JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Application of Hand-Held and Portable Infrared Spectrometers in Bovine Milk Analysis

Poliana M. Santos,[†,§] Edenir R. Pereira-Filho,[§] and Luis E. Rodriguez-Saona*[,†]

[†]Department of Food Science and Technology, The Ohio State University, 110 Parker Food Science and Technology Building, 2015 Fyffe Road, Columbus, Ohio 43210, United States

[§]Department of Chemistry, Federal University of São Carlos, Rodovia Washington Luiz, km 235, São Carlos, SP, Brazil

ABSTRACT: A simple and fast method for the detection and quantification of milk adulteration was developed using portable and hand-held infrared (IR) spectrometers. Milk samples were purchased from local supermarkets (Columbus, OH, USA) and spiked with tap water, whey, hydrogen peroxide, synthetic urine, urea, and synthetic milk in different concentrations. Spectral data were collected using mid-infrared (MIR) and near-infrared (NIR) spectrometers. Soft independent modeling of class analogy (SIMCA) classification models exhibited tight and well-separated clusters allowing the discrimination of control from adulterated milk samples. Partial least-squares regression (PLSR) was used to estimate adulteration levels, and results showed high coefficients of determination (R^2) and low standard errors of prediction (SEP). Classification and quantification models indicated that the tested MIR systems were superior to NIR systems in monitoring milk adulteration. This method can be potentially used as an alternative to traditional methods due to their simplicity, sensitivity, low energy cost, and portability.

KEYWORDS: *hand-held and portable spectrometers, NIR, MIR, milk adulteration, multivariate analysis*

INTRODUCTION

Milk provides essential nutrients (water, carbohydrate, fat, protein, minerals, and vitamins) of great nutritional relevance for humans, particularly during childhood.[1−3] In 2007−2008, the problem of milk adulteration gained substantial notoriety with the addition of melamine.[4] Melamine was intentionally used to increase the concentration of apparent protein content in milk as the Kjeldahl standard protein assay cannot differentiate between protein nitrogen and nonprotein nitrogen. Nowadays, the occurrence of milk adulteration is a major issue in the dairy industry and has been causing concerns among costumers and food manufacturers. Milk is one of the seven most common targets for adulteration,[5] usually accomplished by the addition of water, whey, sodium hydroxide (caustic soda), urea, and melamine, among others.[6,7] The reason for adulteration is generally to increase the volume, mask inferior quality, and replace the authentic substances for the seller's economic gain. In addition, the adulterants used in food fraud are often unconventional and designed to avoid detection through routine analysis.[5] Different strategies have been evaluated in recent years for the identification and quantification of milk adulteration,[8−12] but there is a continuing requirement for rapid, accurate, and automated methods to control milk quality. Fingerprinting approaches for untargeted detection of economic adulteration of milk can offer the dairy industry the ability to detect the presence of known and unknown contaminants. Among these technologies, infrared spectroscopy allows for the rapid, high-throughput, and nondestructive analysis of a wide range of sample types producing a characteristic chemical "fingerprint" with a unique infrared profile.[13] Nuclear magnetic resonance (NMR) spectroscopy as well as a range of mass spectrometry (MS) techniques provides highest selectivity and specificity for screening raw materials[13] but require costly instrumentation and are less amenable to implementation in quality control laboratories at manufacturing facilities.

Miniaturization of vibrational spectroscopy components has allowed the development of portable or hand-held systems[14] offering simplicity, speed, selectivity, and performance similar to those of benchtop instruments found in the laboratory. Portable and hand-held infrared spectrometers (IR) represent an ideal tool in food quality control, providing sensitivity and portability for in situ analysis.[15] Examples of application of portable and hand-held near-infrared (NIR) and mid-infrared (MIR) technologies in food analysis include the prediction of quality parameters in intact nectarines[16] and oranges,[17] quantification of trans fat in edible oils,[18] and monitoring of oil oxidative stability[19] and mineral fortification in whole grain cornmeal.[20] These results have demonstrated that this technique allowed the development of calibration and classification models with excellent performance statistics.

The objective of this study was to examine the feasibility and compare the performance of hand-held and portable IR spectrometers combined with multivariate analysis to identify and quantify milk adulteration by the addition of tap water, whey, synthetic milk, synthetic urine, urea, and hydrogen peroxide. These adulterants were selected on the basis of reported adulterated milk concerns in countries such as Brazil,[6,7] India,[21] and Pakistan[22] by dilution with water and/or addition of low-cost synthetic milk (combination of vegetable oil, urea, detergents, and water), urea to contribute nitrogen and enhance milk's heat stability, synthetic urine as source of nitrogen, hydrogen peroxide as preservative to

Received: September 6, 2012
Revised: January 14, 2013
Accepted: January 22, 2013
Published: January 22, 2013

enhance shelf life, and whey to increase solids. Qualitative and quantitative models were developed by using pattern recognition techniques including soft independent modeling of class analogy (SIMCA) and partial least-squares regression (PLSR) algorithm, respectively.

