Quantitative analysis of melamine in milk powders using near-infrared hyperspectral imaging and band ratio

**Min Huang¹, Moon S. Kim², Stephen R. Delwiche^{*3}, Kuanglin Chao², Jianwei Qin²,
Changyeun Mo⁴, Carlos Esquerre⁵, Qibing Zhu¹**

**1. Key Laboratory of Advanced Process Control for Light Industry (Ministry of Education),
Jiangnan University, Wuxi, 214122, China**

**2. Environmental Microbial and Food Safety Laboratory, Agricultural Research Service,
USDA, Beltsville, MD, 20705, USA**

**3. Food Quality Laboratory, Agricultural Research Service, USDA, Beltsville, MD, 20705,
USA**

**4. National Academy of Agricultural Science, Rural Development Administration, Jueonju-si,
Jeollabuk-do, 560-500, Korea**

5. Department of Biosystems Engineering, University of Dublin, Ireland

Running title: NIR Hyperspectral imaging of melamine in powdered milk

Corresponding author information:

Name: Stephen Delwiche

Affiliation: Food Quality Laboratory, Agricultural Research Service, USDA.

Address: Building 303, BARC-East, Beltsville, MD, 20705, USA

Email address: stephen.delwiche@ars.usda.gov

Telephone number: +1-301-504-8450 ext 236

Fax: +1-301-504-9466

^{*} Corresponding author. Tel.: +1-301-504-8450 ext 236. E-mail address: stephen.delwiche@ars.usda.gov

Abstract

Since 2008, the detection of the adulterant melamine (2,4,6-triamino-1,3,5-triazine) in food products has become the subject of research due to several food safety scares. Near-infrared (NIR) hyperspectral imaging offers great potential for food safety and quality research because it combines the features of vibrational spectroscopy and digital imaging. In this study, NIR hyperspectral imaging was investigated for quantitative evaluation of melamine particles in nonfat and whole milk powders. Melamine was mixed into milk powders in a concentration range of 0.02–1.00% (w/w). A NIR hyperspectral imaging system was used to acquire images (938–1654 nm) of melamine powder, whole milk powder, nonfat milk powder, and mixtures of melamine and each of the milk powders. Two optimal bands (1447 nm and 1466 nm) were selected by a linear correlation algorithm with pure milk and pure melamine. Band ratio ($B_{1447/1466}$) images coupled with a single threshold were used to create resultant images to visualize identification and distribution of the melamine adulterant particles in milk powders. The identification results were verified by spectral feature comparison between separated mean spectra of melamine pixels and milk pixels. Linear correlations (r) were found between the number of pixels identified as containing melamine and melamine concentration in nonfat milk and whole milk powders, which were 0.980 and 0.970 or higher, respectively. The study demonstrated that the combination of NIR hyperspectral imaging and simple band ratioing was promising for rapid quantitative analysis of melamine in milk powders.

Keywords: Food safety; Hyperspectral imaging; Milk powder; Melamine; Band ratio; Image classification.

1. Introduction

Recent recalls involving infant food and milk products contaminated with melamine (2,4,6-triamino-1,3,5-triazine) have aroused widespread food safety concerns. Melamine is a nitrogen-rich chemical substance (66% nitrogen by weight) that is commonly used as an industrial chemical in the production of melamine formaldehyde resins for manufacturing laminates, coatings, commercial filters, glues or adhesives (Jawaid et al., 2013). Melamine is sometimes illegally added to food products such as milk, infant formula, frozen yogurt, pet food, biscuits, candy, and coffee drinks to increase apparent protein content (Chan et al., 2008; WHO, 2011). Although melamine has low toxicity, its consumption may lead to kidney stones, eventual renal failure, and ultimately death (Brown et al., 2007; Tsai et al., 2010). In 2004, an outbreak of food adulteration with melamine led to renal failure in dogs and cats in Asia (Brown et al., 2007). In 2007, US scientists confirmed that pet food adulteration with melamine caused the illnesses and death of thousands of cats and dogs (Puschner et al., 2007). In 2008, consumption of melamine-contaminated infant formula and related dairy products in China caused more than 51,900 infants and young children to suffer from kidney illness, including six child deaths (Sun et al., 2010). The European Union (EU) set a maximum residue limit for melamine in dairy products and high-protein foods at 2.5 mg/kg, and the US Food and Drug Administration (FDA) set it as 0.25 mg/kg in milk and dairy products (Filazi et al., 2012). The Ministry of Health of China updated the dairy standards in 2010 and stressed that addition of melamine to dairy products is prohibited (Guo et al., 2011).

