes again, the set was imaged a second time, then the samples were remixed and prepared again, and the set was imaged a third time, resulting in 9 sample-set hypercubes as shown in Figure 2.C., or 108 individual-sample hypercubes.

2.4 Hyperspectral imaging and reflectance spectrum analysis

As shown in Figure 3.A, hyperspectral images can include signal noises such as that from the effects of illumination and detector sensitivity. In order to eliminate these noises, the original hyperspectral images were corrected using the following formula [23].

$$R_r(\lambda) = \frac{R_h(\lambda) - R_d(\lambda)}{R_w(\lambda) - R_d(\lambda)} \times 100 \quad \text{(Equation 1)}$$

Where R_r is the relative reflectance image, R_h is the original hyperspectral image, R_d is the dark current image acquired by removing the lighting source, R_w is the reference image obtained for a white diffuse reflectance panel (Spectralon™, SRT-

99-120, Labsphere, NH, USA), and λ is wavelengths.

After converting all the original hyperspectral images to relative reflectance images (Figure 3.B) by applying Equation 1, mask images were created to separate the background spaces and the petri dish rims from the areas of the powder samples in the hyperspectral images. The biggest difference in reflectance intensity between the powder pixels and background/dish pixels occurred at the 1151.0 nm waveband, therefore the spectral image at this waveband was used as a threshold band to generate the mask template. The mask template as shown in Figure 3.C was applied to the hyperspectral images at each of the 144 waveband images between 992.0 nm and 1681.2 nm, in order to extract from the hyperspectral images only the desired region of interest (ROI), encompassing only the powder sample areas, for more effective analysis. Figure 3.D shows an example of the ROI for the 1473.8 nm waveband image for the first imaging of the first set of samples (set I-1). For this image, the average number of pixels per circular powder area was 12,039 pixels. The control software for hyperspectral image acquisition was developed on a MS (Microsoft) Visual Basic (Version 6.0) platform in the MS Windows operating systems, using a SDK (Software Development Kit) provided by the camera manufacturer. Software for image processing and masking was developed using Matlab (R2007b, MathWorks, Natick, MA, USA).

2.5 PLSR and regression coefficient

The PLSR model was developed to correlate the hyperspectral reflectance spectrum data (independent variables) with melamine concentration (dependent variables) in milk powder. Equation 2 was applied to obtain the dependent variable

to be used for the development of the PLSR model.

$$R(\lambda)_{x,y}^{New} = [R(\lambda)_{x,y}^{Me} \times \frac{C^{me}}{100}] + [R(\lambda)_{x,y}^{Mi} \times (1 - \frac{C^{me}}{100})] \quad \text{(Equation 2)}$$

Where $R(\lambda)_{x,y}^{new}$ is new reflectance spectrum of a milk-melamine mixture calculated from a linear combination of pure milk spectrum and pure melamine spectrum, $R(\lambda)_{x,y}^{Me}$ and $R(\lambda)_{x,y}^{Mi}$ are reflectance spectra of pure melamine and milk, respectively, and C^{me} is 11 melamine concentration levels (0%, 0.02%, 0.04%, 0.06%, 0.08%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8% and 1%). The subscripts (x and y) indicate spatial location with the ROI.

Nine different PLS regression models for melamine detection were developed based on different spectral pretreatment methods for smoothing and noise reduction. The spectral preprocessing treatments that were applied included the following: maximum normalization (Max), range normalization (Range), mean normalization (Mean), standard normal variate (SNV), mean scattering correction (MSC), 1st and 2nd Savitzky-Golay (SG) derivatives (number of smoothing: 3 points, polynomial: 2nd degrees) (1st D and 2nd D), SG smoothing (SM).

PLSR model developed by each pretreatment method has a regression coefficient which is expressed as shown in Equation 3.

$$C = B_1X_1 + B_2X_2 + B_3X_3 + \dots + B_nX_n + B_0 \quad \text{(Equation 3)}$$

Where C is the predicted melamine concentration, $B_1 \sim B_n$ values are regression coefficients for each 144 wavelengths reading, X_n is the reflectance computed by each spectra preprocessing methods and B_0 is the regression

coefficient for intercept [24].

