A Non-destructive method to assess freshness of raw bovine milk using FT-NIR spectroscopy

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Abstract A non-destructive method to analyze the freshness of raw milk was developed using a FT-NIR spectrometer and a fiber optic probe. Diffuse transmittance spectra were acquired in the spectral range 833–2,500 nm from raw milk samples collected from Northwest A&F University Animal Husbandry Station. After each spectral acquisition, quality parameters such as acidity, pH, and lactose content were measured by traditional detection methods. For all milk samples, PLS (partial least square regression), MLR (multiple linear regression), and ANN (artificial neural networks) analyses were carried out in order to develop models to predict parameters that were indicative of freshness. Predictive models showed R^2 values up to 0.9647, 0.9876 and 0.8772 for acidity, pH, and lactose content, respectively (validation set validations). The similarity analysis and classification between raw milk freshness during storage was also conducted by means of hierarchical cluster analysis. Over an 8 day storage period, the highest heterogeneity was evident between days 1 and 2.

Keywords Non-destructive evaluation · Raw milk · Freshness assessment · FT-NIR

Introduction

The dairy industry has relied primarily on refrigeration to maintain the quality of raw milk during storage and transportation. However, the shelf life of raw milk is limited by the presence of enzymes, which are either endogenously present

from the cow (e.g. plasmin, EC 3.4.21.7) or produced by psychrotrophic bacteria growing in the milk (e.g. lipoprotein lipase, LPL, EC 3.1.1.34). Plasmin and LPL are biochemically active at refrigeration temperatures and can cause slow degradation of milk proteins and lipids, (Law 1979). Changes in the internal constituents of raw milk during storage are mainly related to nutrient exchanges by bacteria between the inner and outer milk fractions (Baldo et al. 1991). Bacteria can also decompose lactose into lactic acid and consequently cause the acidity to increase and lactose content to decrease. The degree of change in a given sample during storage depends primarily on the variables time and temperature. Raw milk stored under different conditions can be qualitatively different to the consumer, in terms of freshness and taste.

Current methods to determine milk freshness are based on the measurement of sample acidity by titrating 10 mL of milk to a phenolphthalein end-point using sodium hydroxide (GB 19301–2010 2010 China), pH using a calibrated pH meter, and lactose content was determined by potassium permanganate titration (GB 5413.33–2010 2010 China). These methods are not only were time-consuming, but also accompany labor intensive inputs for operation. Therefore, a more precise and less invasive detection method to monitor milk freshness were developed to process samples rapidly in a high-throughput manner that was also non-destructive. FT-NIR spectroscopy technology offered the possibility to quickly and non-destructively analyze different constituents and properties of diversified food products without complex sample preparation. The focus of on-line and off-line applications was the measurement of the fat, protein (Luinge et al. 1993) and starch (Bao et al. 2007). This technique has made continuous progress in the improvements of the sensors and instrumentations capable of supporting complex calculations based on multivariate statistical methods such as principal component analysis (PCA), partial least squares regression (PLS) (Nelson et al. 1996, 2006), Multiple linear regression (MLR), artificial

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Table 1 Analysis for measured values of acidity, pH, and lactose content of milk samples

	Acidity (°T)	pH	Lactose content (%)
Range	16.0~24.8	6.95~6.45	4.70~3.09
Average	19.1	6.78	3.71
Rank difference	8.8	0.50	1.61

neural networks (ANN) (Guo et al. 2000; Roy and Pratim Roy 2009). Vis-NIR transmission spectroscopy technology has been used in the non-destructive measurement of egg freshness and milk shelf-life, but not on non-destructive measurement of milk freshness. Giunchi et al. (2008) using FT-NIR spectroscopy to assess the freshness of shell eggs, and their results demonstrated that NIRS predictive models for air cell height, thick albumen height and Haugh unit were obtained with R^2 of 0.722, 0.789 and 0.676, respectively. Vallejo-Cordoba et al. (2006) used FT-NIR spectroscopy and artificial neural networks to predict the shelf-life of milk and obtained a predictive model with a standard error of the estimate within 2 days of the milk's shelf-life, which demonstrated the applicability of an artificial neural network.