66 Various methods for the detection of melamine in foods for human consumption and animal feeds
67 have been reported in recent years, including high performance liquid chromatography (HPLC) (Ehling
68 et al., 2007; Muñoz-Valencia et al., 2008), mass spectrometry (MS) (GB/T 22388-2008; Filigenzi et al.,
69 2008), enzyme-linked immunosorbent assay (ELISA) (Garber, 2008; Yin et al., 2010), capillary
70 electrophoresis (CE) (Chen and Yan, 2009), chemiluminescence (CL) (Wang et al, 2009), and
71 molecularly imprinted polymer film (MIP) (Pietrzyk et al., 2009). For these chromatographic
72 approaches, sample pretreatment is relatively time-demanding and labor-intensive.

73 Near-infrared (NIR) and Raman spectroscopy have been applied to melamine detection in milk
74 (Mauer et al., 2009; Balabin and Smirnov, 2011; Liu et al., 2009) because of high penetrability,
75 nondestructive behaviour, and ease of pretreatment. However, spectroscopic assessments with relatively
76 small point-source measurements do not provide the spatial information of melamine particles. NIR
77 hyperspectral imaging technology combines the features of imaging and spectroscopy to simultaneously
78 acquire both spatial and spectral data, which is valuable for investigating the location where the
79 components being studied are distributed and for monitoring of particular banned chemicals (Huang et
80 al., 2014). Several studies in recent years have investigated the imaging of melamine in feed materials
81 and milk powders (Fernández Pierna et al., 2014; Huang et al., 2014; Fu et al., 2014). Although these
82 studies provided detection results for melamine particles in which imaging allowed for the visualization
83 of the distribution of melamine particles within images of milk powder mixture samples prepared with
84 various melamine concentrations, there was no exploration of the relationship between melamine

concentration and the number of pixels identified as containing melamine. To establish efficient imaging systems, essential spectral wavelengths are first sought through a variety of strategies, such as Principal Component Analysis, (Linear) Discriminant Analysis, Decision Boundary, Projection Pursuit, and kernel methods (Landgrebe, 2002). These algorithms treat the raw pixel spectra as input vectors in high dimensional spaces and rely upon linear or nonlinear mapping to a feature space by optimizing certain criterion. Band ratio, the ratio of spectral values at two different bands, offers the potential of substantially reducing processing time during classification (Kim et al., 2007). Therefore, the objective of this study was to develop band ratio methodology for the detection and quantitative prediction of melamine-adulterated milk powders using NIR hyperspectral imaging.

2. Materials and methods

2.1 Sample preparation

Nonfat milk powder (Organic Valley, La Farge, WI, USA) and whole milk powder (Hoosier Hill Farm, Fort Wayne, Indiana, USA) were purchased from a local supermarket. Melamine was obtained from Sigma-Aldrich Company (St. Louis, MO, USA) with 99% purity. The ranges in particle size of milk powder and melamine were 5–39 μm and 7–39 μm , respectively. For each of the two milk types, ten milk-melamine mixtures were prepared with melamine concentrations (w/w) of 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, 0.20%, 0.40%, 0.60%, 0.80% and 1.00%. Twenty-five g of dry powder mixture were placed in a plastic vessel (89 mm diameter $\times$ 102 mm height) and mixed with an acoustic mixer (Resodyn Acoustic Mixers, Model LabRAM, Butte, MT, USA). The intensity, frequency and mixing

time were set as 100%, 61.9 Hz and 10 min, respectively, to enhance the uniform distribution of the melamine particles within the dry milk. Next, each milk-melamine mixture was put in a custom-designed aluminum plate (15 mm height, 50 mm width, 50 mm length) with one square well (2 mm depth, 30 mm width, 30 mm length) which was spray painted in flat black. The well was slightly overfilled by milk-melamine mixture powder using a spoon, without compressing the dry powder, and then leveled across the top using a card to smooth the sample surface and remove excess powder. A well contained approximately 1.0 g of sample mixture. A total of four replicates (sets) for each mixture were prepared. Likewise, three preparations each of whole milk powder and nonfat milk powder, and two preparations of pure melamine were imaged. **Blends of whole milk and non-fat milk powders were not examined due to the powdered milk industry practice of keeping powdered milk types separate.**