The performance of each PLSR model was evaluated using a full cross-validation method. The determination coefficient for calibration (R_c^2), standard error of calibration (SEC), determination coefficient for the validation (R_v^2), standard error of prediction (SEP), and the number of factors (F) of the prediction models were calculated to compare the performance. The spectral pretreatment methods and PLSR model development were conducted using Matlab (R2007b, MathWorks, Natick, MA, USA) and the Unscrambler software v9.2 (CAMO Software AS, Oslo, Norway).

3. Results and discussion

The average reflectance spectra of the samples are shown in Figure 4.A. The mean spectrum of pure melamine shows very different spectral characteristics compared to the mean spectra of the other 11 samples. The pure melamine spectrum crosses the spectra of the milk and melamine-milk samples near 1459.4 nm with a very steep slope compared to the other spectra. Three large absorption peaks at 1473.8 nm, 1497.8 nm and 1531.6 nm can be observed for pure melamine. Peaks in this wavelength region are known as the first overtone of NH_2 symmetric and antisymmetric stretching [19].

Figure 4.B provides a closer view of the mean spectra of the pure milk and melamine-milk samples within the range of the large absorption peaks for pure melamine. The 11 mean spectra in that range do show visible separation from each other, but they do not appear to be well ordered according to melamine concentration level. This spectral characteristic can be inferred from the weak

absorption intensity by melamine containing a very small amount in milk powder, light scattering by the difference in particle size between melamine and milk powder. Hence, additional analysis is needed to identify melamine concentration in milk using specific absorption peaks of melamine.

Single-band images at the three wavebands corresponding to three major absorption peaks of melamine, 473.8 nm, 1497.8 nm and 1531.6 nm, are shown for the 12 samples in data set I-1 in Figure 5. The intensity of the pure melamine sample in each of the three images is obviously much lower than that of the pure milk sample and the melamine-in-milk mixture samples. However, there is no obviously visible difference in intensity in the images between the pure milk sample and the mixture samples. Thus, it is necessary to develop a novel method to detect the melamine particles.

From each of the 9 data sets (I-1,2,3; II-1,2,3; III-1,2,3), the average spectrum for the pure milk ROI and the average spectrum for the pure melamine ROI were calculated, for a total of 18 spectra. Equation 2 was applied to these spectra and the results of calibration and validation of the developed PLSR models (using different spectral pretreatment methods) are shown in Table 1.

The 10-factor no-pretreatment PLSR model (Raw) resulted in calibration R_c^2 and SEC values of 0.962 and $\pm 0.066\%$, respectively, and validation R_v^2 and SEP values of 0.943 and $\pm 0.081\%$, respectively. The optimal prediction model was found to be an 8-factor PLSR model using a 1st Derivative pretreatment method, which produced R_v and SEP values of 0.956 and $\pm 0.071\%$, respectively.

The regression coefficient values for the PLSR model developed using the original reflectance spectra (RAW) and for the model developed using the 1st

derivative pre-treated spectra (1st D) are shown in Figures 6 and 7, respectively. Highly positive or highly negative coefficients indicate wavebands that strongly influence the PLSR model, and some of the wavebands corresponding to these highly positive or negative coefficients match the signature absorption bands of melamine. In case of regression coefficient of 1st D pretreatment method shown the best PLSR model results, it can be observed that only two of the band increased peak wavelength of the entire 144 bands. In the case of the 1st D model, which showed the best results of all the PLSR models, the plot of regression coefficients shows significantly large max/min values at only 2 of the 144 wavebands, at the 1478.6 nm and 1468.9 nm bands respectively.

The regression coefficient values of each of the PLSR models were applied to the hypercube images of each of the 9 data sets and the converted images were reconstructed as two- dimensional PLS images. Figure 8 shows the PLS images for data set III-2 resulting from RAW model (a) and the other 8 models using the different spectral pre-treatments (b through i). In these PLS images, the suspected melamine particles in the milk powder are still difficult to find.

