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DEVELOPMENT OF NEAR-INFRARED SPECTROSCOPIC SENSING SYSTEM FOR
ONLINE REAL-TIME MONITORING OF MILK QUALITY DURING MILKING
(搾乳時乳質のオンラインリアルタイム評価のための
近赤外分光センシングシステムの開発)

Hokkaido University
Graduate School of Agricultural Science
Division of Environmental Resources
Doctor Course

IWEKA Patricia Nneka

August, 2019

DEVELOPMENT OF NEAR-INFRARED SPECTROSCOPIC SENSING SYSTEM FOR ONLINE REAL-TIME MONITORING OF MILK QUALITY DURING MILKING

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

Hokkaido University
Graduate School of Agriculture
Division of Environmental Resources

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August, 2019

DEDICATION

This research is dedicated to

God Almighty for His grace and mercy upon my life, to my husband Yemi Babatope Abadariki and to my children, Ebunoluwa Israel Abadariki and Toluwani Joan Abadariki in gratitude for their gifts of inspiration.

Abstract

Milk and dairy products have played a major role to the human diet in many different countries across the globe since the beginning of time. The dairy cow was domesticated over 6,000 years ago. Therefore, it is not a surprise that over many years, significant attention has been paid to improving milk quality. Studies have been carried out to improve the yield, the compositional quality and hygienic quality, and have attempted to reduce the level of adulterants which can gain access to it. The chain of people involved in the milk industry extends from milk production- farmers, veterinarians and farm advisors-through transport to processing-quality controllers, manufacturers- and on to retailers, legislators, nutritionists, dairy educators and consumers. All will be interested in milk quality indicators which are frequently measured for commercial purposes, business, legal conditions and for nutritional reasons.

However, Dairy farming is very demanding, most especially for extensive dairy farmers since they manage their livestock in groups. This is because it requires lots of tasks such as feeding, milking, livestock management feed crop production and manure treatment. Fat, protein and lactose are the three major milk constituents and are essential indicators for milk quality. Individual cow management system is also important so as to enable dairy farmers to monitor the milk quality and physiological situation of each cow. This type of cow management is needed to upgrade the production of high-quality milk.

Near-infrared spectroscopy (NIRS) is a nondestructive system for collecting qualitative information on foods and agricultural products. NIRS has already been used for the evaluation of the physiochemical properties of rice and wheat and has also been used to determine milk quality. But, it has been hard to use NIRS for real-time on-line monitoring of milk quality of each cow during milking.

Therefore, the general objectives of this study were (1) to develop a near-infrared (NIR) spectroscopic sensing system for online real-time monitoring of milk quality during milking, (2) to examine the factors limiting the excellent performance of calibration models and to propose possible solutions to achieving high precision and accuracy of the calibration models for each milk quality constituents, (3) to determine milk progesterone concentration using NIR spectroscopic sensing system, and (4) to examine the measurement accuracy of the NIR sensing system installed into an automatic milking system. I also focused on investigating the effect of cow individuality, milking season and calving time on the accuracy of calibration models developed for each milk quality indicator using the NIR sensing system. The explained variance for the determination of milk quality versus number of PLS factors, scores and loading weights for the first three PLS factors were examined. Individual cow milking trend for each milk quality indicator was analyzed.

ABSTRACT

The results obtained showed that cow individuality, milking season and calving times could affect the accuracy of calibration models. The results obtained showed that the precision and accuracy level of the model for fat and moisture content was very high and relatively the same for each milking season considered (autumn, winter and spring). The results showed that the accuracy to determine milk fat and moisture content was high and almost constant for each season. However, the values of the standard error of prediction (SEP) indicated that the accuracy of protein lactose, milk urea nitrogen (MUN) and somatic cell count (SCC) were fluctuated in each season. Similar result was obtained for each calving time. Lactation stage of the two cows was throughout the autumn, winter and spring season. The lactation stage affected the constituent contents of the milk and amount of milk production. However, the SEP value for each milk constituent for each season was different and also was different for each calving time. As for the effect of cow individuality on the performance of the calibration models developed for each milk quality indicator, the result obtained was not good due to cow individuality.

The performance of the NIR spectroscopic sensing system was sufficiently high. Sufficient levels of precision and accuracy for predicting the three major milk constituents were indicated by the high values of coefficient of determination (r^2) and small values of SEP compare with the range of each constituent and by the negligible values of the bias (almost zero). Also, the difference in milk constituents between seasons may average 0.4% for fat and 0.2% for protein. MUN concentration was high in warm season compare to cold season which is due to the fact that cows are fed with feed of high protein content in warm season. Variation of lactation yield and genetic quality of cows fed diets containing similar level of nutrition, especially of protein, may also affect MUN content. High SCC which is related to seasonal environmental changes showed peak levels during warm season compare to other seasons. High SCC is routinely used by dairy farmers to detect mastitis. Therefore, it is important to monitor the quality of milk constituents, MUN and SCC in real time during milking for different seasons as this would ensure proper individual cow management. The optimal PLS factors for determination of milk quality was estimated as the number of factors after which explained variance no longer increased significantly.

Explained variance (EV) against the number of PLS factors (NPF) for milk quality determination is as follows: EV = 99% for fat; 73% for protein; 47% for lactose; 59% for MUN and 81% for SCC. The explained variances obtained in this study were sufficiently high for the determination of each milk quality indicator.