2.2 Instrument and experiment

An in-house developed line-scan hyperspectral imaging system was used to acquire hyperspectral images from samples (Fig. 1). This system consisted of a hyperspectral imaging unit, a DC regulated light source and a sample handling unit. The hyperspectral imaging unit was made up of an InGaAs focal-plane-array (FPA) camera with 320×256 pixels (Xenics, Model Xeva-1.7-320, Leuven, Belgium), an imaging spectrograph (SWIR Hyperspec, Headwall Photonics, Fitchburg, MA, USA) connected with a 25-mm zoom lens (Optec, Model OB-SWIR25/2, Parabiago, Italy) as well as a computer for commanding the camera and acquiring images. The DC light source was composed of two external 150 W quartz tungsten halogen illuminators (Dolan Jenner, Model DC-950, Boxborough, MA, USA), with

each illuminator connected to a low-OH fiber optic bundle whose terminus was configured in a 0.25 m line of single fibers oriented perpendicular to the direction of movement of the scanned object. The sample handling unit consisted of a motorized uniaxial stage (Velmex, Model XN10-0180-M02-21, Bloomfield, NY, USA). The effective NIR spectral range of the spectrograph-camera combination consists of 150 pixels (150 spectral bands) in the spectral dimension over a wavelength range of 937.5–1653.7 nm, with an average wavelength spacing of 4.8 nm. The NIR imaging system was calibrated both spectrally and spatially by following the procedures described in Kim et al. (2012).

In this study, the line-to-line distance was set to 0.144 mm to match the pixel-to-pixel distance along the line, which included 320 pixels. The camera exposure time was set at 2.5 ms and a total of 250 scans were acquired in 50 seconds. The camera digitized raw energy readings in 14-bit resolution. Finally, each sample's spectral data was stored as a hyperspectral image cube of dimensions $320 \times 250 \times 150$ for later analysis.

Before the acquisition for sample images, a dark current image was acquired to remove the dark current effect of the array detector from the image data. The dark current image was captured by placing a cap over the end of the lens. A 99% diffuse reflectance standard (SpectralonTM, SRT-99-120, Labsphere, North Sutton, NH, USA) was used as a white reference image. These images were used to calculate the relative reflectance of the samples, which used the difference between energy readings from the sample and dark current divided by the difference between energy readings from the white reference and the dark current.

3. Calculation

3.1 Image preprocessing

For individual sample images, regions of interest (ROIs) consisted of 25600 pixels (160 pixels × 160 line scans square encompassing image center) were determined to exclude the background region from further analyses. A mean spectrum of the ROI of each sample concentration was calculated to investigate the general trend of spectral changes with melamine concentrations.

3.2 Band ratio method

In this study, the aim was to identify the pixels containing melamine particles at a sufficiently high enough level (described below) to be counted as “melamine rich.” It was assumed that each compound exhibited unique wavelength-dependent reflectance characteristics, and these spectral features served as the fingerprint for identification or classification (Abrams, 1983). The milk or melamine particle has distinct spectral features in the wavelength range under investigation. For instance, melamine has lower reflectance in the 1450–1550 nm region compared to milk powder. The band ratio techniques have been shown to enhance the spectral differences between samples and reduce the effect of heterogeneous illumination conditions. The ratio image is generalized using the following equation:

$$B_{t/k} = \frac{R_t}{R_k} \quad (1)$$

where $B_{t/k}$ represents a quotient of spectral reflectance, and R_t and R_k are reflectance intensities at t nm and k nm, respectively.

The best pair of wavelengths for distinguishing milk and melamine particles was determined by a linear correlation routine that examined all two-band combinations of pure milk powder and pure melamine. The two-band ratios with the highest correlation coefficients were determined as the optimal wavelength pairs to detect melamine in the milk-melamine mixtures. Using the best band pair, a threshold was set to determine whether the portion of the sample ROI represented by a pixel was melamine rich, also termed a pixel containing melamine. The threshold value was determined by calculating the standard deviation (sd) of the best band ratio of the four pure milk sets and then subtracting 3 times the sd from the pure milk set with the smallest ratio. Binary images (melamine rich or melamine deficient) were subsequently created by applying the threshold value to each pixel's best band ratio, with pixels below or above the threshold designated as melamine rich or deficient, respectively. To investigate the potential of the resultant images for quantitative analysis of the melamine concentration, a linear regression analysis was applied to the number of melamine rich pixels (regressor variable) and the concentration of experimental milk-melamine mixtures (dependent variable). The image processing, spectral preprocessing operation and model development and analyses were performed in MATLAB (R2013, MathWorks, Natick, MA, USA) equipped with the PLS Toolbox (Eigenvector Research Inc, Manson, WA, USA).