The aim of this research was to investigate and validate FT-NIR spectroscopy to predict the freshness of raw milk, and test differences between spectral data at different days of storage through a hierarchical cluster analysis.

Materials and methods

Samples

Forty eight samples of milk were collected from Northwest A&F University Animal Husbandry Station, and stored at 4 °C immediately. Six samples was taken per day for 8 days

Fig. 1 Near infrared spectra of raw milk stored in refrigeration from one to eight days **Fig. 2** X-loadings versus the wavelength (nm) for the measurements of milk samples). PC1, principal component 1; PC2, principal component 2

and then measured. The adopted test intervals make it possible to obtain a well-balanced range of variability for the freshness parameters.

Spectral measurements

Diffusion transmittance spectra in relation to transmittance were obtained by means of an NIR spectrometer (Bruker Optics, Germany MPA) and a fiber optic sampling probe (Bruker Optics). For each sample, spectral data were acquired at room temperature (18–20 °C) in the 833–2,500 nm range with 2 nm of resolution (with an average of 64 scans).

Destructive measurements

Acidity, pH, and lactose content of milk were also measured after NIR acquired the milk spectra. The acidity was measured by acid–base titration (GB 19301–2010 2010 China), the pH was measured by a calibrated pH meter (PHS-3C, Shanghai),

Table 2 Comparison of calibration models using three different methods

Modeling methods	Acidity		pH value		Lactose content	
	R ² / %	SECV / °T	R ²	SECV	R ² / %	SECV / %
PLS	96.46	0.396	96.09	0.0207	87.52	0.255
MLR	95.08	0.433	94.47	0.023	84.87	0.266
ANN	97.09	0.380	96.45	0.0202	88.22	0.238

and the lactose content was assayed by potassium permanganate titration (GB 5413.33–2010 2010 China).

Data analysis

Principal component analysis (PCA)

PCA (SAS8.0, SAS Software Institute) was performed on diffusion transmittance data acquired during storage in order to assess the variance contribution of x-variables (wavelengths) and the extraction of the principal components.

Partial least square regression (PLS)

PLS predictive models of freshness parameters were established by using absorbance units (Abs) spectra: $\text{Abs} = \log(1/T)$, where T is the diffusion transmittance. In particular, PLS regressions (PLS2, OPUS ver.5.5, Bruker Optics) were performed in order to predict the acidity, pH and lactose content of a given sample.

Multiple linear regressions (MLR)

MLR predictive models of freshness parameters were set up by using the extracted principal components via PCA and MLR (SAS8.0, SAS Software Institute) was also performed in order to predict the acidity, pH and lactose content.

Artificial neural networks (ANN)

ANN was performed in order to set up calibration models. The principal components acquired from PCA were applied as ANN (MATLAB7.0, U.S. Math Works Corporation) inputs and the freshness parameters were applied as outputs. The training set models were optimized using the Levenberg-Marquardt method and were composed of a three-layer network structure.

Three modeling methods such as PLS, MLR, and ANN were conducted in order to set up calibration models of freshness parameters (by using 75 % of the dataset). The optimal calibration models were selected by the highest R² value and the minimum value of the standard error (SECV) obtained using the leave-one-out cross validations. These optimal calibration models were then evaluated in terms of R² and standard error of prediction (SEP) by performing a test set validation (by using 25 % of the dataset).

Cluster analysis

Hierarchical cluster analysis (OPUS ver.5.5, Bruker Optics) was carried out to explore the degree of spectral similarity between different days of storage. Mean spectral values obtained by averaging the original milk spectra of each day (6 spectra) for the whole acquisition range (833–2,500 nm) were considered. The spectral distances were calculated by performing the standard algorithm based on the Euclidian

Fig. 3 Correlation between predicted values and measured values of acidity

Fig. 4 Correlation between predicted values and observed sample pH

distance. Furthermore the growth of heterogeneity factor H was determined according to Ward's algorithm.