MATERIALS AND METHODS

Milk Samples. Milk samples were purchased at a local supermarket (Columbus, OH, USA). Samples were adulterated by the addition of tap water, whey, synthetic milk,[23] synthetic urine,[24] urea, and hydrogen peroxide. All milk samples were spiked with targeted adulterant solutions and followed the same dilution process ranging from 3 to 50% v/v. The concentration of adulterant spiked into milk samples was calculated and ranged from 1.87 to 30 g/L for whey, from 0.78 to 12.5 g/L urea for synthetic urine and urea, from 0.05 to 0.8 g/L urea for synthetic milk, and from 0.009 to 0.15 g/L for hydrogen peroxide.

Overall, milk samples from the same Ohio producer and including different production lots (4−9) were adulterated with one of six adulterants (tap water, whey, urea, hydrogen peroxide, synthetic milk, and synthetic urine) at five levels of adulteration, resulting in 30 adulterated samples per lot. Control milk samples were evaluated from all production lots using three independent replicates per bottle, giving a total of 27 (9 (lots) × 3 (replicate)) control samples. Samples were stored in a freezer at −15 °C before the analysis. Besides direct measurements of milk samples, a fat extraction procedure was evaluated to remove matrix interferences by mixing equal amounts (v/v) of milk and chloroform; samples were centrifuged at 13000 rpm for 8 min (model 5415, Eppendorf, Westbury, NY, USA), and the supernatant (water-soluble phase) was applied onto the attenuated total reflectance (ATR) diamond crystal and vacuum-dried to form a film (dried sample).

Mid-Infrared Spectroscopy Measurements. *Hand-Held System.* Attenuated total reflectance (ATR-MIR) spectra were recorded on an interferometer-based hand-held MIR spectrometer (4200 FlexScan FTIR; Agilent Technologies Inc., Danbury, CT, USA), with a ZnSe beamsplitter, DTGS detector, and single-reflection diamond ATR sampling interface. The absorbance spectrum was obtained by rationing the sample single-beam spectrum against that of a blank optical path (reference spectrum). Two different strategies were investigated for the analysis of milk samples in this system. Spectra were obtained by (1) direct measurements of the *fluid samples*, or (2) milk samples were dried under vacuum for 1 min until a thin film was obtained (*dried sample*). For each spectrum an average of 64 scans were performed at a resolution of 4 cm^{-1}, over the 4000−650 cm^{-1} frequency range. A total of 837 spectra (31 (samples) × 9 (lots) × 3 (replicate)) were collected for the fluid milk, whereas 90 (6 (samples) × 5 (lots) × 3 (replicate)) spectra were collected for each adulterant using the dried milk samples.

Portable System. A Cary 630 FTIR spectrometer (Agilent Technologies Inc.) equipped with a liquid transmission, ZnSe beamsplitter, and DTGS detector was used to analyze fluid milk. Aliquots of fluid milk samples (0.025 mL) were placed on the zinc selenide (ZnSe) crystal, and MIR spectra were collectrf in the absorbance mode by using a DialPath transmission technology in the portable MIR selected at a 30 μm fixed path length created by an optical head that rotates into position, sandwiching the sample between two IR transparent ZnSe windows. The spectrum was collected in the frequency range of 4000−650 cm^{-1} using a 4 cm^{-1} resolution, and 64 interferograms were co-added to improve the signal-to-noise ratio. A total of 372 spectra (31 (samples) × 4 (lots) × 3 (replicate)) were collected for fluid milk samples. Infrared spectra of background and samples were observed on a personal computer using Agilent MicroLab PC software.

Near-Infrared Spectroscopy Measurements. *Benchtop System.* Fluid milk (1 mL) was dispensed in a 3 mm diameter Petri dish base (Perkin-Elmer, Norwalk, CT, USA) placed on the reflectance accessory for direct measurement by transflection using an aluminum diffuse reflector (Perkin-Elmer). The reflector contained integral spacers that allowed two passes of the beam through the sample to provide a total path length of 0.5 mm. Diffuse transflectance measurements were collected by using an Excalibur 3500 Fourier-Ttransform IR spectrometer (Varian Inc., Palo Alto, CA, USA) with a quartz beam splitter and a lead selenide (PbSe) detector equipped with a NIR integrating sphere diffuse reflectance accessory (Integrat IR, Pike Technologies, Madison, WI, USA) integrating sphere. Spectra were collected at 4 cm^{-1} resolution using the frequency range from 10000 to 4000 cm^{-1} and displayed in terms of absorbance. Interferograms of 64 scans were co-added. For each fluid sample, a total of three independent spectra were measured. The absorbance spectrum was obtained by rationing the sample spectrum against that of a blank optical path (reference spectrum). The instrument was continuously purged with CO_2 free dry air from a CO_2RP140 dryer (Domnick Hunter, Charlotte, NC, USA).

Hand-Held System. Fluid milk aliquots of each sample (0.2 mL) were placed into a glass vial, and NIR spectra were collected in a dispersive hand-held NIR (microPHAZIR, Thermo Fisher Scientific Inc., Wilmington, MA, USA) equipped with a single indium gallium arsenide (InGaAs) detector and using a liquid adapter accessory (Thermo Fisher Scientific Inc.). All diffuse reflectance spectra were computed at an optical resolution of 11 nm, spectral range from 1600 to 2400 nm (6250−4170 cm^{-1}), and 64 scans to increase the signal-to-noise ratio. Three replicate spectra were obtained from 31 samples (from 7 different lots), and a total of 756 spectra were used in the chemometrics analysis. A background reading was taken on an empty glass vial.