Therefore, threshold values were applied to the PLS images to create binary images for detecting melamine pixels. Table 2 shows the threshold values used for each model in each data set; these values were determined from pixel intensities in the pure-milk and the pure-melamine samples. For example, the threshold value of 2.364 for the RAW model in data set I-1 was the maximum value from among all the pixels in the pure-milk ROI for the RAW PLS image. In Table 2, starred (*) threshold values are global threshold values that were applied to detect the melamine pixels for all 9 data sets. In the binary detection images, pixels that were above the threshold value are shown in yellow as melamine pixels, and pixels that were below

the threshold value are shown in blue as milk pixels.

Figure 9 shows binary images highlighting melamine pixels in yellow in each of the sample ROIs for the 9 PLSR models produced for data set III-2. To calculate a quantitative detection result for each type of PLSR model (RAW or different pretreatments), the number of melamine pixels were counted for the sample ROIs of matching melamine concentration and then expressed as a percentage of the total number of sample ROI pixels. As shown in Table 3, melamine particles were detected by all 9 models for samples of all concentrations, regardless of concentration or spectral pretreatment methods. However, the detected concentration results matched more closely to the actual melamine concentrations for the lower-concentration samples ($< 0.06\%$). For the samples of higher concentrations, all models except the 2nd D model tended to overestimate detection above the actual melamine concentrations.

The melamine particles were distributed throughout the powder mixture samples, each of which had a depth of 7mm (contained in Petri dish), and thus the samples were not presented as a single-layer structure for imaging. The NIR hyperspectral imaging system can detect melamine particles both below and at the surface of the powder sample. The particle size of melamine is generally less than 0.01 mm in diameter which is smaller than the spatial resolution ($0.132 \text{ mm}^2 / \text{pixel}$) of the hyperspectral imaging system used in this study. The pixels containing a fraction of melamine exhibit spectral characteristics of melamine and are detected as melamine pixels. This can contribute to the over estimation of melamine concentrations in relatively higher concentration levels under investigation. Figure 10 illustrates the curve-linear relationship and the over estimation of concentration due to the above factors.

Figure 11 shows the original reflectance spectra of 875 pixels that were identified as melamine pixels in the binary detection images for the 1st derivative (1st D) PLSR model, data set III-2 (i.e., yellow pixels in Figure 9.g). The reflectance spectra show three major absorption peaks in the region related to melamine and can explain melamine-milk powder mixture samples in melamine particles. These reflectance spectra do not match specific spectral characteristics of the pure melamine (i.e., Figure 4.A) because the absorption peaks of melamine have been weakened with the inclusion of melamine in milk powder, with the detection of both milk and melamine together within pixels given the spatial resolution (larger than melamine particle size) of the imaging detector. These results suggest that with additional refinement, NIR hyperspectral imaging technique based on regression coefficient of PLSR model could become a significant and useful method to detect melamine in milk powders and to visualize the distribution of melamine particles.

4. Conclusions

A NIR hyperspectral imaging system in the spectral range of 990-1700 nm was used for acquiring hyperspectral images of melamine-milk powder mixture samples. The acquired hyperspectral images were converted to the relative reflectance images to remove signal noise. The PLSR model was developed to correlate the hyperspectral reflectance spectrum data (independent variables) with melamine concentration (dependent variables) in melamine-milk powder mixture samples. Regression coefficient values calculated from the developed PLSR models using various pretreatment methods were applied to the hyperspectral images and two-dimensional PLS images were reconstructed. PLS images were converted to the binary images to discriminate the melamine pixels from milk pixels for the melamine-

milk powder mixture samples. The binary images were able to detect suspected melamine particles in melamine-milk powder mixture samples for each melamine concentration level. These results suggest that a NIR hyperspectral imaging technique combined with PLSR model can be an effective tool for detecting the melamine adulteration particles in milk powder at 0.02% melamine concentration.

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Table 1. The results of calibration and validation of developed PLSR models by each pretreatment method.

	Raw	Normalization			SNV	MSC	1 st D (S.G)	2 nd D (S.G)	SM
		Mean	Max	Range					
R_c²	0.962	0.960	0.960	0.960	0.956	0.958	0.970	0.954	0.961
SEC	0.066	0.067	0.067	0.067	0.071	0.069	0.058	0.072	0.067
R_v²	0.943	0.940	0.940	0.940	0.935	0.940	0.956	0.938	0.941
SEP	0.081	0.083	0.083	0.083	0.087	0.084	0.071	0.084	0.082
F	10	10	10	10	9	9	8	6	10

468 **Table 2.** Threshold values applied to PLS images.