The precision and accuracy of progesterone concentration at every 20 s during milking and at one milking time were almost the same. In other words, we were able to determine

progesterone concentration at each milking time with almost the same accuracy as the predicted progesterone concentration in real-time milking. Therefore, by taking records of this predicted progesterone value at every milking time and monitoring the continuous changes in progesterone concentration, it is possible to predict each cow ovulation status and diagnose the early pregnancy of each cow.

Also, the precision and accuracy of the NIR spectroscopic sensing system when installed into a robotic milking system was good. Thus, the installation of NIR spectroscopic sensing system developed in our study into a milking robot system would facilitate the monitoring of milk constituents and diagnosis of mastitis of individual cows in real-time during milking. The NIR sensing system could provide dairy farmers and veterinarians useful information on milk quality and physiological status of each cow and thus, give them assessment control for improving dairy farm management. The application of this NIR sensing system could take dairy farm management to the next level of dairy precision farming on the basis of individual cow information.

Acknowledgement

Table of Contents

TABLE OF CONTENT

List of tables

List of figures

LIST OF FIGURES

1.   Chapter I: General Introduction

1.1 Introduction to near-infrared spectroscopy

This chapter is an introduction to the field of near-infrared spectroscopy in food science and technology and explains the significance of their study. First, this section summarizes the definition of near-infrared spectroscopy (NIRS), origin of NIRS and NIR spectra, Origin of NIR absorption, principles of NIRS, Molecular vibrations, quantum theory, instrumentation of NIRS, data analysis and application of NIRS in food system. It also outlined the importance of milk quality control in dairy management, mastitis, feed components and milk quality and the importance of their study for obtaining high quality milk is considered. Finally, after a comprehensive review of the literature related to the utilization of NIRS for the determination of milk quality, the problem, objectives and hypothesis of the research were defined.

1.1.1   What is near-infrared spectroscopy?

Near-infrared spectroscopy (NIRS) is a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum (from about 700 nm to 2500 nm). NIR spectroscopy is a relatively new analytical technique that has been utilized for the prediction of product properties since the 1960s. Prior to that time spectrophotometers were available that could measure NIR spectra; however, the NIR region of the electromagnetic spectrum was rarely utilized as it was considered that it contained no relevant structural information (Barton 2004). Burns and Margoshes (1992) described many of the problems encountered by spectroscopists who wanted to work with the NIR region; these included an absence of sharp peaks, an abundance of overlapping and shoulder peaks, a dramatic loss in sensitivity (2 to 3 orders of magnitude relative to the mid-infrared), and the difficulty of making band assignments owing to the presence of numerous overtone and combination bands.

1.1.2   Characteristics of near-infrared spectroscopy

The earliest analytical applications of NIR spectroscopy were reported in the early 1960s by Karl Norris of the U.S. Department of Agriculture (Norris, 1989 & Barton 2004). For example, (Ben-Gera, & Norris, 1968) used NIR spectroscopy to determine the moisture, crude protein, and oil concentrations of cereal grains and oil-bearing seeds. By the early 1990s the analytical applications of NIR spectroscopy were widespread, with many different industries (agriculture, food, paper, petro-chemical, polymer, and textile) utilizing the technology (Ciurczak, 1992). The rapid growth of the technology can be attributed to the emergence of high precision spectroscopic instruments with very high signal-to-noise ratios to detect minute differences in the reflectance spectra, and high-speed computers to carry out the complex

calculations involved in multivariate analysis (Norris, 1989). In addition, NIR spectroscopy has several advantages over traditional laboratory techniques, including:

- Rapid speed and reliability of determinations;
- Rapid and easy sample preparation;
- Multiplicity of analysis with one operation;
- Operation by unskilled personnel; and
- Non-destructive analysis (Norris, 1989; Schultz & Burns, 1990)