4. Results and discussion

4.1 NIR Hyperspectral reflectance spectra

Representative mean spectra (for sample ROI, 25600 pixels) of pure milk, pure melamine, and milk-melamine mixtures at ten different concentrations of melamine are shown in Figure 2a for nonfat (NF) milk powders and Figure 2b for whole milk (WM) powders, respectively. Considerable differences, particularly the absorption peaks related to the first and second N-H functional group, can be clearly observed between the spectra of pure melamine and pure milk powders. For the mean melamine spectrum, reflectance minima (absorption peaks) near 1524 nm and 1490 nm correspond to the first overtone of N-H symmetric and anti-symmetric stretching vibration, respectively. The second overtone of N-H stretching vibration is centered at 1010 nm. The greatest spectral difference between melamine and milk was observed at around 1466 nm, which is attributed to aromatic amine structures (Mauer et al., 2009), and showed the strongest absorption in the melamine spectrum. For the NF milk spectra, the reflectance intensity is higher than that of the WM spectra. A visual comparison of the nonfat and whole milk spectra revealed that the whole milk spectra had a stronger absorption peak around 1212 nm, which was due to the second overtone of C-H stretching vibration attributed to a greater presence of saturated fat structures (Nagarajan et al., 2006).

As shown in Figure 2a and 2b, the mean spectra of milk-melamine mixtures are very similar to the spectrum of the pure milk samples, and exhibit no prominent melamine absorption peaks in either NF or WM under the relatively low melamine concentrations investigated. This observation suggests that melamine particles may be better detected using individual pixel spectrum-based evaluation. As shown in Figure 2c, the mean reflectance decreased as the melamine concentrations increased in the 1466 nm

melamine absorption peak region for both NF and WM samples, with the mean spectra of the 1% mixture showing the lowest reflectance intensity.

The panels of Figure 3a and 3b show, for NF and WM, respectively, hyperspectral images of pure milk, pure melamine and 10 concentration levels of milk-melamine mixtures at the four melamine (N-H absorption) peaks, illustrating the intensity differences among pure melamine, pure milk, and milk-melamine mixtures with different melamine concentrations. As seen from the four different bands, the reflectance intensities of images at 1466.3 nm, 1490.3 nm and 1523.9 nm are similar and lower than that of images at 1009.6 nm. The images of pure milk and milk-melamine mixtures are much brighter than the pure melamine images because of the stronger melamine absorption. However, it is visually difficult to distinguish the melamine rich pixels in mixtures due to the pixel-to-pixel spectral intensity variations. The proposed band ratio algorithm can potentially enhance the contrast between the milk and melamine in the sample images.

Figure 4 shows the individual pixel spectra obtained from 25600 pixels of the 0.6% milk-melamine mixture sample for both NF (4a) and WM (4b) mixtures. High spectral variation occurred at around 1500 nm, with some of the spectra exhibiting strong melamine absorption features presumably due to a high relative proportion of melamine contained in those pixels. We rely on this spectral trend as the basis for determining the melamine concentration of a mixture by counting the number of rich pixels.