Data sets	Raw	Normalization			SNV	MSC	1 st D (S.G)	2 nd D (S.G)	SM	
		Mean	Max	Range						
I	1 st	2.364	2.236	2.156	2.162	2.154	2.009	2.649	4.565*	2.136
	2 nd	2.506	2.457	2.334	2.181	2.304	2.142	2.126	4.053	1.965
	3 rd	2.364	2.304	2.263	2.151	2.084	1.978	2.489	4.148	2.521*
II	1 st	2.010	1.883	1.834	1.722	1.759	1.599	2.645	4.036	1.841
	2 nd	2.763	2.647	2.467	2.317	2.481*	2.372	2.084	4.121	2.220
	3 rd	2.888*	2.796*	2.665*	2.536*	2.440	2.379*	2.505	4.072	2.241
III	1 st	2.510	2.369	2.347	2.404	2.257	2.148	2.968*	4.284	2.214
	2 nd	2.357	2.253	2.228	2.232	2.192	2.064	2.736	4.382	2.083
	3 rd	2.433	2.326	2.251	2.231	2.304	2.126	2.276	4.174	2.160

469 *The maximum (global) threshold value of each pretreatment methods

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471 **Table 3.** Melamine detection results (in percent) for each pretreatment methods.

Concentration (%)	Raw	Normalization			SNV	MSC	1 st D (S.G)	2 nd D (S.G)	SM (S.G)
		Mean	Max	Range					
0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02
0.04	0.02	0.03	0.03	0.03	0.04	0.03	0.03	0.01	0.03
0.06	0.05	0.05	0.06	0.06	0.07	0.06	0.05	0.02	0.05
0.08	0.10	0.10	0.12	0.12	0.14	0.13	0.10	0.05	0.10
0.10	0.11	0.12	0.13	0.14	0.15	0.14	0.13	0.05	0.13
0.20	0.19	0.19	0.22	0.24	0.26	0.25	0.23	0.08	0.23
0.40	0.44	0.46	0.51	0.54	0.59	0.57	0.49	0.21	0.52
0.60	0.84	0.86	0.97	1.03	1.16	1.10	0.95	0.37	1.08
0.80	1.31	1.33	1.55	1.68	1.98	1.87	1.44	0.50	1.73
1.00	1.96	1.99	2.25	2.35	2.86	2.69	2.14	0.81	2.57

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Figure Captions:

Figure 1. Schematic diagram of NIR hyperspectral imaging system and its core components.

Figure 2. (A) Hyperspectral imaging of samples: distances/angle for sample-to-camera, sample-to-light, and illumination set up; (B) sample array and scanning direction; and (C) representation of the acquired hypercube image.

Figure 3. (A) Original hyperspectral image, (B) relative reflectance image, (C) mask image, and (D) ROIs of masked relative reflectance image

Figure 4. Mean spectrum for each sample concentration as calculated from ROIs of data set I-1.

Figure 5. Spectral images at 1473.8 nm, 1497.8 nm, and 1531.6 nm of samples in data set I-1. a) pure melamine, b) pure milk powder, c) melamine 1%, d) melamine 0.8%, e) melamine 0.6%, f) melamine 0.4%, g) melamine 0.2%, h) melamine 0.1%, i) melamine 0.08%, j) melamine 0.06%, k) melamine 0.04%, l) melamine 0.02%.

Figure 6. Regression coefficients of PLSR model developed using the original (RAW) reflectance spectra ($B_0=3.828939$).

Figure 7. Regression coefficient values of PLSR model developed using 1st derivative (1st D) pretreated spectra ($B_0=1.247808$)

Figure 8. PLS images for detection of melamine particles (pixels) based on regression coefficients calculated by PLSR model for data set III-2.

Figure 9. Binary images for detection of melamine particles (pixels) based on regression coefficients calculated by PLSR model (data set III-2).

Figure 10. Curve-linear relationship between the detected melamine (pixels) and actual melamine concentrations.

Figure 11. Original reflectance spectra correspond to all the pixels detected as melamine particles (pixels) of binary images for melamine detection based on regression coefficients (data set III-2, 1st derivative pretreatment method).

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