INTRODUCTION

Near-infrared spectroscopy (NIRS) is a nondestructive technique used for obtaining qualitative and quantitative information pertaining to foods and agricultural commodities. NIRS has been used for the evaluation of physicochemical properties of rice and wheat (Kawamura et al., 2002; Natsuga et al., 2006), and it has already been practically used for the automatic inspection system of rice quality (Kawamura et al., 2003). NIRS has also been used to determine milk quality (Sato et al., 1987; Tsenkova et al., 2001; Kawamura et al., 2007; Kawasaki et al., 2008; Iweka et al., 2016; 2017). However, in recent times, there has been a strong need for a novel system that can be used by dairy farmers to assess the quality of milk of an individual cow during milking. A near-infrared (NIR) spectroscopic sensing system was designed on an experimental basis for the online assessment of the three major milk constituents (fat, protein, and lactose), solids not fat (SNF), milk urea nitrogen (MUN), and somatic cell count (SCC). This system was used to obtain the NIR spectra of non-homogenized milk during milking over a wavelength range of 700 to 1,050 nm. Calibration models for predicting three major milk constituents, SNF, MUN and SCC of non-homogenized milk were developed, and the precision and accuracy of the models were validated. The coefficients of determination, standard errors of prediction, and bias values showed high levels of precision and accuracy for the prediction of the considered parameters. The results indicated that the developed NIR spectroscopic sensing system can be used to assess milk quality in real-time during milking. This system can provide dairy farmers with information concerning milk quality and physiological condition of each cow, and can therefore optimize dairy farm management.

Keywords : calibration models, milk constituents, milk urea nitrogen, solids not fat, somatic cell count

MATERIALS AND METHODS

Near-infrared spectroscopic sensing system

An experimental online NIR spectroscopic sensing system was designed for assessing the quality of milk of each cow during milking. The system consisted of an NIR spectrum sensor, NIR spectrometer, milk flow meter, milk sampler and a laptop computer (Figs. 1 and 2). The system was fixed between a teatcup cluster and a milk bucket of the milking system. Non-homogenized milk from the teat-cup cluster flowed continuously across a bypass into the milk chamber of the NIR spectrum sensor. Excess non-homogenized milk flowed past the milk flow meter and was then released through a line tube into the milk bucket (Figs. 1 and 2). The NIR spectrum sensor consisted of three Halogen lamps, namely, halogen A, B and C. The optical axes of halogen lamps A and B and the optical fiber were set at the same level, and the optical axis for halogen lamp C was set at 5 mm higher than the optical fiber (Fig. 3). The volume of milk sample in the milk chamber was approximately 30 mL. The spectrum sensor acquired NIR absorbance spectra through the milk. Absorbance spectra were obtained in the wavelength range of 700 nm to 1,050 nm at 1-nm intervals every 20 seconds during milking (Table 1). The milk flow rate was simultaneously recorded every 20 seconds during milking.

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Cows and milk samples

Two Holstein cows belonging to the Hokkaido University dairy barn were employed for milking. Table 2 presents the information of the two cows employed in the experiment. The cows were milked during different lactation periods. The measurement period was from May 7, 2018 to May 18, 2018. Measurements were performed in two consecutive milkings, one milking in the evening and milking in the following morning, for approximately two weeks during the experiment. A pipeline milking system was used for milking the cows at the Hokkaido University dairy barn. The two cows were milked at the same milking time and measurements were obtained for sixteen milkings. Milk spectra data and milk flow rates were recorded, and then the milk samples were collected from the milk sampler every 20 seconds during milking. The experiment was conducted to determine the variation in milk spectra caused by cow individuality and lactation stage (Table 2).