Multivariate Data Analysis. Multivariate analysis was used to develop qualitative and quantitative models with MIR and NIR data sets. The analysis was evaluated by using Pirouette software (version 4.0, Infometrix Inc., Woodville, WA, USA). Before the analysis, the spectra were normalized, the second derivative was calculated (Savitzky−Golay polynomial filter with a 25-point window), and then the data were mean centered.

Classification of control (unadulterated milk) from adulterated milk was done using the SIMCA algorithm. SIMCA is a supervised pattern recognition classification technique that requires previous knowledge about the category membership of samples.[25] The number of significant PCs was defined by means of a cross-validation procedure (leave-one-out). SIMCA assumes that the residuals are normally distributed and calculates object residual standard deviations (RSDs) and class RSDs using an F test.[26] Probability clouds (α = 0.05) are built around the clusters, allowing SIMCA to be used as a predictive modeling system. In addition, to provide a means of classifying objects, residuals provide valuable information regarding class homogeneity, separation between classes (interclass distance), and the relative strength of any given variable to model the structure of a class or to discriminate between classes (discriminating power). SIMCA's interclass distance (ICD) describes quantitatively the similarity or dissimilarity of the different classes, it being generally accepted that samples can be differentiated when ICD > 3.[27] The discriminating power of variables was used to eliminate noise from the data set, such that variables have both low discriminating power and modeling power. For development of SIMCA models, the data set was divided into a training set (75%) and a validation set (25%). SIMCA performance was examined in terms of discriminating power, class projections, misclassifications (percentage of samples correctly allocated to their real groups), and ICD.

PLSR was evaluated to quantify the concentration of adulterant in milk. Models were either obtained using the known levels of adulterants (g/L) or based on the dilution levels (v/v) as dependent variable. Prior to the analysis, the data set was divided into a training set (75%) and a validation set (25%). The validation set contained the same broad range of milk samples, in terms of concentration of adulterant spiked into milk samples, as the calibration set. Performances of the regression models were examined in terms of standard error of prediction (SEP) and coefficient of determination of calibration (R^2cal) and validation (R^2val). SEP is an estimate of the predictive ability of the model, and the coefficient of determination gives the proportion of variability of the property that is described by the model. X residuals and leverage were used for the evaluation of

