Rapid identification of adulteration of milk by near infrared spectroscopy

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Introduction

Food quality and safety is very important for human health. Cow milk adulteration is one of the most common types of food fraud. It is simply achieved by adding less-expensive materials, such as water, wheat, dextrin and melamine, into natural milk. The Kjeldahl nitrogen method has been widely applied to determine protein content of milk. Melamine, an organic chemical material, is illegally added to milks to increase nitrogen (protein) content of the milk because it contains six nitrogen atoms (about 67 % nitrogen). Long-term or repeated intake of melamine will cause damage to the kidneys and bladder, and lead to kidney-stones in a human or animal body.

The standard methods of detecting melamine are HPLC, LC-MS/MS and GC-MS or GC-MS/MS.[1] But these procedures are tedious and time consuming. The methods are not suitable for on-line and quick detection of adulterated milk. Near Infrared spectroscopy (NIRS) in combination with chemometrics is widely employed in food analysis, including milk examination.[2–5] The near infrared (NIR) technique has also been successfully applied to classify and discriminate matrices of food,[6] and it exhibits good performance on qualitative pattern recognition. Kasemsumran *et al*.[7] also indicate that NIR spectroscopy can be used to detect water or whey adulterants and their contents in milk samples. In the present work, we try to establish an NIR model for rapidly detecting adulteration of milk by adding water, melamine and dextrin.

Materials and methods

Samples

110 raw milk samples were collected from 110 operations in Shanghai. 110 adulterated milk samples were prepared by adding different amounts (1 %, 5 % and 10 %) of aqueous (water)

solutions containing dextrin and melamine (WDM solution). These adulterated milk samples contained 1 ppm, 5 ppm and 10 ppm of melamine, respectively.

Method for evaluating quality of NIR spectra

In order to evaluate the quality of the NIR spectra of a raw milk sample, we repeatedly tested a sample and named the spectra as repeat spectra. In theory, all repeat spectra should completely coincide with one another, i.e. the standard variance spectra of repeat spectra (SVSRS) should be a horizontal line with zero value. In practice, zero values of SVSRS are impossible to achieve because of measurement errors, instrument and environment noise, and other factors. We judged the effect of these factors on spectral quality according to SVSRS, which was calculated as follows:

$$SVSRS(j) = \sqrt{\frac{\sum_{i=1}^{n}(X_{ij} - \overline{X_j})^2}{n-1}}, (j = 1,2,\cdots p) \tag{1}$$

where p is the spectrum point number, n the times of repeatedly testing a sample, X_{ij} value of the j-th spectrum point of the i-th repeat spectrum and $\overline{X}_j$, the j-th value of the average spec-