1.2 Infrared spectroscopy

Infrared (IR) spectroscopy is a technique based on the absorption of the electromagnetic radiation in the infrared region (Figure 1.1). The IR spectrum can be divided into three sub-regions, namely near-IR (NIR; 12500 – 4000 cm-1), mid-IR (4000 – 400 cm-1) and far-IR (400 – 10 cm-1). Photon energies of IR radiation are not high enough to excite electrons, but may excite molecular vibrations (and the associated molecular rotations) of covalently bonded atoms and groups. At room temperature, a molecule is generally in its ground electronic state where it sits in its ground vibrational state. The absorption occurs when the energy of the incident photons exactly matches that of a vibrational energy transition in the molecule (Dardenne, 2004). The absorption of energy occurs to excite the molecule to a particular higher vibrational state. A change in the dipole moment of molecule during a vibration is also necessary to induce the absorption process, i.e. $\delta\mu/\delta q \neq 0$ ($\delta\mu/\delta q$ = change of the dipole moment μ with distortion of the normal co-ordinate q). When a bond with an electric dipole moment is exposed to IR radiation, it will increase its amplitude of vibration. Bonds with a dipole moment that oscillate at discrete 9 resonant frequencies when exposed to IR radiation are called IR-active. If the resonant frequency of the bond matches the frequency of the incident radiation, interaction will occur leading to absorption. An IR absorption spectrum of a sample is obtained by passing the IR radiation through the sample and the fraction of the radiation absorbed at each frequency is determined. The frequency at which any peak in the absorption spectrum appears corresponds to the frequency of a normal mode of bond vibration in the molecule (Cen & He, 2007). The fundamental vibrational modes are most often observed in the mid-IR region of the spectrum (4000 – 400 cm-1).
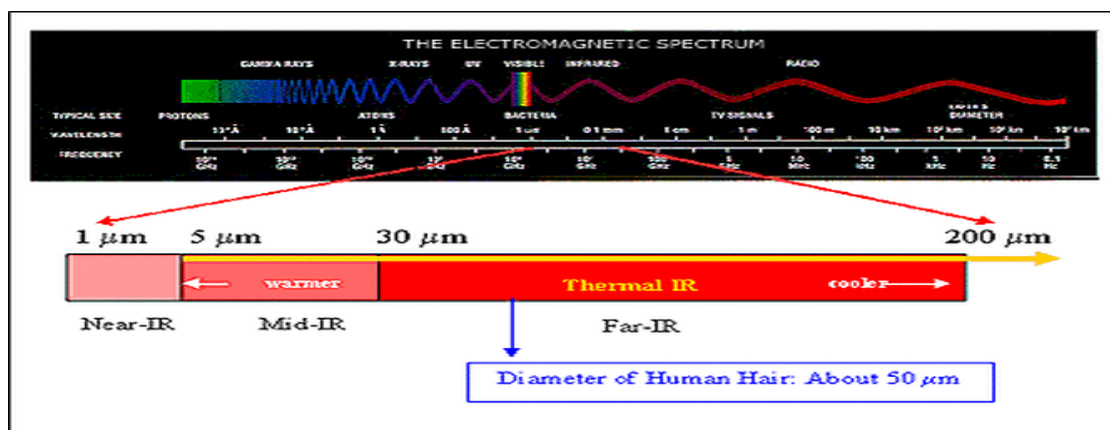
Figure 1.1   Electromagnetic spectrum
Reprinted from Pasquini, (2003)

1.2.1   Origin of near-infrared spectroscopy (NIRS)

The discovery of near-infrared energy is ascribed to William Herschel in the 19th century, but the first industrial application began in the 1950s. In the first applications, NIRS was used only as an add-on unit to other optical devices that used other wavelengths such as ultraviolet (UV), visible (Vis), or mid-infrared (MIR) spectrometers. In the 1980s, a single-unit, stand-alone NIRS system was made available, but the application of NIRS was focused more on chemical analysis. With the introduction of light-fiber optics in the mid-1980s and the monochromator-detector developments in early-1990s, NIRS became a more powerful tool for scientific research ("X. Near infrared spectroscopy," n.d.).
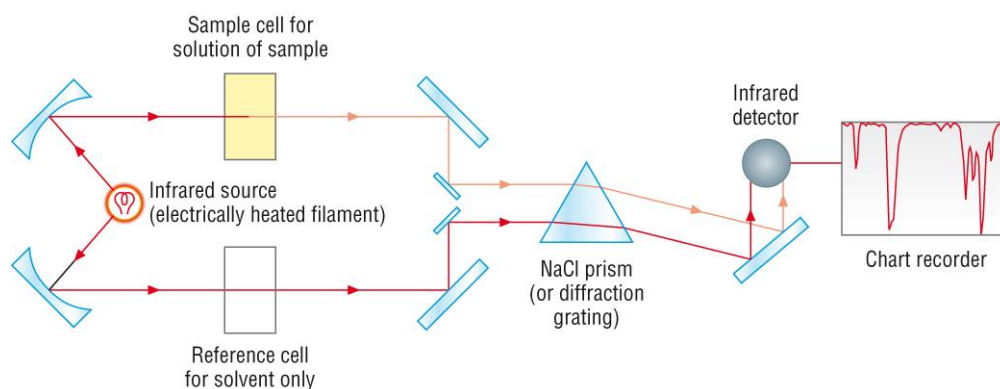
Figure 1.2   Schematic diagram of NIR spectroscopy mechanism
Reprinted from (Workman & Weyer, 2007)

The first (near) infrared spectra were measured in 1881 by Abney and Festing using photographic plates. Not only did they produce the first spectra but they also suggested, correctly, that the absorptions were related to the chemical composition of the liquids they investigated. The most important pioneer of IR spectroscopy was William W. Coblentz. In 1905 he published the result of a large study of compounds whose spectra he had recorded from 1000 nm to 16,000 nm. Coblentz's work was a breakthrough in that researchers were able to relate the

character of groups of atoms within molecules as being related to specific absorptions in the mid-IR (2500–50,000 nm). These absorptions are the result of interactions with the fundamental vibrations of the chemical bonds associated with the atoms of the groups (O'Neil et al., 2010; Smith, 1982).

1.2.2 Origin of near-infrared spectra

The infrared region of the electromagnetic spectrum ranges from 700 to 106 nanometers (nm) (10 to 14 300 cm-1) and is divided into near-, middle-, and far-infrared. A summary of each region is given in Table 1 (Osborne et al., 1993). The most useful region for quantitative (and to a lesser extent qualitative) analysis by reflectance in the near-infrared is 1200 to 2500 nm (8333-4000 cm-1). For wavelengths below 1200 nm the weak absorption bands make reflectance measurements difficult and for those above 2500 nm the bands become too strong (Norris, 1989). Spectra that occur in the near-infrared region consist largely of overtone and combination bands of the fundamental stretching vibrations of O-H, N-H, and C-H functional groups (Osborne, et al., 1993; Shenk et al., 1992).