Results and discussion

Chemical measurements

The quality of the quantitative analysis model largely depended on the detection accuracy of measured values along with the coverage of parameters used to test the samples. The duration values measured for freshness for samples over 8 days are shown in Table 1. The acidity of milk samples ranged from 16.0 ° T to 24.8 ° T over the 8 d storage period. On the contrary, pH and lactose content decreased from 6.95 to 6.45 and 4.70 % to 3.09 %, respectively. Observed values of acidity, pH, and lactose content composed high, medium, low levels and were normally distributed and mean-centered. Take together, this indicates that the selected milk samples were highly representative and therefore it was possible to set up near-infrared quantitative analysis models accurately.

Near infrared spectral analysis

Near infrared spectra of raw milk refrigerated from day one to eight is shown in Fig. 1. The spectra of raw milk over eight days shared similar absorbance patterns in the range of 12,000–4,000 cm^{-1} , as the main absorption materials in raw milk are primarily due to protein, fat and water molecules. Milk proteins showed an extensive absorption peak in the ranges of 6,369–6,097 cm^{-1} , 4,878–4,831 cm^{-1} and 4,587 cm^{-1} . The wide bands in this region may be due to the overlapping effects of O–H stretching, N–H stretching, as well as C–H stretching and C–H deformation (4,878–4,831 cm^{-1} , 4,587 cm^{-1}). Fat had a strong absorption in absorbance of 8,250 cm^{-1} , 7,184 cm^{-1} , 5,784 cm^{-1} ,

5,672 cm^{-1} , 4,664 cm^{-1} , which can be attributed to the combination of O–H bending and C–O stretching (4,600–4,800 cm^{-1}). Water molecules were detected at approximately 6,896 cm^{-1} and 10,416 cm^{-1} caused by initial –OH (H_2O) stretching (~6,896 cm^{-1}) and a secondary –OH (H_2O) stretch occurring at (~10,400 cm^{-1}). The peaks that occurred around 5,160 cm^{-1} were the result of asymmetric stretching and bending from H_2O ; and the presence of lactic acid and lactose (affect the freshness of milk) which had absorption peaks in the range of 6,410–5,714 cm^{-1} and 4,784–4,566 cm^{-1} . The raw spectral data were further processed, to avoid the influence of the high frequency random noise, baseline drift, uneven samples, light scattering and other effects. After the first derivative treatment, it can be concluded that during storage, lactic acid and lactose content in sample affected the near-infrared spectral absorption and scattering coefficients.

PCA were then performed on diffusion transmittance data of 48 milk samples during storage. Results show that the accumulative reliabilities of the first four components were more than 99.80 %, so the first four components were retained in lieu of the raw milk variables. It also showed that the highest variance between the samples was relative to the

Fig. 5 Correlation between predicted and observed values of lactose content

Table 3 Prediction using the ANN model

Sample number	Acidity (°T)			pH			Lactose content (%)		
	Measured value	Predicted value	Bias	Measured value	Predicted value	Bias	Measured value	Predicted value	Bias
1	16.30	16.21	−0.09	6.92	6.860	−0.060	4.70	4.94	0.24
2	17.00	16.78	−0.22	6.90	6.875	−0.025	4.37	4.62	0.25
3	17.80	17.75	−0.05	6.83	6.820	−0.010	4.15	4.44	0.29
4	18.50	19.00	0.50	6.80	6.780	−0.020	4.00	3.83	−0.17
5	18.90	19.27	0.37	6.75	6.749	−0.001	3.80	3.60	−0.20
6	19.00	19.59	0.59	6.75	6.725	−0.025	3.76	3.90	0.14
7	20.20	19.90	−0.30	6.74	6.717	−0.023	3.69	3.87	0.18
8	20.30	19.76	−0.54	6.74	6.716	−0.024	3.57	3.45	−0.12
9	20.31	20.12	−0.19	6.72	6.700	−0.02	3.46	3.36	−0.10
10	20.70	20.80	0.10	6.70	6.685	−0.015	3.15	3.36	0.21
11	22.00	21.50	−0.50	6.63	6.628	−0.002	3.42	3.66	0.24
12	23.00	23.20	0.20	6.47	6.498	0.028	3.40	3.18	−0.22

wavelength ranges 1,350–1,720 nm and 1,887–2,275 nm (Fig. 2).