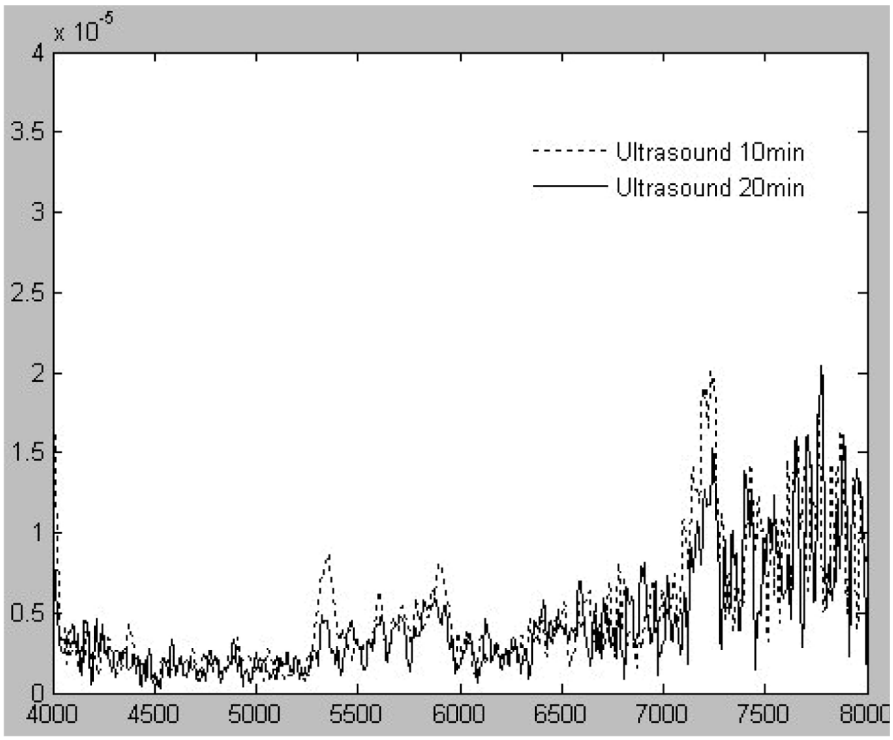

Figure 1. Standard variance spectra of a sample treated under different ultrasound times.

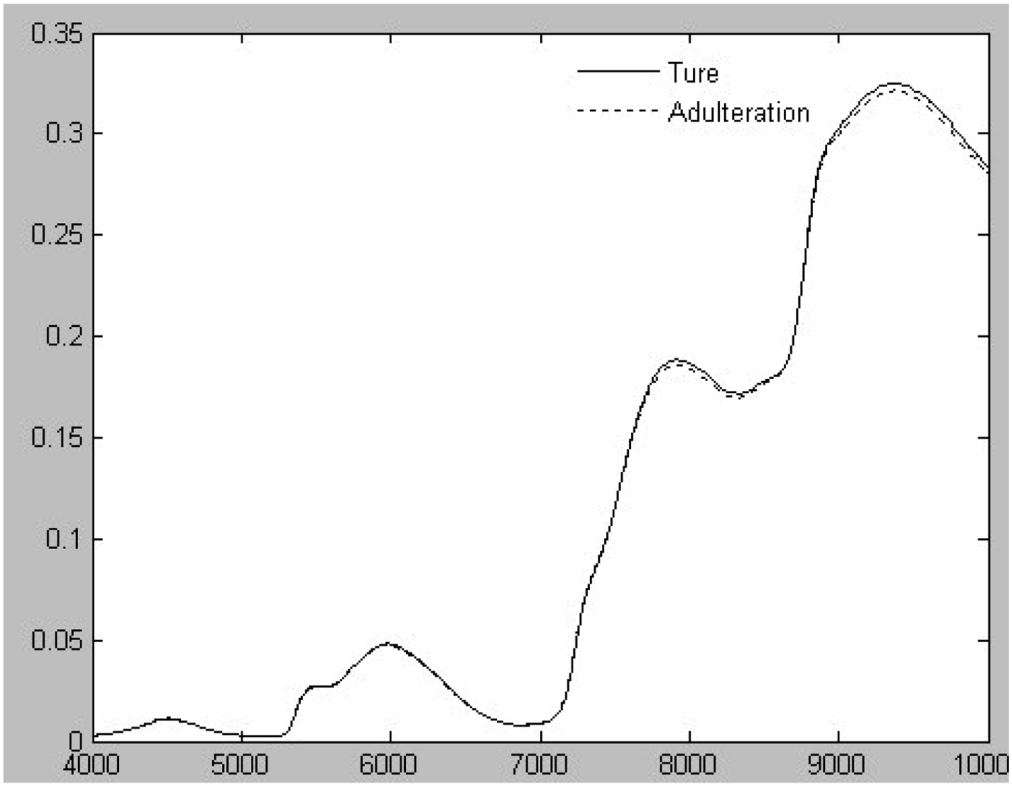

Figure 2. Reflectance average spectra of true (solid line) and adulterated (dotted line) milk samples.

trum of repeat spectra. By means of SVSRS, we chose the best measurement conditions and test mode for NIRS application.