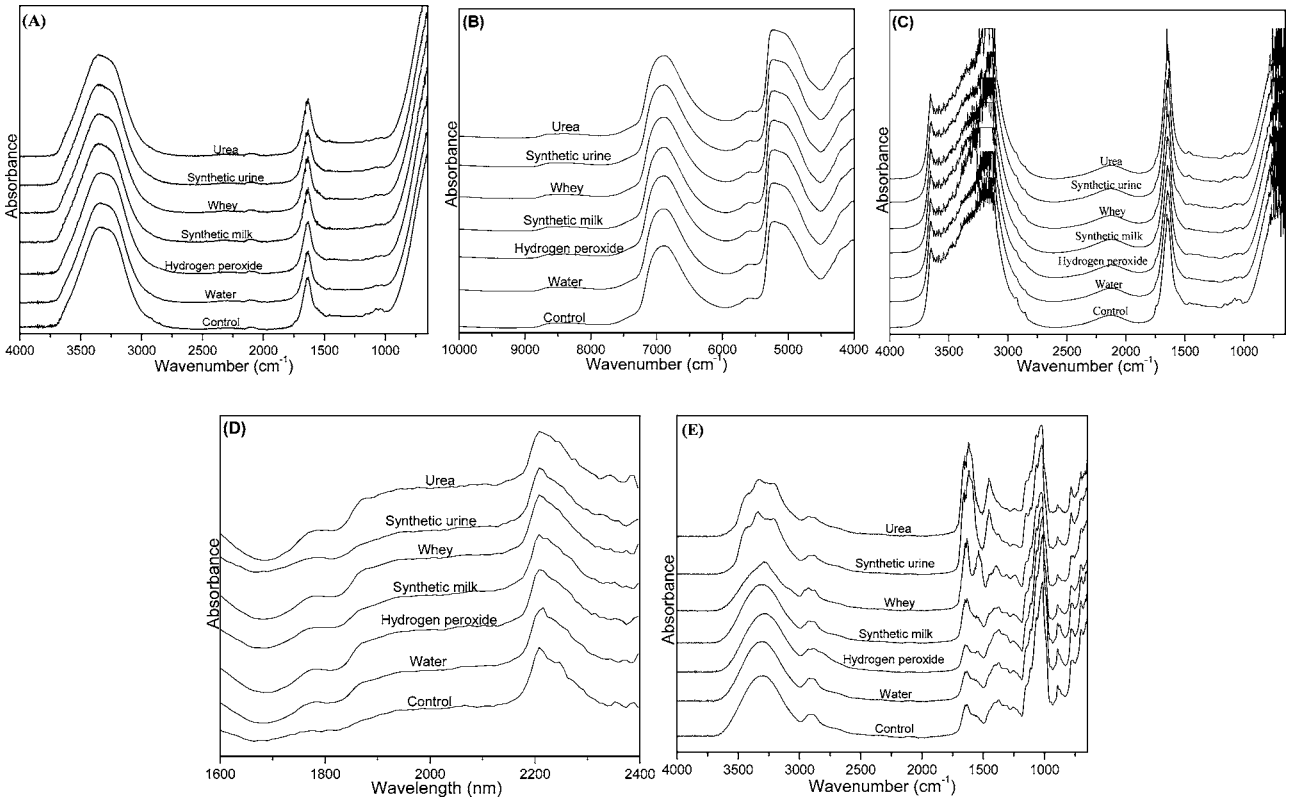

Figure 1. Spectra of control samples and milk spiked with water, hydrogen peroxide, synthetic milk, whey, and urea obtained in the (A) hand-held MIR (fluid milk), (B) benchtop NIR, (C) portable MIR, (D) hand-held NIR, and (E) hand-held MIR (dried milk) systems. Milk samples were spiked with the highest tested level of adulterant, that is, 50% v/v.

outliers. Any observation with abnormal residual or leverage was reanalyzed and eliminated if necessary, after which the calibration model was repeated.

RESULTS AND DISCUSSION

Spectral Characteristics. MIR and NIR spectra of control and milk spiked with water, whey, synthetic milk, synthetic urine, urea, and hydrogen peroxide are shown in the Figure 1. MIR (Figure 1A) and NIR (Figure 1B) spectra obtained with fluid milk samples showed two prominent absorption bands centered at 3300 and 1639 cm^{-1} (MIR) and at 7700 and 5000 cm^{-1} (NIR), associated with the strong O−H stretching vibrations (MIR) and the NIR overtone/combination modes of water.[28,29] The use of the DialPath transmission technology in the portable MIR (Figure 1C) showed signal saturation in the O−H absorption bands centered at 3300 cm^{-1} but an increase in the intensity of signal in the information-rich region from 1600 to 900 cm^{-1}. Spectra obtained with hand-held NIR (dispersive system) (Figure 1D) exhibited a major broad band centered at 2250 nm (O−H combination band) due to the short spectral range (from 1600 to 2400 nm) of the spectrometer.

The dried film milk extraction protocol resolved spectral features previously masked by water and fat signal (Figure 1E). Major spectral differences between the control samples and adulterated milk were evidenced from 1600 to 1200 cm^{-1}. Spectra of milk adulterated with whey showed two new prominent absorption bands at 1635 and 1530 cm^{-1} associated twith amide I (C═O stretching) and amide II (N—H bending/C—H stretching) group vibrations, respectively.[30] Whey is a mixture of β-lactoglobulin, α-lactalbumin, bovine serum albumin, and immunoglobulins[31] that causes qualitative and quantitative spectral changes to the milk protein profile as evidenced in Figure 1E. Samples adulterated with urea, synthetic milk, and urine showed a strong absorption at 1615 cm^{-1} attributed to the C═O absorption from urea (CH_4N_2O) and at 1454 cm^{-1} associated with the NH_4^+ deformation indicating urea decomposition.[32] Spectra of milk adulterated with hydrogen peroxide did not exhibit any visual difference from the control milk and can be attributed to the low levels of this substance used in the process to spike the control milk. Hydrogen peroxide is a strong oxidizing agent that is used to prevent physical changes over the lifetime of the product.[33] The dried film approach was not adequate for determining the water addition in milk samples because it was evaporated during the process of sample preparation. MIR spectra of the dried milk films also showed additional absorption bands at 3700−3200 cm^{-1} associated with O—H stretching vibrations of bound water, at 3000−2800 and 1852 cm^{-1} associated with the C—H stretching vibrations of residual lipids, at 1067 cm^{-1} of the phosphate group (O═P—O) from casein, and several absorption bands in the complex fingerprint region (1200−800 cm^{-1}) attributed to C—H bending, C—O—H in-plane bending, and C—O stretching vibrations of lipids, organic acids, amino acids, and carbohydrate derivatives.[34,35]

Classification Analysis. SIMCA classification performance showed that the tested NIR system exhibited poor discrimination power when compared to the MIR system. The SIMCA 3D plot obtained with the hand-held NIR system (Figure 2A) resolved only two clusters discriminating fluid milk samples adulterated at the highest levels, that is, ≥ 50% v/v, with an ICD of 2.9. Overall, SIMCA's ICD value for control and