4.2 Identification and distribution of melamine in milk

216 The band ratio algorithm was applied to individual pixel spectra to identify melamine rich pixels in
217 the mixture samples described in Section 2.3. The band ratio values from all possible two-band ratios
218 were calculated and used to develop the discriminant model for pure milk and pure melamine particles.
219 One ratio using 1447 nm and 1466 nm produced the highest correlation coefficient (absolute value) of
220 the discriminant model for both NF and WM mixture samples. The band ratio ($B_{1447/1466}$) images of NF
221 and WM are shown in Figure 5a and 5b, respectively. The mixture samples with higher melamine
222 concentrations reveal more dark dots than those with lower concentrations because of more melamine
223 particles. Threshold values were used to qualitatively determine and classify the pixels as either
224 melamine deficient or melamine rich. For pure milk and pure melamine, the $B_{1447/1466}$ values had two
225 distinct distributions where NF and WM ranged from 0.915 to 1.011 and from 0.918 to 0.990,
226 respectively, which were much larger than those for pure melamine ranging from 0.385 to 0.635. The
227 three-standard-deviation-down threshold values determined for NF and WM samples were 0.905 and
228 0.910, respectively, with pixels having values less than these thresholds being designated as melamine
229 rich. The resultant binary images for NF and WM mixture samples are shown in Figure 5c and 5d for
230 all the samples under investigation (sets 1-4). Both NF and WM showed similar classification results.
231 The spatial distribution of the melamine particles in milk powders are clearly displayed in these
232 resultant images. Pixels with values larger than 0.905 for NF and 0.910 for WM were classified as
233 predominantly milk powder, and appear as light blue areas in the classification images. Red dots
234 scattered inside the light blue areas represent the melamine rich regions isolated from the milk

background. No melamine rich pixels (false positives) are found in either the pure NF or pure WM binary images. Also, no milk powder (i.e., melamine deficient, false negatives) pixels are found in the pure melamine binary images. The number of melamine rich pixels progressively increased with melamine concentration for the ten mixture samples. Table 1 shows the numbers of pixels identified as melamine rich by the $B_{1447/1466}$ value for the NF and WM melamine-milk powder mixtures. The results of the ratio algorithm in this investigation, in comparison to analyses based on spectral similarity (Fu et al., 2014), exhibited no false negatives for the lowest concentrations (0.02-0.08%) of the mixture samples. This may also be attributable to a more thorough level of mixing by the acoustic mixing device in this study as opposed to that by a simple shaker in the earlier study.

To further assess the band ratio results, spectral features of the pixels classified as melamine and milk pixels were compared with the recent paper of Fu et al. (2014). The mean spectra of melamine rich pixels and melamine deficient pixels classified by the $B_{1447/1466}$ threshold calculated for each mixture sample, for all concentrations of the NF and WM mixture samples, are shown in Figure 6a and 6b, respectively. Because of the nearly identical classification results of the four sets, only the results for one set are shown, and the spectral region is limited to 1440–1560 nm to highlight the traits of the distinct melamine absorption features. Individual pixels classified as melamine rich under investigation (dashed lines) exhibited strong absorption peaks around 1466 nm and 1490 nm, while those of milk (melamine deficient) pixels (solid lines) do not show the features. These observations suggest that the band ratio method based on NIR hyperspectral imaging can accurately classify/detect pixels with

melamine in a range of relatively low mixture concentrations (as low as 0.02%). Note that the spectrum of each pixel identified as melamine rich only exhibited a partial resemblance to pure melamine spectrum owing to integration of the reflectance and scattering response of both melamine and milk particles.

4.3 Quantification of melamine

The number of the melamine rich pixels in Figure 5 generally increases with the increasing concentration of the melamine. Table 2 shows the correlation coefficients between number of melamine rich pixels and melamine concentration for each of the four sets. Both NF and WM mixtures show a linear relationship with the average correlation coefficients (r) of 0.990 and 0.988, respectively. Figure 7 shows the response between number of melamine rich pixels detected by band ratio and the experimental concentrations of milk-melamine mixture for NF (7a) and WM (7b). The correlation coefficients (r) between the melamine concentrations and the pixel numbers of melamine were 0.994 and 0.990 for NF and WM mixtures of set 1, respectively, with sets 2-4 demonstrating similar results. The high correlation suggests great potential for using the resultant images for quantitative assessment of melamine adulterant concentrations in milk powders.

This method, with a studied mass concentration range of 200 to 10000 mg/kg, is operating at melamine concentrations that are well above the common international limit of 2.5 mg/kg. The choice of a higher range was based on the supposition that deliberate adulteration of milk powder with melamine is economically motivated. At a nominal protein content of 25.0%, whole milk powder that

is adulterated at the studied range will result in powder with apparent protein content range of 25.1 to 29.0%. Despite possessing contamination levels that are orders of magnitude above allowable, the economic profit from such a range is either quite low or, at the lowest end of the range, insignificant and, in either case, not worth the risk.