NIR spectra acquisition

Prior to NIR measurement, every sample was placed in an ultrasonic cleaner (180 W, 40 KHz) for 5–20 min at 40°C to homogenise it. The milk samples were put into a glass-surface vessel at a level of 2/3 volume. The reflectance mode was used to detect NIR spectra of the 220 samples in the region of 4000–10000 cm^{-1} (NIRFlex-N500, InGaAs detector, diffuse reflectance accessory, Buchi Co., Swiss). Each spectrum was the average of 60 scanned interferograms at 8 cm^{-1} resolutions. All the spectra were recorded as log(1/R), using a ceramic reference standard.

Spectral analysis

One-fourth of the samples, uniformly selected, were used as a validation sample set. Four classification methods, discriminant partial least squares (DPLS),[8] LDA, KNN and Improved and simplified KNN (IS-KNN) method[9] were applied to classify the cow milk, and milk adulterated

Table 1. Average classification results of four methods based on NIR spectra after different pretreatment.

Sample set	DPLS			LDA			KNN			IS-KNN		
	AR%	Pretr.	LV	AR%	Pretr.	PC	AR%	Pretr.	K	AR%	Pretr.	PC
Set 1	66.36	Original	16	61.36	Original	11	67.27	SNV	3	89.09	SNV	44
Set 2	57.64	Original	17	74.33	SNV	5	78.47	SNV/ MSC	3	93.75	SNV	31
Set 3	59.46	SNV	20	77.08	Firstder.	6	78.38	SNV/ MSC	13	85.81	SNV	37
Set 4	52.08	SNV/ MSC	19	76.39	Firstder.	5	76.39	SNV/ MSC	11	81.94	SNV	34

Footnote: Set 1 consists of the 110 true milks and 110 adulterated milks; Set 2, 3 and 4 consists of the 110 true milks and 34 adulterated milks with high WDM concentration, 38 adulterated milks with medium WDM concentration, 34 adulterated milks with low WDM concentration, respectively; AR= average number of samples correctly classified /total validation sample number; Pretr. means pretreatment method of NIR spectra; LV, PC and K stand for number of latent variables in DPLS, principal components in LDA and IS-KNN and nearest neighbor samples in KNN, respectively.

by water, dextrin and melamine, with original, first derivative, MSC and SNV pretreated NIR spectra, in the region of 4000–10000 cm^{-1}. All algorithms were compiled on MATLAB ver. 7.0 (The Math-Works, USA). Results and Discussion

Effect of sample pretreatment on quality of spectra

Figure 1 confirms that standard variance spectra of the sample tested repeatedly 5 times, and ultrasonically treated for 20 min at 40°C, was the most suitable. Accordingly all samples were put in the ultrasonic cleaner for 20 min at 40°C before collecting the NIR spectra.

Table 2. ISKNN average classification results calculated from SNV NIR spectra.

Sample set	Wavelength (cm^{-1})	AR%	Number of validation samples					
			True milk for validation			Adulterated milk for validation		
			Correct	Wrong	ARt%	Correct	Wrong	ARf%
Set 1	4000–10000	89.09	24.25	3.25	88.18	24.74	2.76	89.96
Set 2	4000–10000	93.75	26.00	1.75	94.55	7.75	0.75	91.18
Set 3	4000–10000	85.81	23.25	4.25	84.55	8.50	1.00	89.47
Set 4	4000–10000	81.94	23.25	4.25	84.55	6.25	2.25	73.53

Footnote: The data in column 4, 5 and 7, 8 are the average numbers of samples; ARt and ARf is average validation accuracy ratio for true and adulterated milk samples, respectively.

Validation results by the four pattern recognitions

Figure 2 illustrates that there was no obvious difference between true and adulterated milk samples, except in the region of 8000–10000 cm^{-1}.

The comparison of IS-KNN and PLS-DA, LDA and KNN with different pretreatments of spectra indicated that IS-KNN with SNV spectra gave the best classification (see Table 1).

Table 2 shows that when all adulterated milks with different WDM concentration were compared with true milks, the total average validation accuracy was 89 %. The average validation ratio for total samples, true and adulterated milks in set 2, 3 and 4 decreased with the decrease of WDM concentration in adulterated milks.

The data reported in this paper indicate that it is feasible to discriminate adulterated milk by NIR reflectance spectra after appropriate sample and spectra pretreatments.

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