Milk Fat Analysis by Fiber-optic Spectroscopy

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ABSTRACT : We have evaluated the application of spectroscopy using an insertion-type fiber-optic probe and a sensor at wavelengths from 400 to 1,100 nm to the measurement of milk fat content on dairy farms. The internal reflectance ratios of 183 milk samples were determined with a fiber-optic spectrophotometer at 5°C, 20°C and 40°C. Partial least squares (PLS) regression was used to develop calibration models for the milk fat. The best accuracy of determination was found for an equation that was obtained using smoothed internal reflectance data and three PLS factors at 20°C. The correlation coefficients between predicted and reference milk fat at 5°C, 20°C and 40°C were r=0.753, r=0.796 and r=0.783, respectively. The predictive explained variances (Q^2) of the final model, moreover, were more than 0.550 at all temperatures, and the regression coefficients of determination (R^2) were more than 0.6 (60%). Our results indicate that milk has different internal reflectance measured in the range of visible and near infrared wavelengths (400 to 1,100 nm), depending on its fat content. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 4 : 580-583)***

Key Words : Cow, Fiber-optic Spectroscopy, Milk Fat

INTRODUCTION

Milk composition could be used for animal management and is an important factor for economical value. Therefore, rapid, cost-effective methods has been investigated to evaluate milk (Tsenkova et al., 1999; Lee et al., 2004). As the content of milk fat forms the basis of the milk trade, much attention has been paid to increasing yields from dairy cows. It is recognized that dietary factors and inadequate rumen fermentation affect the yieldness. The inadequate fermentation reduces profitability, even if the cows do not display clinical symptoms. Therefore, monitoring milk fat on farms is a useful and practical method for identifying problems in the feeding system, and for detecting chronic diseases at an early stage.

Milk fat is analyzed chemically, or instrumentally by infrared (IR) analysis: however, these methods are not suitable for on-farm analysis. Recently, some authors have analyzed the absorbance of dairy products in the near infrared (NIR), and have reported that it is strongly correlated with fat content (Wittrup and Norgaard, 1998; Tsenkova et al., 2000). However, measuring absorbance at wavelengths above 1,100 nm requires a specialized illumination.

Phillips et al. (1995) reported a correlation between milk fat and color, and it has been shown that the Hunter color depends on fat content (Quiñones et al., 1997). These findings suggest the possibility of analyzing milk fat using visible light. However, there have been no reports of milk fat analysis using the reflectance of visible light. In addition, such reflectance may be affected by milk temperature, because the milk fat globule are particles of variable size surrounded by membrane, and its physical state is temperature-dependent (Prindiville et al., 2000; Shi et al., 2001). We have therefore investigated the relationship between milk fat content and the internal reflectance ratio obtained by fiber-optic spectrophotometry with the aim of developing a milk fat calibration model and determining the appropriate temperature for analysis.

MATERIALS AND METHODS

Milk samples

A total of 183 milk samples from 92 Holstein cows were collected twice a week. One hundred-ml samples were taken during morning and evening milkings with a milk meter (MC6B®, Orion, Suzaka, Japan), kept at 4°C, and aliquots of the samples were analyzed for milk fat and protein content by the reference method (AOAC official methods 989.05, 972.16 FG). The milk fat content of the samples analyzed as reference varied from 2.72 to 5.92% (4.21±0.05, mean±SEM).

Fiber-optic spectra

The internal reflectance ratios (Irie, 1999) from 400 to 1,100 nm wavelength of the same samples were measured at 1-nm intervals with a fiber-optic spectrophotometer (HRS6500®, OPTO Research, Tokyo, Japan), and the data downloaded to a portable personal computer via an IEEE interface, and displayed on a monitor. The samples were

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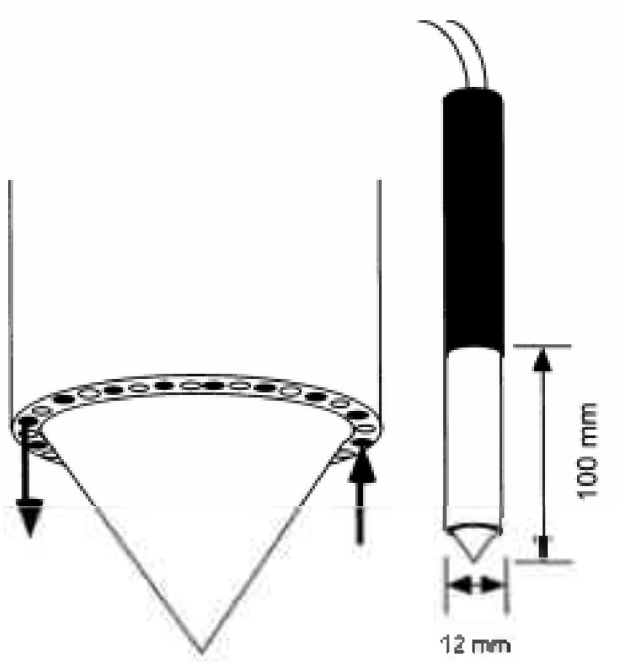

Figure 1. The insertion-type probe of the fiber-optic spectrophotometer. Magnified figure of the tip of the probe (left). Arrows show light path.

kept in polyethylene bottles (50 ml) and their internal reflectance ratios measured with an insertion-type probe (Figure 1). To determine the appropriate measuring temperature, we measured the internal reflectance ratios of the 183 samples at 5°C, 20°C and 40°C in a water bath. Each measurement took less than one second.