5. Conclusion

This research demonstrated that NIR hyperspectral imaging coupled with a simple band ratio classification algorithm can be used to detect melamine adulterant in both nonfat milk and whole milk powder. The NIR hyperspectral imaging system can provide spectral and spatial information to identify and map the melamine particles mixed into milk powders. Optimal two-band selection for pure milk and pure melamine is an important step to develop the classification model for milk-melamine mixtures. These band ratio images can then be used to establish a single threshold for deciding whether a pixel is rich or deficient in melamine. Resultant binary images can be used to visualize the spatial distribution of melamine adulterant regions in the investigated milk powders. A highly linear relationship between the number of melamine rich pixels and the melamine concentration demonstrates the potential of the NIR hyperspectral imaging method for quantitative analysis of the melamine in milk powders over a concentration range of 0.02 to 1.00% (w/w). We suggest that the detection algorithm developed in this study could be adapted to other cases of single-chemical adulteration of milk powder and other powdered food for rapid and accurate authentication.

292

293 **Acknowledgements**

294 In addition to base funding from the USDA-ARS, this work was supported by a grant from the
295 Agenda Program (PJ009399), Rural Development Administration, Suwon, Korea, the National Natural
296 Science Foundation of China (Grant no. 61271384, 61275155), the 111 Project (B12018) of China, and
297 the Qing Lan Project.

298

299 **References**

- 300 Abrams, J.M. (1983). Remote sensing for porphyry copper deposits in Southern Arizona. *Economic*
301 *Geology*, 78, 591-604.
- 302 Balabin, R.M. & Smirnov, S.V. (2011). Melamine detection by mid-and near-infrared (MIR/NIR)
303 spectroscopy: a quick and sensitive method for dairy products analysis including liquid milk,
304 infant formula, and milk powder. *Talanta*, 85(1), 562-568.
- 305 Brown, C.A., Jeong, K.S., Poppenga, R.H., Puschner, B., Miller, D.M., Ellis, A.E., Kang, K.I., Sum, S.,
306 Cistola, A.M., & Brown, S.A. (2007). Outbreaks of renal failure associated with melamine and
307 cyanuric acid in dogs and cats in 2004 and 2007. *Journal of Veterinary Diagnostic*
308 *Investigation*, 19(5): 525-531.
- 309 Chan, E.Y.Y., Griffiths, S.M., & Chan, C.W. (2008). Public-health risks of melamine in milk products.
310 *Lancet*, 372, 1444-1445.
- 311 Chao, Y.Y., Lee, C.T., Wei, Y.T., Kou, H.S., & Huang, Y.L. (2011). Using an on-line
312 microdialysis/HPLC system for the simultaneous determination of melamine and cyanuric acid
313 in non-dairy creamer. *Analytica Chimica Acta*, 1, 56-61.
- 314 Chen, Z. & Yan, Z. (2009). Simultaneous determination of melamine and 5-hydroxymethylfurfural in
315 milk by capillary electrophoresis with diode array detection. *Journal of Agricultural and Food*
316 *Chemistry*, 57(19), 8742-8747.

317 Ehling, S., Tefera, S. H. O. I. P., & Ho, I. P. (2007). High-performance liquid chromatographic method
 318 for the simultaneous detection of the adulteration of cereal flours with melamine and related
 319 triazine by-products ammeline, ammelide, and cyanuric acid. *Food additives and*
 320 *contaminants*, 24, 1319-1325.

321 Fernández Pierna, J.A., Vincke, D., Dardenne, P., Yang, Z., Han, L., & Baeten, V. (2014). Line scan
 322 hyperspectral imaging spectroscopy for the early detection of melamine and cyanuric acid in
 323 feed. *Journal of Near Infrared Spectroscopy*, 22(2), 103-112.

324 Filazi, A., Sireli, U.T., Ekici, H., & Karagoz, A. (2012). Determination of melamine in milk and dairy
 325 products by high performance liquid chromatography. *Journal of Dairy Science*, 95(2), 602 -
 326 608.

327 Filigenzi, M.S., Puschner, B., Aston, L.S., & Poppenga, R.H. (2008). Diagnostic determination of
 328 melamine and related compounds in kidney tissue by liquid chromatography/tandem mass
 329 spectrometry. *Journal of Agricultural and Food Chemistry*, 56(17), 7593 - 7599.