Establishment of quantitative analysis model concerning acidity, pH and lactose content

Establishment of calibration models

Two unusual spectrums were deleted for model construction, and three kinds of modeling methods such as PLS, MLR and ANN were then taken to establish quantitative calibration models for 34 samples. Cross validations of the results for the calibration models are shown (Table 2). The best calibration models were obtained for 34 milk samples: acidity, pH, and lactose content were predicted with SECV values of 0.380

°T, 0.0202 and 0.238 %, respectively (with R^2 values of 0.9709, 0.9645 and 0.8822, respectively) and were analyzed by ANN. Lower R^2 and higher SECV emerged for the calibration models using PLS and MLR.

The performance of the calibration model for acidity, pH and lactose by ANN were optimal. Therefore, ANN can be used to establish milk freshness quantitative calibration models and a calibration spectrum zone selection at 833~2,500 nm. The regression analysis of predicted and measured values for acidity, pH and lactose content calibration models are shown in Figs. 3, 4 and 5.

Validation and evaluation of the best calibration models

Artificial neural networks was performed to predict sample acidity, pH, and lactose content of the validation set (12 samples) and the results are shown in Table 3. Acidity, pH,

Fig. 6 Hierarchical cluster analysis for mean spectral values

and lactose content were predicted with SEP values of 0.355 °T, 0.0202 and 0.205 %, respectively (with R^2 values of 0.9647, 0.9876 and 0.8772, respectively).

Sufficient levels of precision and accuracy for predicting the three milk freshness parameters were indicated by the high R^2 values, small SEP values and having negligible values of bias. Validation of the calibration model indicated that the NIR spectroscopic sensing system could be successfully used to assess milk freshness during storage.

Cluster analysis

Figure 6 shows a dendrogram with the spectral distances and heterogeneity factor H of the cluster analysis conducted on the average spectral values. The hierarchical trees appeared to group the spectral data into four relatively distinct clusters which corresponded to eight days of storage. The highest heterogeneity emerged between the clusters between day 1 and 2 and also for those at days 3, 4, 5, 6, 7 and 8.

Comparing the dendrogram and the measured values, it was concluded that milk samples were at the early stages of freshness in the first two days, at a transition stage of decline in the following four days, and in a phase of accelerated deterioration in the last two days. Based on this data, the classification of milk freshness threshold can be determined. When the acidity of milk was in the range of 16–18 °T, pH was in the range of 6.82–6.95 and lactose content was in the range of 3.96 %–4.70 %, the milk was considered fresh. When the acidity of milk was in the range of 18–20.7 °T, pH in the range of 6.71–6.82 and lactose content was in the range of 3.42–3.96 %, the milk was considered of marginal quality. When the acidity of milk was more than 20.7 °T, pH was less than 6.71 and lactose content less than 3.42 %, the milk was considered unusable.

Conclusion

Diffusion transmittance FT-NIR spectroscopy, conducted using a fiber optic probe in direct contact with raw milk, was able to analyze raw milk samples during storage. By using these prediction models, raw milk freshness can be assessed quickly and conveniently. Cluster analysis grouped the spectral data according to different days of storage. The classification of milk freshness threshold, via comparison with the dendrogram and measured values, was determined. Milk freshness thresholds for samples unfit for human consumption are reached when sample acidity is greater than 20.7 °T, pH is

less than 6.71 and the lactose content is less than 3.42 %. Average standard errors of prediction (SEP) obtained from the artificial neural networks (ANN) for acidity, pH and lactose content were 0.355 °T, 0.0202 and 0.205 %, respectively. The R^2 values indicated that these models were suitable for a preliminary screening.

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