Table 1.1   A summary of the infrared region of the electromagnetic spectrum

Region	Characteristic transitions	Wavelength range (nm)	Wavenumber range (cm^{-1})
Near-infrared (NIR)	Overtones combinations	700-2500	14300-4000
Middle-infrared (MIR)	Fundamental vibrations	$2500-5 \times 10^4$	4000-200
Far-infrared	Rotations	$5 \times 10^4 - 10^6$	200-10

1.2.3 Origin of near-infrared absorption bands

In the past ten years, near-infrared spectroscopy has become very popular technique for a wide range of analyses in various industries (Bokobza, 2007). The usefulness of this technique is mainly attributed to allowing the rapid and nondestructive analysis of bulk material. On the other hand, improvements in instrumentation, and especially the development of Chemometric software (Viscarra, 2008; Geladi, 2003), have contributed to the great expansion of the current state of popularity of this technique.

1.2.4 Infrared absorptions

For a molecule to show infrared absorptions it must possess a specific feature, i.e. an electric dipole moment of the molecule must change during the vibration. This is the selection rule for infrared spectroscopy.
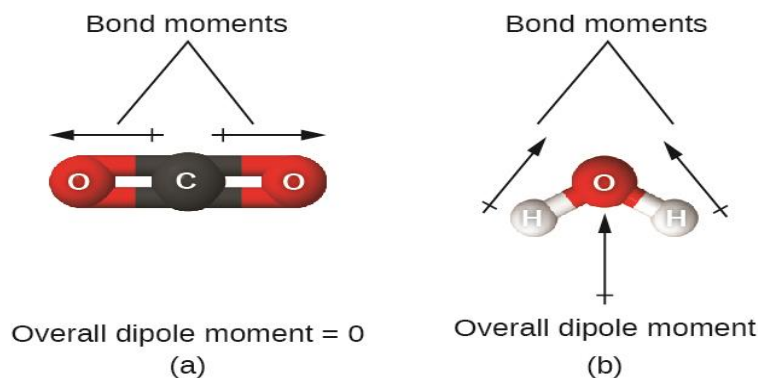
Figure 1.3 Illustrates an example of an 'infrared-active' molecule, a heteronuclear diatomic molecule. Reprinted from Workman & Weyer, (2007)

Infrared absorptions are not infinitely narrow and there are several factors that contribute to the broadening. For gases, the Doppler effect, in which radiation is shifted in frequency when the radiation source is moving towards or away from the observer, is a factor. There is also the broadening of bands due to the collisions between molecules. Another source of line broadening is the finite lifetime of the states involved in the transition. From quantum mechanics, when the Schrodinger equation is solved for a system which is changing with time, the energy states of the system do not have precisely defined energies and this leads to lifetime broadening. There is a relationship between the lifetime of an excited state and the bandwidth of the absorption band associated with the transition to the excited state, and this is a consequence of the Heisenberg Uncertainty Principle. This relationship demonstrates that the shorter the lifetime of a state, then the less well defined is its energy.

Molecular vibrations can be excited via two physical mechanisms: the absorption of light quanta and the inelastic scattering of photons (Figure 1.3) (Song, et al., 2015). Direct absorption of photons is achieved by irradiation of molecules with polychromatic light that includes photons of energy matching the energy difference between two vibrational energy levels, the initial (i, e.g., ground state) and the final vibrational state (first excited state).

As these energy differences are in the order of 0.5 and 0.005 eV, light with wavelengths longer than 2.5 mm, that is infrared (IR) light, is sufficient to induce the vibrational transitions. Thus, vibrational spectroscopy that is based on the direct absorption of light quanta is denoted as IR absorption or IR spectroscopy. The physical basis of IR light absorption is very similar to light absorption in the ultraviolet (UV)–visible (vis) range, which causes electronic transitions or combined electronic–vibrational (vibronic) transitions. Thus, UV–vis absorption spectroscopy can, in principle, also provide information about molecular vibrations. However, for molecules in the condensed phase at ambient temperature, the vibrational fine structure of the absorption

spectra is only poorly resolved, if at all, such that vibrational spectroscopy of biomolecules by light absorption is restricted to the IR range.
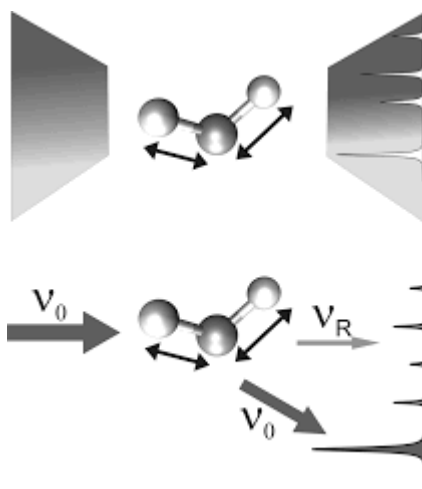
Figure 1.4 Vibrational Raman transitions correspond to inelastic scattering (vR; thin arrow) of the incident monochromatic light (v0) whereas the elastic scattering (v0) is represented by the thick arrow. Reprinted from Müller & Zumbusch, (2007)

Illustration of the excitation of molecular vibrations in IR (top) and Raman (bottom) spectroscopy. In IR spectroscopy, the vibrational transitions are induced by absorption of light quanta from a continuous light source in the IR spectral region.