The log-transformed reflectance ratio data were smoothed over 25 point intervals, and transformed to second derivatives with the Salvitzki-Golay second-order polynominal filter (Salvitzki-Golay). A milk fat calibration model was developed at each temperature by partial least squares (PLS) regression with the Chemish version 3.55, which was provided by the Computer Aided Chemistry Forum, University of Tokyo (Chemish). PLS factors were determined from the spectra and the corresponding reference data including protein data by a cross-validation method using the leave-one-out method. The PLS model was evaluated by the predictive explained variances (Q^2), which was accepted as a measure of accuracy of determination and was calculated as follows.

$$Q^2 = 1\text{-}PRESS/s^2$$

where $PRESS=\Sigma(Y\text{-}Ypred)^2$, Y: reference data, Ypred: predicted data by the PLS model, s^2: variance of Y.

The differences in internal reflectance ratio at different temperatures were compared statistically by MANOVA followed by Tukey-Kramer's multiple range test.

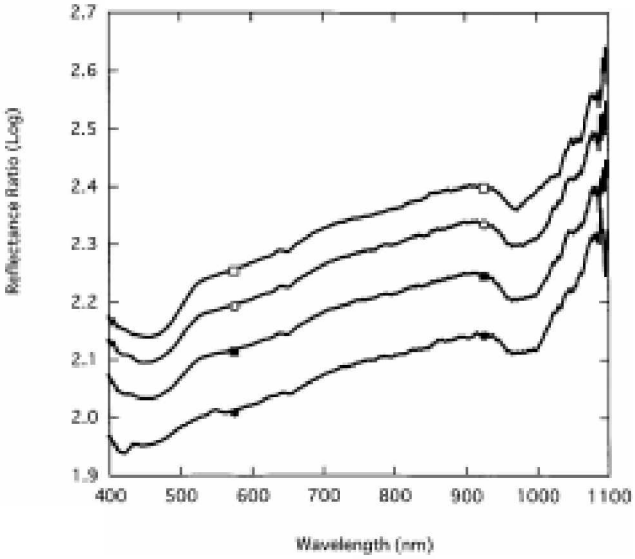

Figure 2. Typical spectra of internal reflectance ratio from 400 to 1,100 nm at four levels of milk fat content at 5°C and all data were log transformed; (●) 2.72%, (■) 3.55%, (○) 4.65%, (□) 5.92%.

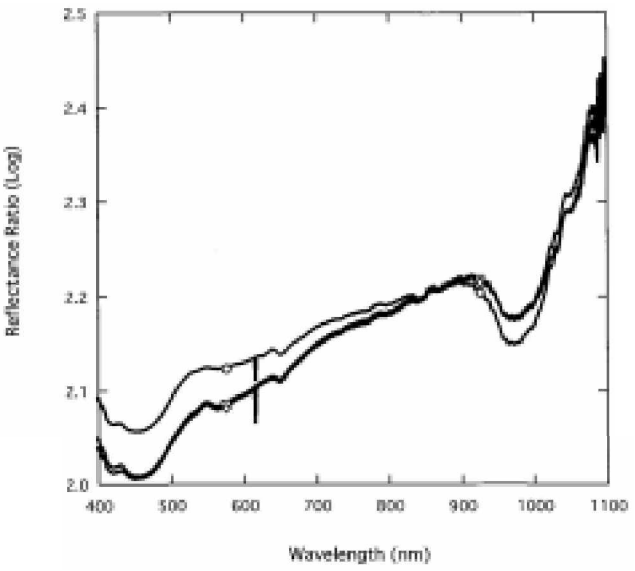

Figure 3. The mean internal reflectance ratio of samples at different temperatures: (◊) 5°C, (=) 20°C and (○) 40°C.

RESULTS AND DISCUSSION

The spectral distribution curves obtained at four levels of milk fat content were similar, and the internal reflectance ratio increased with milk fat content (Figure 2), indicating that milk brightness increases with milk fat content. The Hunter color of milk changes as the fat content of the milk increases: the milk becomes whiter, less green and less blue (Phillips et al., 1995; Quiñones et al., 1997). In addition, increased scattering of the light by fat globules may add to internal reflectance. As a result of these factors we found that the internal reflectance ratio from 400 to 1,100 nm rose

as milk fat content increased. It can therefore be concluded that the internal reflectance ratio obtained with visible light is suitable for measuring milk fat content.