Reference analyses

In this study, we measured three major milk constituents (fat, protein and lactose), solids not fat (SNF), milk urea nitrogen (MUN) and somatic cell count (SCC) of non-homogenized milk as milk quality indicators. These indicators were considered owing to the valuable information they provide in terms of milk quality. For example, milk with high fat content and low SCC is considered to be of high quality. The three milk constituents, SNF and MUN were determined using a MilkoScan instrument (Foss Elec-

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**Table 1** Specifications of the near-infrared (NIR) spectroscopic instrument.

| Devices                        | Specifications                        |
|--------------------------------|---------------------------------------|
| NIR spectrum sensor            | Absorbance spectrum sensor            |
| Light source                   | Three halogen lamps                   |
| Optical fiber                  | Quartz Fiber                          |
| Milk chamber surface           | Glass                                 |
| Volume of milk sample          | Approx. 30 mL                         |
| Distance between optical axis  | 55 mm                                 |
| and milk level                 |                                       |
| NIR spectrometer               | Diffraction grating spectrometer      |
| Optical density                | Absorbance                            |
| Wavelength range               | 700–1,050 nm, 1-nm internal           |
| Wavelength resolution          | Approx. 6.4 nm                        |
| Photocell                      | CMOS linear array, 512 pixels         |
| Thermal controller             | Heater and cooling fan                |
| Data processing computer       | Windows 7                             |
| A/D converter                  | 16 bit                                |
| Spectrum data acquisition      | Every 20 s                            |

**Table 2** Information concerning cows used for milking in the experiment.

| Cow number | Date of birth | Date of latest calving | Parity |
|------------|---------------|------------------------|-------|
| 1256       | Mar. 07, 2013 | Aug. 01, 2017          | 3     |
| 1257       | Mar. 12, 2013 | Oct. 04, 2017          | 3     |
tric, Hillerod, Denmark) and SCC was determined using a Fossomatic instrument (Foss Electric) as reference analyses.

Chemometric analyses

Chemometric analyses were carried out to develop calibration models for each milk quality indicator and to validate the precision and accuracy of each model. The total reference analyses data were randomly divided into two data sets such that two-thirds of all data were used as the calibration set and the remaining data (one-third) was used as the validation set. Spectra data analysis software (The Unscrambler ver. 10.3, Camo AS, Trondheim, Norway) was used for the analyses. The statistical regression method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data.

RESULTS AND DISCUSSION

Changes in milk fat and flow rate during one milking time

Figure 4 shows an example of the changes that occurred in the reference milk fat content and milk flow rate every 20 seconds during one milking time. These results revealed that the milk fat content and milk flow rate fluctuated during milking which in this case lasted about 5 minutes for one milking time. As usual, the time range for one milking time for each cow was between 3 to 6 minutes with an average of 5 minutes. Milk content fluctuations also occurred in other milk quality indicators such as milk protein, lactose, SNF, MUN and SCC. Accordingly, we needed to acquire the data of milk spectra and milk flow rate, and milk sample for reference analysis every 20 seconds during milking.

Near-infrared spectra

NIR spectra data pretreatments such as multiplicative scatter correction, Savitzky-Golay smoothing and second derivatives methods were performed. However, there were no differences in the accuracy and precision of the calibration models between with pretreatments and without pretreatments. Therefore, original NIR absorbance spectra without pretreatments were used in this study.

Figure 5 is an example of the original NIR absorbance spectra set for non-homogenized milk from a cow during one milking time. The two peaks in the spectrum around 740 nm and 840 nm indicate the overtone absorption by C-H strings and C-C strings that are related to the typical absorption band of fat content in milk. The peak of the absorbance spectrum in the wavelength of approximately 960 nm indicates the second-overtone absorption by water molecules. The PLS method uses all of the NIR spectra data obtained ranging from 700 to 1,050 nm wavelength to develop calibration models for each milk quality indicator. PLS regression factors (latent variables) are defined by using absorbance from 700 to 1,050 nm wavelength. However, loading weights of each wavelength are different for each milk quality indicator.

Loading weight and explained variance

Multivariate calibration in PLS consists of model indicators which includes the number of PLS regression factors. Loading weight of the first three PLS factors to predict fat content is shown Fig. 6. The explained variance of first, second and third PLS factors were 62.2%, 23.5% and 6.0% respectively. The first PLS factor had the high-
est contribution to predict fat content. Positive loading weights of PLS factor 1 were found in the wavelength range of 700 to 1,050 nm. The wavelength around 960 nm was influenced by water absorption. This result indicates that PLS factor 1 explained fat variance including water influence. PLS factors 2 and 3 also explained fat variance including water influence with their negative and positive loading weights. However, their contribution to predict fat content was much smaller than the PLS factor 1.