In contrast to IR spectroscopy, the scattering mechanism for exciting molecular vibrations requires monochromatic irradiation. A portion of the incident photons is scattered inelastically such that the energy of the scattered photons (hv_R) differs from that of the incident photons (hv_0). According to the law of conversation of energy, the energy difference corresponds to the energy change of the molecule, which refers to the transition between two vibrational states. Thus, the energy differences lie in the same range as the transitions probed by the direct absorption of mid-IR quanta, although photons of UV, visible, or near-infrared light are used to induce scattering (Hildebrand, 2007).

The principle sources of information in vibrational spectroscopy are the energies of the vibrational transitions and the strength of their interaction with the IR or UV–vis radiation, i.e., the band intensities. Classical mechanics constitutes the basis for describing the relationship between vibrational frequencies and the molecular structure and force fields whereas quantum mechanics is indispensable for understanding the transition probabilities and thus the intensities of vibrational bands in the IR spectra.

1.2.5 Instrumentation of infra-red spectroscopy

A number of technologies exist that can be used to separate the polychromatic NIR spectral region into monochromatic frequencies for both qualitative and quantitative purposes. Broadband, discrete filter photometers, or light-emitting diode (LED) based instruments provide

spectral coverage over a narrow spectral region (50–100 nm). Diffraction grating, interferometer, diode-array or acousto-optic tunable filter (AOTF) based instruments provide full-spectral coverage. Selection of the appropriate technology is usually based upon the required analyte sensitivity and selectivity, reliability, ease-of-use, and implementation needs. Furthermore, agricultural, pharmaceutical and chemical manufacturers demand cost-effective and efficient method development and implementation. For instance, NIRS XDS analyzers use a combination of internal performance standards to maintain instrument stability and response and NIST-traceable external standards placed directly at the sample location to precisely match the band pass, photometric and wavelength response for all analyzers at the sample location. By precisely matching the performance for all instruments, a quantitative calibration model or a qualitative library developed on one XDS NIR analyzer can be used to predict quantitative or qualitative results on subsequent analyzers (of similar configuration) or the same analyzer after service (lamp or component change) without requiring a bias or slope adjustment or any other data manipulation (Popp, 2014).

1.3 Principles of near-infrared spectroscopy

Infrared is used to study the vibrational properties of a sample. Molecular vibrations produce absorption bands located in the mid-infrared range (between 400 and 4000 cm^{-1}) where they are the most strong and uncomplicated.

Close to the mid-infrared, the NIR region covers the interval between approximately 4000 and 12500 cm^{-1} (2.5-0.8 µm). This region contains absorption bands corresponding to overtones and combinations of fundamental vibrations.

Infrared radiation absorbed by a molecule causes individual bonds to vibrate in a manner similar to that of a diatomic oscillator (Barton et al., 2002).

1.3.1 Infrared selection rules

An overall change in the dipole moment of the molecule must occur for it to be IR active. The dipole moment is determined by the magnitude of the difference of charge and the distance between two centers of charge. The absorption will be high if the dipole moment associated with the bond vibration is large (as with highly polar groups of atoms with different electronegativities) and vice versa. A vibration which retains the center of symmetry of a non-vibrating molecule (e.g. that found in homonuclear species such as O_2) is termed IR inactive. IR active vibrations are categorized into two types of vibrations, namely bending and stretching vibrational modes, which are classified depending upon the change of the molecular shape during the vibration with respect to its center of symmetry. A stretching mode involves a change in the bond length, whereas the bending modes are characterized by the change in the angle of

the bond. Anhamonicity of the vibrations in the IR can cause the occurrence of overtone and combination bands. Overtones (the excitation of a vibration to a double or higher frequency) and combinations that are the sum or difference of two or more fundamental bands are observed in the near-IR region of the spectrum (12500 – 4000 cm-1). These features however are less intense than the corresponding fundamental vibrational modes in the mid-IR region of the spectrum (Popp, 2014).

1.3.2 Molecular vibrations

Vibrational spectroscopies such as NIR spectroscopy are techniques that yield complementary information in order to characterize and identify the molecular structure of materials. These vibrational spectroscopic techniques, however, are governed by different selection rules (Pasquini, 2003b).

The position of a molecule in three-dimensional space can be described using x, y and z co-ordinates for each atom relating to the three mutually orthogonal independent axes in space. Thus, each molecule containing N atoms has 3N degrees of freedom. Molecular motions consist of combinations of translations, rotations and vibrations; three of these fundamentals are pure translations that involve moving atoms simultaneously in the same direction, hence there is no change in the shape of the molecule and its bond lengths. Linear molecules have two rotational fundamental motions around the three orthogonal axes of the molecules (again, not altering any distances between the atoms) and non-linear molecules have three. Thus, each nonlinear molecule has 3N-6 vibrational degrees of freedom and each linear molecule has 3N-5, giving rise to the fundamental vibrations of the molecule (Ciurczak, 1992b).