Figure 3 shows the average internal reflectance ratio of samples at 5°C, 20°C and 40°C. There was no significant difference in the means of the internal reflectance ratios at wavelengths between 5°C and 20°C. The internal reflectance ratio from 400 to 600 nm at 40°C, however, was significantly greater than at 5°C or 20°C ($p<0.05$, Table 1) in agreement with a previous report that color variation is greatest at 37°C (Cano-Ruiz and Richter, 1998). The internal reflectance ratio of milk can be affected by the physical characteristics of the fat globule. Since nucleation of milk fat occurs at 24°C, whereas it is liquid above about 40°C (Rudan and Barbano, 1998; Shi et al., 2001), it consists of a mixture of solid and liquid phases between 24°C and 40°C. The milk fat globule, moreover, is covered with casein (Sharma et al., 1996), the structure of which changes with temperature (Curley et al., 1998). The difference in the pattern of spectra at 40°C compared with both 5°C and 20°C may therefore reflect these physical changes. Thus corresponding reference protein values were calculated in PLS regression as an independent variable.

A milk fat calibration model was developed using the internal reflectance ratios determined by fiber-optic spectroscopy and reference protein values (Table 2). The predictive explained variances (Q^2) were maximum at 20°C with log transformed data and smoothed log data. These data sets resulted in the highest correlation coefficient between predicted and reference milk fat. The Q^2 values of the PLS models at 5°C and 40°C also were more than 0.550, and the correlation coefficients were similar to those at 20°C. The usefulness of the PLS model need to be addressed for on-farm milk fat analysis.

The prediction accuracy of the NIR method is poorer in the wavelength range 700 to 1,100 nm than between 1,100 and 2,400 nm (Tsenkova et al., 1999). Our results were less accurate than the NIR method, as Table 2 shows, because the fiber-optic method primarily detects changes in milk brightness and light scattering, whereas the NIR and IR

Table 1. Mean internal reflectance ratios at different wavelengths compared by MANOVA

Spectral region	Source	Value	Df	F value	p value
400 -	Temperature	0.0291	2	7.4106	0.0007
500 nm	Wavelength	189.1654	100	775.5780	<0.0001
(blue)	Wavelength×temperature	0.3292*	200	3.0457	<0.0001
500 -	Temperature	0.01868	2	4.7531	0.0090
580 nm	Wavelength	132.1363	80	710.2324	<0.0001
(green)	Wavelength×temperature	0.2986*	160	4.4620	<0.0001
580 -	Temperature	0.01234	2	3.1405	0.0441
600 nm	Wavelength	21.0111	20	514.7714	<0.0001
(yellow)	Wavelength×temperature	0.5331*	40	9.0557	<0.0001
600 -	Temperature	0.0034	2	0.8623	0.4228
800 nm	Wavelength	959.8564	200	1,487.7774	<0.0001
(red)	Wavelength×temperature	0.0525*	400	5.2127	<0.0001
800 -	Temperature	0.0022	2	0.5684	0.5668
1,100 nm	Wavelength	2,984.7208	300	2,089.3046	<0.0001
	Wavelength×temperature	0.0130*	600	5.4312	<0.0001

* Wilks' lamda.

Table 2. Selected results of Fiber-optic spectra calibration

Temperature	Spectral data transformation	Smoothing interval or derivative windows	PLS[1] factors	Predictive explained variance (Q^2)	Coefficient of determination (R^2)	Correlation coefficient between predicted and reference milk fat (r)
5°C	Log (REF[2])		5	0.563	0.633	0.753
	Smooth	25	6	0.553	0.649	0.748
	Second derivative	25	7	0.236	0.707	0.538
20°C	Log (REF)		3	0.632	0.662	0.795
	Smooth	25	3	0.633	0.671	0.796
	Second derivative	25	5	0.203	0.696	0.500
40°C	Log (REF)		6	0.599	0.699	0.777
	Smooth	25	6	0.609	0.681	0.783
	Second derivative	25	4	0.270	0.688	0.546

[1] PLS: partial least square. [2] REF: internal reflectance ratio.

methods detect changes in absorbance reflecting alterations in the structure of casein and fat (Curley et al., 1998; Irie, 1999; Tsenkova et al., 1999). These previous investigators showed that fat has absorbance bands at 630 and 930 nm, and we also detected an absorbance band around 630 nm. Furthermore, temperature affected the assessment of milk fat with visible light (Figure 3). However, our fiber-optic analysis showed similar correlation coefficient at 5°C, 20°C and 40°C, while the IR and near NIR procedures are carried out at 40°C. These reflectance-temperature relationships should be the subject of further research. However, the fiber-optic method is convenient because it does not need pretreatment and the device is small and inexpensive. The accuracy of fat content determination was higher in the model based on smoothed data than on the second derivative transformed data, although these treatments of the spectral data did not improve the calibration for fat in the NIR (Tsenkova et al., 1999). This difference between the effects of data treatment may depend on the wavelength and method: absorbance versus reflectance. The spectra obtained by the fiber-optic method are relatively flat and contain less noise than those obtained by IR and NIR.

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