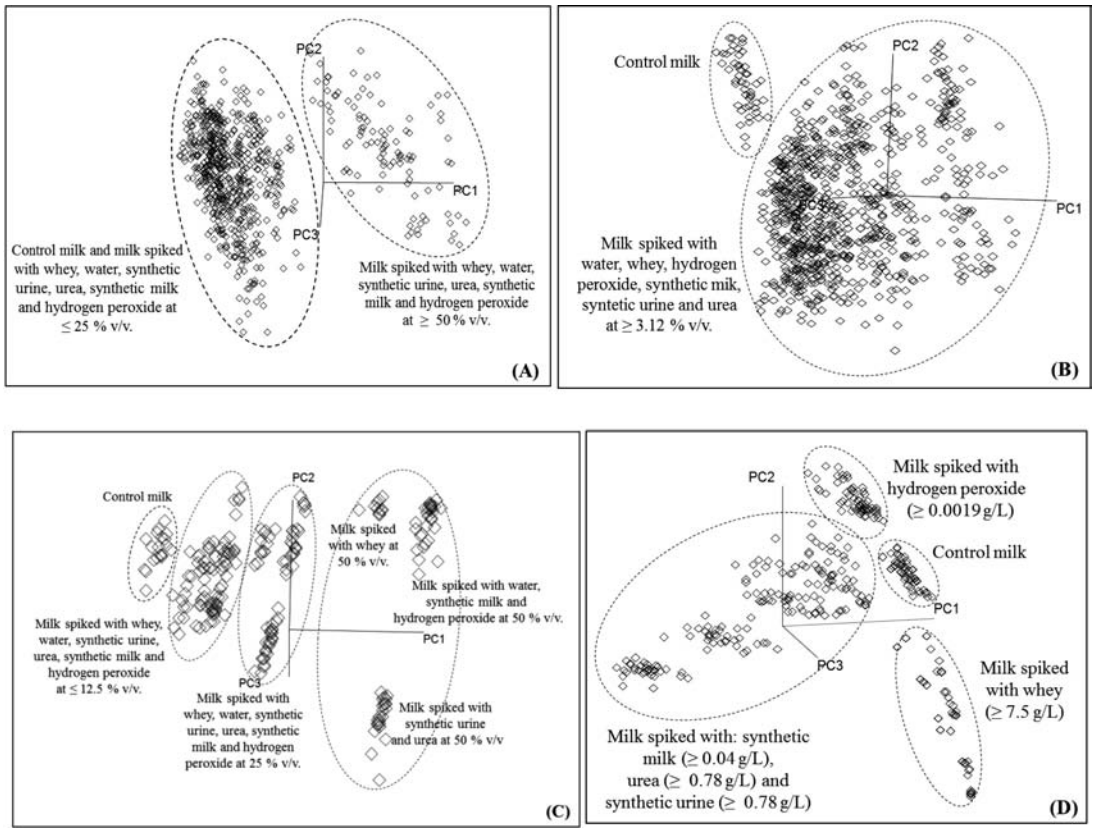

Figure 2. SIMCA 3D projections for data sets obtained in (A) hand-held NIR, (B) hand-held MIR (fluid milk), (C) portable MIR, and (D) hand-held MIR (dried milk) systems.

adulterated milk samples was 0.7, indicating similarity among the classes. Model validation using an independent set of samples gave 80% (control milk) and 56% (adulterated milk) correct classification, which means that about half of adulterated samples were incorrectly classified as control sample. The performance results for benchtop NIR showed marked variability of replicated measurements probably due to scattering effects of the milk samples. Milk contains light-scattering particles in the form of fat globules and protein micelles, and these particles can introduce light-scattering problems in NIR spectroscopy.[36] These effects prevented application of the benchtop NIR system to develop a qualitative model to discriminate milk samples.

SIMCA models obtained with the hand-held 4200 FlexScan (Figure 2B) and portable Cary 630 (Figure 2C) MIR systems showed good separation between the control and adulterated fluid milk samples. SIMCA class projections showed improved classification of control milk from adulterated samples by using the transmission (30 μm path length) as compared to the attenuated total reflectance (ATR) technique, with the former allowing clustering of spiked samples according to the level of adulteration. SIMCA's ICD value was 0.9 for control and adulterated milk samples by using the hand-held FlexScan system, showing that the model could not discriminate the milk samples. However, the portable Cary 630 system showed ICD values $\geq$ 4.0, permitting the discrimination ($p < 0.05$) of control samples from adulterated milk. It is important to note that with the portable Cary 630 system the clustering was dominated by the dilution procedure of samples (3−50% v/v), explaining the discrimination of samples on the basis of milk dilution and not only according to the presence of adulterant. ICD values ranged from 2.6 (for the lowest detectable level of adulterant) to 14.4 (for the highest detectable level of adulterant). Thus, our results showed that dilution of milk at levels $\geq$3% could be detected as tampered product. Model validation using an independent set of samples showed 70 and 100% correct classification of fluid milk by using the hand-held FlexScan and portable Cary 630 system, respectively.

The SIMCA model based on MIR (hand-held 4200 FlexScan) spectra obtained from dried milk sample (Figure 2D) exhibited well-separated clusters between control and adulterated milk samples, allowing clustering samples according to the type of adulterant used in the milk spiking process. Discrimination power showed that the major bands that contributed to the development of the classification models were in the region from 1800 to 1450 cm^{-1} associated with spectral changes in the milk protein profile related with the addition of the adulterants (whey, urea, and hydrogen peroxide). ICD values ranged from 2.7 to 8.7 among the control and adulterated milk samples (Table 1), permitting the discrimination ($p < 0.05$) of milk samples according to the type of adulterant. The SIMCA model discriminated the control milk samples from the milk spiked with whey, hydrogen peroxide, and synthetic milk when the concentrations of adulterants were >7.5, 0.0019, and 0.04 g/L, respectively. Synthetic urine and urea adulteration was detected at the lowest levels evaluated (0.78 g/L) from the control milk. ICD values among the samples adulterated with synthetic milk, synthetic urine, and urea were <2.0, indicating spectral similarities due to the presence of urea. SIMCA, using spectra collected from dried films, did not differentiate control samples from milk diluted with water at any level tested in this experiment.