The number of stretching modes is equal to the number of bonds in the molecule; since acyclic molecules have N-1 bonds, there will be N-1 bond stretching modes and the remaining vibrations are bending modes. The stretching and bending modes comprise the vibrational spectrum of the molecule and for which all atoms move in-phase and with the same vibrational frequency. The numbers of fundamental bands observed are dependent upon the degeneracy of the vibrations and their positions in the spectrum are 4 governed by the vibrational frequencies. Overtone and combination vibrations may produce bands which are less intense than the fundamental vibrations in the Raman and IR spectra (Ozaki   et al., 2007).

To generate the IR spectrum, different frequencies of infrared light are passed through a sample, and the transmittance of light at each frequency is measured. The transmittance is then plotted versus the frequency of the light (which is presented in the somewhat unusual units of cm^{-1}). Different functional groups produce bond absorptions at different locations and intensities on the IR spectrum. Recognizing where the absorptions generated by the common functional

groups occur will help you to interpret IR spectra. This table lists the locations and intensities of absorptions produced by typical functional groups as explained by (Workman & Weyer, 2007).

Table 1.2 Infrared absorptions of common functional groups

IR Absorptions of Common Functional Groups		
Functional Group	*Absorption Location (cm⁻¹)*	*Absorption Intensity*
Alkane (C–H)	2,850–2,975	Medium to strong
Alcohol (O–H)	3,400–3,700	Strong, broad
Alkene (C=C)	1,640–1,680	Weak to medium
(C=C–H)	3,020–3,100	Medium
Alkyne		
(C≡C)	2,100–2,250	Medium
(C≡C–H)	3,300	Strong
Nitrile (C≡N)	2,200–2,250	Medium
Aromatics	1,650–2,000	Weak
Amines (N–H)	3,300–3,350	Medium
Carbonyls (C=O)		Strong
Aldehyde (CHO)	1,720–1,740	
Ketone (RCOR)	1,715	
Ester (RCOOR)	1,735–1,750	
Acid (RCOOH)	1,700–1,725	

Source: Reprinted from (Workman & Weyer, 2007)