330 Fu, X., Kim, M.S., Chao, K., Qin, J., Lim, J., Lee, H., Garrido-Varo, A., Pérez-Marín, D., & Ying, Y.
 331 (2014). Detection of melamine in milk powders based on NIR hyperspectral imaging and
 332 spectral similarity analysis. *Journal of Food Engineering*, 124, 97-104.

333 GB/T 22388-2008. Determination of melamine in raw milk and dairy products. *Chinese National*
 334 *Standards*.

- 335 Garber, E.A.E. (2008). Detection of melamine using commercial enzyme-linked immunosorbent assay
336 technology, *Journal of Food Protection*, 71,590-594.
- 337 Guo, Z., Gai, P., Hao, T., Wang, S., Wei, D., & Gan, N. (2011). Determination of melamine in dairy
338 products by an electrochemiluminescent method combined with solid-phase extraction. *Talanta*,
339 83, 1736-1741.
- 340 Huang, Y., Min, S., Duan, J., Wu, L., & Li, Q. (2014). Identification of additive components in
341 powdered milk by NIR imaging methods. *Food Chemistry*, 14, 278-283.
- 342 Jawaid, S., Talpur, F.N., Sherazi, S.T.H, Nizamani, S.M., & Khaskheli, A.A. (2013). Rapid detection of
343 melamine adulteration in dairy milk by SB-ATR-Fourier transform infrared spectroscopy.
344 *Food Chemistry*, 141, 3066-3071.
- 345 Kim, M.S., Chen Y.R., & Cho, B.K. (2007). Hyperspectral reflectance and fluorescence line-scan
346 imaging for online detect and fecal contamination inspection of apples. *Sensing and*
347 *Instrumentation for Food Quality and Safety*, 1(3), 151-159.
- 348 Kim, M.S., Delwiche, S.R., Chao, K., Garrido-Varo, A., Perez-Marin, D., Lefcourt, A.M., & Chan, D.
349 2012). Visible to SWIR hyperspectral imaging for produce safety and quality evaluation.
350 *Sensing and Instrumentation for Food Quality and Safety*, 5:155-164.
- 351 Landgrebe, D. (2002). Hyperspectral image data analysis. *IEEE Signal Processing Magazine*, 19, 17-28.

352 Liu, Y., Chao, K., Kim, M.S., Tuschel, D., Olkhovyk, O., and & Priore, R. (2009). Potential of Raman
 353 spectroscopy and imaging methods for rapid and routine screening of the presence of melamine
 354 in animal feed and foods. *Applied Spectroscopy*, 63(4): 477-480.

355 Mauer, L.J., Chernyshova, A.A., Hiatt, A., Deering, A., & Davis, R. (2009). Melamine detection in
 356 infant formula powder using near- and mid infrared spectroscopy. *Journal of Agricultural and*
 357 *Food Chemistry*, 57(10), 3974-3980.

358 Muñiz-Valencia, R., Ceballos-Magaña, Rosales-Martinez, D., Gonzalo-Lumbreras, R., Santos-Montes,
 359 A., Cubedo-Fernandez-Tapiella, A., & Izquierdo-Homillos, C. 2008). Method development
 360 and validation for melamine and its derivatives in rice concentrates by liquid chromatography.
 361 Application to animal feed samples. *Analytical and Bionanalytical Chemistry*, 392, 523-531

362 Nagarajan, R. Singh, P., & Mehrotra, R. (2006). Direct determination of moisture in powder milk using
 363 near infrared spectroscopy. *Journal of Automated Methods and Management in Chemistry*,
 364 Article ID 51342, 1-4.

365 Pietrzyk, A., Kutner, W., Chitta, R., Zandler, M.E., D'Souza, F., Sannicolo, F., & Mussini P.R. (2009).
 366 Melamine acoustic chemosensor based on molecularly imprinted polymer film. *Analytical*
 367 *Chemistry*, 81, 10061-10070.

368 Puschner, B., Poppenga, R.H., Lowenstine, L.J., Filigenzi, M.S., & Pesavento, P.A (2007). Assessment
 369 of melamine and cyanuric acid toxicity in cats. *Journal of Veterinary Diagnostic Investigation*,
 370 19, 616-624.

371 Sun, F., Ma, W., Xu, L., Zhu, Y., Liu, L., Peng, C., Wang, L., Kuang, H., & Xu, C. (2010). Analytical
 372 methods and recent developments in the detection of melamine. *Trends in Analytical*
 373 *Chemistry*, 29(11), 1239-1249.