Table 1. SIMCA Interclass Distance of Control and Adulterated Milk[a]

	control milk	whey	synth milk	synth urine	urea	H_2O_2
control milk	0.00					
whey	8.66	0.00				
synth milk	2.68	9.42	0.00			
synth urine	4.79	9.60	1.83	0.00		
urea	3.65	8.61	1.64	0.48	0.00	
H_2O_2	2.69	6.14	3.40	5.31	5.15	0.00

[a]These distances were collected in the hand-held 4200 FlexScan MIR from 1800 to 1450 cm^{-1} region with dried sample.

Misclassification performance of the validation set showed that 100 and 96% of dried milk samples were correctly classified as control or adulterated, respectively. None of the adulterated samples were predicted as control sample, and seven adulterated samples (4%) were not classified in any category. These results confirmed the ability of the model developed to discriminate milk samples not only on the basis of the presence of adulteration but also according to the type of adulterant used in the adulteration process.

Determination of the Levels of Adulterants in Spiked Milk Samples. PLSR analysis generated quantitative calibration models using the same spectral regions identified in the classification analysis (Table 2). Because the SIMCA classification for fluid milk indicated that the dilution process of control milk was driving the clustering of samples, we developed PLSR models based on the dilution levels instead of the concentration of specific adulterants. PLSR models showed a good correlation between the infrared estimated concentrations and the spiked adulterant levels for the fluid milk approach (Table 2). According to the statistic results, all MIR models showed performance superior to that of the hand-held NIR (microPhazir) system, with high coefficients of determination (R^2val > 0.92) and low SEP (Table 2). Because of the scattering effects observed with the benchtop NIR, quantitative models were not developed. The best performance models for fluid milk were obtained using the portable Cary 630 MIR system, with high R^2val values (>0.98) and low SEP, similar in magnitude to SECV (Table 2), indicating that the calibration model has accurate and reliable analytical performance. PLSR scatter plot (Figure 3) showed a good fit between the reference levels and MIR predicted values for the level of adulteration.

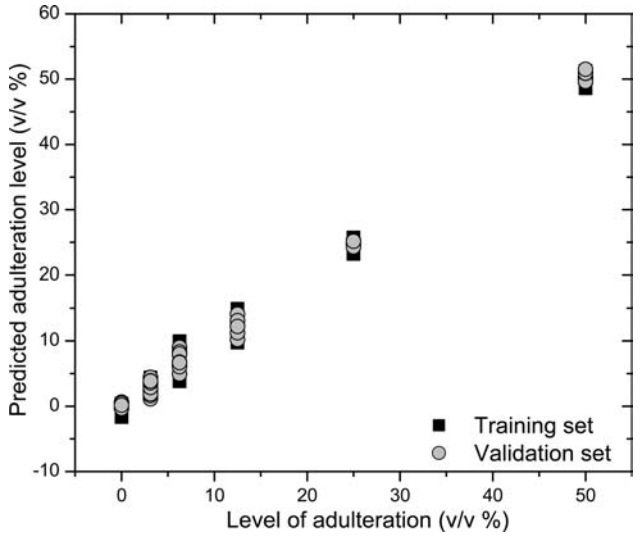

Figure 3. PLSR calibration curve for training (squares) and validation (circles) sets of control and milk spiked with tap water, whey, synthetic milk, synthetic urine, urea, and hydrogen peroxide. The spectra were collected in the portable Cary 630 MIR from fluid milk.

Conversely, PLSR models obtained with spectra collected from dried films were developed using the concentration of adulterant spiked into milk samples, as the effect of water was eliminated by vacuum-drying in the samples. The prediction ability of the models showed SEP values of 1.18 (whey), 0.009 (hydrogen peroxide), 0.028 (synthetic milk), 0.412 (synthetic urine), and 0.232 g/L (urea) using the FlexScan 4200 ATR portable spectrometer. These low SEP values can be associated with the presence of distinct and specific absorption signals for each adulterant used in the spiking process. Our regression modeling showed better prediction abilities than those described in the literature.[37,38]

Conclusion. In this paper the feasibility of using portable and hand-held IR spectrometers in conjunction with multivariate analysis was demonstrated for monitoring milk adulteration by tap water, whey, synthetic milk, synthetic urine, urea, and hydrogen peroxide. The procedure is simple, fast, robust, and conveniently adaptable to in situ analysis.

Table 2. Calibration and Cross-Validation Results of Multivariate Models Developed by Using Hand-Held and Portable NIR and MIR Spectrometers

	no. of milk lots	no. of samples	factors	SEC	SECV	SEP	R^2cal	R^2val
hand-held MicroPHAZIR Rx NIR[a] (1708−2300 nm)								
all adulterants	8	744	5	4.63	4.69	4.74	0.92	0.92
portable Cary 630 MIR[a] (1300−950 cm^{-1})								
all adulterants	4	372	4	0.74	0.76	0.83	0.98	0.98
hand-held 4200 FlexScan MIR[a] (1800−800 cm^{-1})								
all adulterants	9	837	5	4.05	4.12	4.18	0.94	0.92
hand-held 4200 FlexScan MIR[b] (1800−800 cm^{-1})								
whey	5	90	5	1.03	1.16	1.18	0.98	0.98
H_2O_2	5	90	4	0.008	0.009	0.009	0.96	0.94
synth milk	5	90	5	0.023	0.027	0.028	0.98	0.98
synth urine	5	90	4	0.333	0.364	0.412	0.98	0.98
urea	5	90	5	0.175	0.210	0.232	0.98	0.98