Table 1.3 Interaction of chemical bonds with specific NIR spectrum region

Wavelength (nm)	Description	Functional group	Wavelength (nm)	Description	Functional group
713	C–H str. fourth orvertone	benzene	1510	N–H str. first orvertone	protein
738	O–H str. third orvertone	ROH	1520	O–H str. first orvertone	ROH
740	C–H str. fourth orvertone	CH_3	1520	N–H str. first orvertone (intramol.H–bond)	$CONH2$
746	C–H str. fourth orvertone	CH_2			
747	O–H str. third orvertone	ArOH	1528	O–H str. first orvertone (intramol.H–bond)	starch
760	O–H str. third orvertone	H_2O			
762	C–H str. fourth orvertone	CH_2	1530	N–H str. first orvertone	RNH_2
779	N–H str. third orvertone	RNH_2	1533	C–H str. first orvertone	=CH
790	N–H str. third orvertone	$ArNH_2$	1540	O–H str. first orvertone (intramol.H–bond)	starch
806	N–H str. third orvertone	RNH_2			
808	$2 \times$ N–H str.$+2 \times$ N–H def.$+2 \times$ C–N str.	RNHR'	1570	N–H str. first orvertone	–CONH–
815	N–H str. orvertone	RNHR'	1580	O–H str. first orvertone (intramol.H–bond)	starch, glucose
832	$2 \times$ N–H str.$+2 \times$ N–H def.$+2 \times$ C–N str.	RNHR'			
840	$3 \times$ C–H str.$+2 \times$ C–C str.	benzene	1620	C–H str. first orvertone	$=CH_2$
874	C–H str. third orvertone	benzene	1645	C–H str. first orvertone	R–CH–CHO
880	C–H str. third orvertone	$CHCl_3$	1660	C–H str. first orvertone	cis–RCH=CHR'
900	C–H str. third orvertone	CH_3	1685	C–H str. first orvertone	aromatic
910	C–H str. third orvertone	protein	1695	C–H str. first orvertone	CH_3
913	C–H str. third orvertone	CH_2	1705	C–H str. first orvertone	CH_3
928	C–H str. third orvertone	oil	1725	C–H str. first orvertone	CH_2
938	C–H str. third orvertone	CH_2	1740	S–H str. first orvertone	–SH
970	O–H str. second orvertone	ROH, H_2O	1765	C–H str. first orvertone	CH_2
990	O–H str. second orvertone	starch	1780	C–H str. first orvertone	cellulose
1000	O–H str. second orvertone	ArOH	1820	O–H str.$+2 \times$ C=O str.	cellulose
1015	$2 \times$ C–H str.$+3 \times$ C–H def.	CH_3	1900	O–H str.$+2 \times$ C=O str.	starch
1020	$2 \times$ N–H str.$+2 \times$ amide I	protein	1900	C=O str. second orvertone	–CO–OH
1020	N–H str. second orvertone	$ArNH_2$	1908	O–H str. first orvertone	ROH
1030	N–H str. second orvertone	RNH_2	1920	C=O str. second orvertone	CONH
1037	$2 \times$ C–H str.$+2 \times$ C–H def.$+(CH_2)_n$	oil	1940	O–H str.+O–H def.	H_2O
1053	$2 \times$ C–H str.$+2 \times$ C–H def.$+(CH_2)_n$	CH_2	1950	C=O str. second orvertone	–CO–O–R
1060	N–H str. second orvertone	RNH_2	1960	N–H sym. str.+amide II	$CONH_2$
1080	$2 \times$ C–H str.$+2 \times$ C–C str.	benzene	1980	N–H asym. str.+amide II	protein
1097	$2 \times$ C–H str.$+2 \times$ C–C str.	cyclopropane	2000	$2 \times$ O–H def.+C–O def	starch
1143	C–H str. second orvertone	aromatic	2000	N–H sym. str.+amide II	$CONH_2$, CONHR
1152	C–H str. second orvertone	CH_3	2030	C=O str. second orvertone	$CONH_2$
1170	C–H str. second orvertone	HC=CH	2050	N–H sym. str.+amide II	protein
1195	C–H str. second orvertone	CH_3	2050	N–H asym. str.+amide III	$CONH_2$
1215	C–H str. second orvertone	CH_2	2080	O–H str.+O–H def.	ROH, suclose, starch
1225	C–H str. second orvertone	CH	2100	$2 \times$ O–H def.+C–O str.	starch
1360	$2 \times$ C–H str.+C–H def.	CH_3	2110	N–H sym. str.+amide III	$CONH_2$, CONHR
1395	$2 \times$ C–H str.+C–H def.	CH_2	2132	N–H str.+C=O str.	amino acid
1410	O–H str. first overtone	ROH	2140	=C–H str.+C=C str.	HC=CH
1415	$2 \times$ C–H str.+C–H def.	CH_2	2150	$2 \times$ amide I +amide III	$CONH_2$
1417	$2 \times$ C–H str.+C–H def.	aromatic	2160	$2 \times$ amide I +amide III	CONHR
1420	O–H str. first overtone	ArOH	2180	$2 \times$ amide I +amide III	protein
1430	N–H str. first overtone	$CONH_2$	2190	CH_2 asym.str.+C=C str.	HC=CH
1440	O–H str. first overtone	sucrose, starch	2200	C–H str.+C=O str.	–CHO
1440	$2 \times$ C–H str.+C–H def.	CH	2242	N–H str.+NH_3^+ def.	amino acid
1446	$2 \times$ C–H str.+C–H def.	aromatic	2252	O–H str.+O–H def.	starch
1450	O–H str. first overtone	starch, H_2O	2276	O–H str.+C–C str.	starch
1460	N–H str. first overtone	$CONH_2$	2280	C–H str.+C–H def.	CH_3
1471	N–H str. first overtone	CONHR	2294	N–H str.+C=O str.	amino acid
1480	O–H str. first overtone (intramol.H–bond)	glucose	2310	C–H str.+C–H def.	CH_2
			2323	C–H str.+C–H def.	CH_2
1483	N–H str. first overtone	$CONH_2$	2336	C–H str.+C–H def.	cellulose
1490	N–H str. first overtone	CONHR	2347	CH_2 sym.str.+=CH_2 def.	HC=CHCH_2
1490	N–H str. first overtone (intramol.H–bond)	$CONH2$	2352	C–H def. second orvertone	cellulose
			2380	O–H def. second orvertone	ROH
1490	O–H str. first overtone (intramol.H–bond)	cellulose	2461	C–H str.+C–C str.	starch
			2488	C–H str.+C–C str.	starch
1492	N–H str. first overtone	$ArNH_2$	2500	C–H str.+C–C str.	starch
1500	N–H str. first overtone	NH			

Source: Reprinted from (Workman & Weyer, 2007)

As the starting point for introducing the concept of harmonic vibrations, it is instructive to consider molecules as an array of point masses that are connected with each other by mass-

less springs representing the intramolecular interactions between the atoms (Lee, 2016). The simplest case is given by two masses, m_A and m_B, corresponding to a diatomic molecule A–B. Upon displacement of the spheres along the x-axis from the equilibrium position by Δx, a restoring force F_x acts on the spheres, which according to Hooke's law, is given by

$F_x = -f\,\Delta x$ Equation 1.1

Here f is the spring or force constant, which is a measure of the rigidity of the spring, that is, the strength of the bond. The potential energy V then depends on the square of the displacement from the equilibrium position

$V = 1/2\,f\,\Delta x^2$ Equation 1.2

For the kinetic energy T of the oscillating motion one obtains

$T = 1/2\,\mu\,(\Delta x)^2$ Equation 1.3

where μ is the reduced mass defined by

$\mu = m_A \cdot m_B / m_A + m_B$ Equation 1.4

Because of the conservation of energy, the sum of V and t must be constant, such that the sum of the first derivatives of V and T is equal to zero, as expressed by equation1.7

$0 = dT/dt + dV/dt = 1/2(d(\Delta x^2)/dt) + 1/2\,f\,(d(\Delta x^2)/dt)$ Equation 1.5

Which eventually leads to the Newton equation of motion

$d^2\Delta x/t^2 + f/\mu\,(\Delta x) = 0$ Equation 1.6

Equation 1.8 represent the differential equation for a harmonic motion with the solution given by sine or cosine function, i.e.,