374 Tsai, T.H., Thiagarajan, S., & Chen S.M. (2010). Detection of melamine in milk powder and human
 375 urine. *Journal of Agricultural and Food Chemistry*, 58, 4537-4544.

376 Wang, Z., Chen, D., Gao, X., & Song, Z. (2009). Subpicogram detection of melamine in milk products
 377 using a luminol-myoglobin chemiluminescence system. *Journal of Agricultural and Food*
 378 *Chemistry*, 57 (9), 344-3469.

379 WHO (World Health Organization) (2011). Toxicological and health aspects of melamine and cyanuric
 380 acid. (accessed 01.10.11).
 381 (http://www.whqlibdoc.who.int/publications/2009/9789241597951_eng.pdf).

382 Yin, W., Liu, J., Zhang, T., Li, W., Liu, W., Meng, M., He, F., Wan, Y., Feng, C., Wang, S., Lu, X., &
 383 Xi, R. (2010). Preparation of monoclonal antibody for melamine and development of an
 384 indirect competitive ELISA for melamine detection in raw milk, milk powder, and animal
 385 feeds. *Journal of Agricultural and Food Chemistry*, 58, 8152-8157.

386

387 **Figure Captions**

388 **Fig. 1** Schematic of the NIR hyperspectral imaging system for acquiring reflectance images of
389 powder samples.

390 **Fig. 2** Representative mean spectra of pure milk, pure melamine, and milk-melamine mixtures
391 with different concentrations of melamine (25600 pixels) (a) nonfat milk (NF) and (b)
392 whole milk (WM), and (c) the enlarged view of normalized/mean-centered spectra of
393 milk-melamine mixtures around the melamine peak of 1466.3 nm.

394 **Fig. 3** Representative NIR hyperspectral images at four selected wavelengths for pure milk, pure
395 melamine, and milk-melamine mixtures: (a) nonfat milk and (b) whole milk.

396 **Fig. 4** Individual raw spectra obtained from set 1 of the concentration 0.6% melamine mixture
397 sample (25600 pixels): (a) nonfat milk and (b) whole milk.

398 **Fig. 5** Images of pure milk, pure melamine and milk-melamine mixtures with different
399 concentrations of melamine: (a) band ratio images ($B_{1447/1466}$) of nonfat milk, (b) band
400 ratio images ($B_{1447/1466}$) of whole milk, (c) resultant images for identification of melamine
401 particles of nonfat milk, and (d) resultant images for identification of melamine particles
402 of whole milk.

403 **Fig. 6** Mean spectra of milk pixels and melamine pixels that were identified by band ratio
404 analysis of the NIR hyperspectral images: (a) nonfat milk and (b) whole milk.

405 **Fig. 7** Relationship between the number of melamine pixels detected by band ratio and the
406 experimental concentrations of milk-melamine mixture: (a) nonfat and (b) whole milk.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Table 1 Number of ROI pixels identified as containing melamine by the band ratio $(B_{1447/1466})$ for the melamine-milk powder mixtures of nonfat milk (NF) and whole milk (WM), containing melamine at concentrations between 0.02% and 1.00%

Melamine Concentration		0.02%	0.04%	0.06%	0.08%	0.10%	0.20%	0.40%	0.60%	0.80%	1.00%
NF	Set 1	14	28	16	30	32	84	174	350	391	537
	Set 2	6	12	18	41	44	57	166	246	418	519
	Set 3	13	12	11	25	27	115	149	281	360	371
	Set 4	12	24	28	24	35	58	126	333	382	546
	Average	11	19	18	30	35	79	154	303	388	493
WM	Set 1	4	11	16	34	26	58	217	296	539	611
	Set 2	5	14	24	27	28	71	131	231	480	630
	Set 3	6	17	25	31	27	57	170	311	509	599
	Set 4	17	14	11	13	37	65	204	353	428	563
	Average	8	14	19	26	30	63	181	298	489	601

430 **Table 2 Correlation coefficients of nonfat milk (NF) and whole milk (WM)**

431

Correlation Coefficients		
Milk	NF	WM
Set 1	0.994	0.990
Set 2	0.992	0.974
Set 3	0.986	0.991
Set 4	0.986	0.996
Average	0.990	0.988

432