**Precision and accuracy**

The validation statistics of the NIR sensing system for determination of milk quality are summarized in Table 3.

The correlations between reference and NIRS-predicted values of fat, lactose, protein, SNF, MUN and SCC are shown in Figs. 7 to 12, respectively.

The three major milk constituents are the main determinants of milk quality. The milk constituents can be influenced by the physical condition of each cow and their feed composition. Daily checks for milk constituents while milking can be performed for individual cow management such as monitoring the nutritional intake of each cow. High levels of precision and accuracy for predicting the three milk constituents and SNF of individual cows during milking can be useful for online real-time monitoring of milk constituents and SNF of individual cows during milking.

Milk urea nitrogen (MUN) is a protein feeding efficiency indicator in dairy cows. Extremely low protein content in the diet of a cow leads to poor milk production and extremely high protein content in the diet leads to infertility in cows and consequently results to environmental pollution through urine and fecal output from cows.

| Milk quality items | n1 | n2 | Range    | $r^2$ | SEP | Bias  | RPD  | Regression line |
|-------------------|----|----|----------|------|-----|-------|------|-----------------|
| Fat (%)           | 184| 92 | 1.98–6.26| 0.99 | 0.11| −0.01 | 8.89 | $y = 0.99 x + 0.04$ |
| Protein (%)       | 184| 92 | 2.83–4.20| 0.92 | 0.09| −0.01 | 3.58 | $y = 1.00 x + 0.01$ |
| Lactose (%)       | 184| 92 | 2.44–4.73| 0.79 | 0.22| −0.02 | 2.16 | $y = 0.96 x + 0.16$ |
| SNF (%)           | 184| 92 | 6.27–9.76| 0.89 | 0.22| −0.02 | 3.04 | $y = 1.02 x −0.15$ |
| MUN (mg/dL)       | 184| 92 | 6.80–14.50 | 0.51 | 1.13 | 0.06 | 1.42 | $y = 1.08 x −0.99$ |
| SCC (log SCC/mL)  | 184| 92 | 4.95–6.46 | 0.59 | 0.20 | 0.00 | 1.55 | $y = 1.01 x −0.03$ |

$n_1$: number of calibration samples. $n_2$: number of validation samples.

$r^2$: coefficient of determination value of validation set.

SEP: standard error of prediction. RPD: ratio of SEP to standard deviation of reference data.

Regression line: regression line from predicted value (x) to reference value (y).

SNF: solids not fat. MUN: milk urea nitrogen. SCC: somatic cell count.

![Fig. 7](image7.png) Correlation between reference fat content and NIRS-predicted fat content.

![Fig. 8](image8.png) Correlation between reference protein content and NIRS-predicted protein content.
Sufficient level of precision and accuracy was obtained for determining MUN in our study such that our SEP result for MUN was better than that previously obtained by Kawamura et al. (2007) and Kawasaki et al. (2008). This means that the result of the calibration model for MUN as obtained in this study could be used for monitoring the nutritional status of an individual cow during milking.

Somatic cell count (SCC) is globally recognized as the standard indicator for mastitis diagnosis and milk quality. Milk produced from a healthy cow contains less than 200,000 SCC/mL (=5.30 log SCC/mL) while milk produced from an unhealthy cow usually contains more than 200,000 SCC/mL, which is an indication of mastitis (Smith et al., 1995). Sufficient levels of precision and accuracy were obtained for SCC. The SEP result obtained in our study for SCC was lower compared to the results reported by Kawamura et al. (2004 and 2007) and Kawasaki et al. (2008). Thus, using the calibration model developed in our study, it is possible to predict SCC and monitor the health status of an individual cow during milking.

Non-homogenized milk quality items such as fat, protein, lactose, SNF, MUN and SCC can be monitored online in real time by using the NIR spectroscopic sensing system developed in this study. The system can provide dairy farmers with useful information concerning milk quality and physiological condition of an individual cow during milking. For example, they can manage to change feed composition according to the information of milk constituent contents and MUN. When somatic cell count of milk exceeds the limit they can call a veterinarian. The information provides them with opportunities of feedback control for optimizing dairy farm management. By using the system, dairy farmers could overcome the difficulty in managing individual cow and produce high-quality milk, and precision and smart dairy farming could be realized.
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