[a]PLS models obtained with fluid milk spectra. SEC and SECV values in % (v/v). [b]PLS model obtained with dried milk spectra. SEC and SECV values in g/L.

dx.doi.org/10.1021/jf303814g | J. Agric. Food Chem. 2013, 61, 1205−1211

Portable and hand-held MIR systems allowed the development of classification and quantification models with better discrimination power and prediction ability than NIR systems tested. The portable Cary 630 MIR allowed the quantification of adulteration in milk with high prediction ability (R^2val > 0.98 and SEP < 1%) with total time of <5 min without sample preparation. In addition, the hand-held 4200 FlexScan MIR spectrometer was able to identify the type of adulterant added to the milk due to the distinctive absorption signal in the spectra obtained from dried milk samples.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (614) 292-3339. Fax: +1 (614) 292-0218. E-mail: rodriguez-saona.1@osu.edu.

Funding

We acknowledge Brazilian agencies FAPESP (2009/01345-6), CAPES (1001/11-6), and CNPq and the Ohio Agricultural Research and Development Center for their financial support of this research.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Agilent Scientific for providing the portable spectrometer and technical support.

REFERENCES

(1) Jensen, R. G.; Blanc, B.; Patton, S. Particulate constituents in human and bovine milks. In *Handbook of Milk Composition*; Jensen, R. G., Ed.; Academic Press: San Diego, CA, 1995; pp 50−61.

(2) Hamann, J.; Krosmker, V. Potential of specific milk composition variables for cow health management. *Livest. Prod. Sci.* **1997**, *48*, 201−208.

(3) Dalgleish, D. G. Bovine milk protein properties and the manufacturing quality. *Livest. Prod. Sci.* **1992**, *35*, 75−93.

(4) Jackson, L. S. Chemical food safety issues in the United States: past, present, and future. *J. Agric. Food Chem.* **2009**, *57*, 8161−8170.

(5) Moore, J. C.; Spink, J.; Lipp, M. Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. *J. Food Sci.* **2012**, *77*, R118−R126.

(6) Kartheek, M.; Smith, A. A.; Muthu, A. K.; Manavalan, R. Determination of adulterants in food: a review. *J. Chem. Pharm. Res.* **2011**, *3*, 629−636.

(7) Borin, A.; Ferrão, M. F.; Mello, C.; Maretto, D. A.; Poppi, R. J. Least-squares support vector machines and near infrared spectroscopy for quantification of common adulterants in powdered milk. *Anal. Chim. Acta* **2006**, *579*, 25−32.

(8) Das, S.; Sivaramakrishna, M.; Biswas, K.; Goswami, B. Performance study of a 'constant phase angle based' impedance sensor to detect milk adulteration. *Sensor Actuator A* **2011**, *167*, 273−278.

(9) Hop, E.; Luinge, H. J.; Van Hemert, H. Quantitative analysis of water in milk by FT-IR spectrometry. *Appl. Spectrosc.* **1993**, *47*, 1180−1182.

(10) Mabrook, M. F.; Petty, M. C. A novel technique for the detection of added water to full fat milk using single frequency admittance measurements. *Sensor Actuator B.* **2003**, *96*, 215−218.

(11) Souza, S. S.; Cruz, A. G.; Walter, E. H. M.; Faria, J. A. F.; Celeghini, R. M. S.; Ferreira, M. M. C.; Granato, D.; Sant'Ana, A. S. Monitoring the authenticity of Brazilian UHT milk: a chemometric approach. *Food Chem.* **2011**, *124*, 692−695.

(12) Santos, P. M.; Wentzell, P. D.; Pereira-Filho, E. R. Scanner digital images combined with color parameters: a case study to detect adulterations in liquid cow's milk. *Food Anal. Methods* **2011**, *5*, 89−95.

(13) Ellis, D. I.; Brewster, V. L.; Dunn, W. B.; Allwood, J. W.; Golovanov, A. P.; Goodacre, R. Fingerprinting food: current technologies for the detection of food adulteration and contamination. *Chem. Soc. Rev.* **2012**, *41*, 5706−5727.

(14) Kim, S. S.; Young, C.; Mizaikoff, B. Miniaturized mid-infrared sensor technologies. *Anal. Bioanal. Chem.* **2008**, *390*, 231−237.

(15) Sorak, D.; Herberholz, L.; Iwascek, S.; Altinpinar, S.; Pfeifer, F.; Siesler, H. W. New developments and applications of handheld raman, mid-infrared, and near-infrared spectrometers. *Appl. Spectrosc. Rev.* **2012**, *47*, 83−115.

(16) Sánchez, M. T.; De la Haba, M. J.; Guerrero, J. E.; Garrido-Varo, A.; Pérez-Marín, D. Testing of a local approach for the prediction of quality parameters in intact nectarines using a portable NIRS instrument. *Postharvest Biol. Technol.* **2011**, *60*, 130−135.

(17) Cayuela, J. A.; Weiland, C. Intact orange quality prediction with two portable NIR spectrometers. *Postharvest Biol. Technol.* **2010**, *58*, 113−120.

(18) Birkel, E.; Rodriguez-Saona, L. Application of a portable handheld infrared spectrometer for quantitation of trans fat in edible oils. *J. Am. Oil Chem. Soc.* **2011**, *88*, 1477−1483.

(19) Allendorf, M.; Subramanian, A.; Rodriguez-Saona, L. Application of a handheld portable mid-infrared sensor for monitoring oil oxidative stability. *J. Am. Oil Chem. Soc.* **2012**, *89*, 79−88.

(20) Hassel, S.; Rodriguez-Saona, L. E. Application of a handheld infrared sensor for monitoring the mineral fortification in whole grain cornmeal. *Food Anal. Methods* **2012**, *5*, 571−578.

(21) Rani, R.; Medhe, S.; Raj, K. R.; Srivastava, M. M. High performance thin layer chromatography for routine monitoring of adulterants in milk. *Natl. Acad. Sci. Lett.* **2012**, *35*, 309−313.

(22) Afzal, A.; Mahmood, M. S.; Hussain, I.; Akhtar, M. Adulteration and microbiological quality of milk (a review). *Pak. J. Nutr.* **2011**, *10*, 1195−1202.

(23) Sadat, A.; Mustajab, P.; Khan, I. A. Determining the adulteration of natural milk with synthetic milk using ac conductance measurement. *J. Food Eng.* **2006**, *77*, 472−477.

(24) Laube, N.; Mohr, B.; Hesse, A. Laser-probe-based investigation of the evolution of particle size distributions of calcium oxalate particles formed in artificial urines. *J. Cryst. Growth* **2001**, *233*, 367−374.

(25) Flaten, G. R.; Grung, B.; Kvalheim, O. M. A method for validation of reference sets in SIMCA modeling. *Chemom. Intell. Lab.* **2004**, *72*, 101−109.

(26) Wold, S. Pattern recognition by means of disjoint principal components models. *Pattern Recogn.* **1976**, *8*, 127−139.

(27) Vogt, N. B.; Knutsen, H. SIMCA pattern recognition classification of five infauna taxonomic groups using non-polar compounds analysed by high resolution gas chromatography. *Mar. Ecol.: Prog. Ser.* **1985**, *26*, 145−156.

(28) Laporte, M. F.; Paquin, P. Near-infrared analysis of fat, protein, and casein in cow's milk. *J. Agric. Food Chem.* **1999**, *47*, 2600−2605.

(29) Rodriguez-Saona, L. E.; Koca, N.; Harper, W. J.; Alvarez, V. B. Rapid determination of swiss cheese composition by Fourier transform infrared/attenuated total reflectance spectroscopy. *J. Dairy Sci.* **2006**, *89*, 1407−1412.

(30) Etzion, Y.; Linker, R.; Cogan, U.; Shmulevich, I. Determination of protein concentration in raw milk by mid-infrared Fourier transform infrared/attenuated total reflectance spectroscopy. *J. Dairy Sci.* **2004**, *87*, 2779−2788.

(31) Zhang, F.; Qi, X.; Zou, M.; Li, J. Analysis of alphalactalbumin and betalactoglobulin from the rehydration of bovine colostrum powder using cloud point extraction and mass spectrometry. *J. Anal. Methods Chem.* **2012**, 1−4.

(32) Mortland, M. M. Urea complexes with montmorillonite an infrared absorption study. *Clay Minerals* **1996**, *6*, 143−156.

(33) Silva, R. A. B.; Montes, R. H. O.; Richter, E. M.; Munoz, R. A. A. Rapid and selective determination of hydrogen peroxide residues in milk by batch injection analysis with amperometric detection. *Food Chem.* **2012**, *133*, 200−204.

(34) Subramanian, A.; Prabhakar, V.; Rodriguez-Saona, L. Infrared spectroscopy in dairy analysis. In *Encyclopedia of Dairy Sciences*, 2nd ed.; Fuquay, J. W., Fox, P. F., McSweeney, P. L. H., Eds.; Academic Press: New York, 2011; pp 115−124.
(35) Aernouts, B.; Polshin, E.; Saeys, W.; Lammertyn, J. Mid-infrared spectrometry of milk for dairy metabolomics: a comparison of two sampling techniques and effect of homogenization. *Anal. Chim. Acta* **2011**, *705*, 88−97.
(36) Czarnik-Matusewicz, B.; Murayama, K.; Tsenkova, R.; Ozaki, Y. Analysis of near-infrared spectra of complicated biological fluids by two-dimensional correlation spectroscopy: protein and fat concentration-dependent spectral changes of milk. *Appl. Spectrosc.* **1999**, *53*, 1582−1594.
(37) Kasemsumran, S.; Thanapase, W.; Kiatsoonthon, A. Feasibility of near-infrared spectroscopy to detect and to quantify adulterants in cow milk. *Anal. Sci.* **2007**, *23*, 907−910.
(38) Jha, S. N.; Matsuoka, T. Detection of adulterants in milk using near infrared spectroscopy. *J. Food Sci. Technol.* **2004**, *43*, 313−316.