$\Delta x = A \cdot \cos(\omega t + \varphi)$ Equation 1.7

Where A, ω and φ are the amplitude, circular frequency, and phase, respectively.
Combining Eqn. (1.9) with its second derivative one obtains

$d^2\,\Delta x / dt^2 + \omega^2\,\Delta x = 0$ Equation 1.8

such that comparison with eqn. 1.8 yields

$\omega = \sqrt{f/\mu}$ Equation 1.9

Equation (2.0) describes what one intuitively expects: the circular frequency of the harmonic vibration increase when the rigidity of the spring (or the strength of the bond) increases but decrease with increasing masses of the spheres. In order to express the circular frequency in wavenumbers (in cm-[1]), Eqn, (2.0) has to be divided by $2\pi c$ (with c given in cm s-[1]):

11

$\acute{\upsilon} = 1/2\pi c \sqrt{f/\mu}$ Equation 1.10

In contrast to the straightforward treatment of a two-body system, including a third sphere corresponding to a triatomic molecule clearly represents a conceptual challenge (Cen & He, 2007).
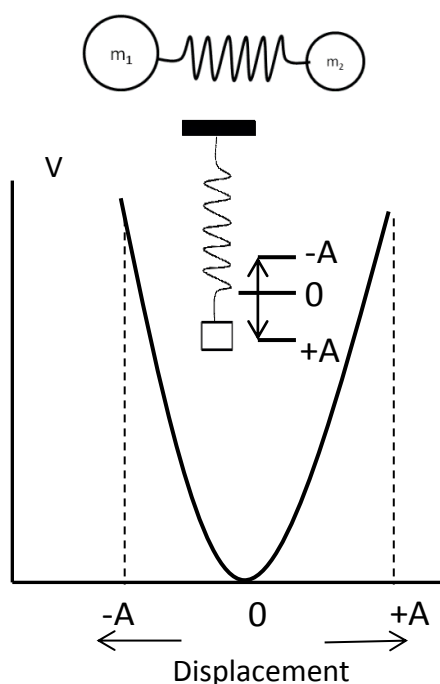
Figure 1.5 Harmonic Oscillator
Reprinted from (Cen & He, 2007)

Figure 1.5 above, is the simplest classical model employed to have a didactic insight on the interaction of radiation and matter (substance) in the NIR spectral region depicts a diatomic molecule as two spherical masses (m_1 and m_2) connected with a spring with a given force constant (k). Hook´s law states that the energy (E) of this system is given by:

$E = (h/2\pi)\sqrt{(k/\mu)}$ where μ is the reduced mass.

The molecular vibration can be described by a simplified model supposing a harmonic oscillator for which the potential energy (V), as a function of the displacement of the atoms (x), is given by:

$V = \frac{1}{2} kx^2$

The potential energy curve of such an oscillator is parabolic in shape and symmetrical about the equilibrium bond length.

1.3.3   Intensities of vibrational bands

Besides the frequencies of a normal mode, the intensity of the vibrational band is the second observable parameter in the vibrational spectrum. The intensity is simply proportional to the probability of the transition from a vibrational energy level n to the vibrational level m,

typically corresponding to the vibrational ground and excited states, respectively. The probabilities of transitions between different states that are induced by the interaction of the molecule with electromagnetic radiation, quantum mechanical treatments are required. Generally, the transition probability P_{nm} is given by the square of the integral

$$P_{nm} = (\psi^*_m /\Omega/ \psi_n) \qquad\qquad (2.3)$$

Where ψ_n and ψ_m are the wave functions for the vibrational states n and m, and Ω is the operator that describes the perturbation of the molecule by the electromagnetic radiation. This operator is different for the physical processes in IR and Raman spectroscopy and is obtained by first-order and second-order perturbation theory, respectively.

1.4 Quantum theory

If a molecule interacts with an electromagnetic field, a transfer of energy from the field to the molecule can occur. According to quantum theory, molecular motion can only have certain discrete energy states, for which a change in state is accompanied by the gain or loss of one or more quanta of energy (Ozawa, 2016). A quantum of energy is described by Planck's equation, $\Delta E = h\upsilon$, where h is Planck's constant and υ is the classical frequency of the molecular motion. The interaction of a molecule with electromagnetic radiation can be analyzed in terms of an energy mechanism. A vibrating molecule will be instantaneously polarized as a photon of incident radiation collides with it and its energy will be raised by $h\upsilon 0$ (the energy being immediately lost again by scattering). The scattered radiation consists of two types; the Rayleigh scattering is strong and has the same frequency as the incident beam, $\upsilon 0$, with no net energy loss and the other type of scattering is called Raman scattering and very weak. Molecules in the ground state give rise to Raman scattering at frequencies $\upsilon 0$-υvib, where υvib is the frequency at which the molecule vibrates, given that a change in polarizability occurs during the vibration. If the molecule happens to be in an excited vibrational state when an incident photon is irradiated, the photon may gain energy when scattered, giving a Raman effect at frequencies $\upsilon 0$+υvib. These are known as the Stokes and anti-Stokes Raman scattering, respectively (Nafie, 2001). The differences between the incident frequency of radiation and inelastic scattered frequencies correspond to the frequency of molecular vibrations present in the molecules of the sample. The excited states are not real and are described as virtual. The Stokes and antiStokes Raman peaks are symmetrically positioned about the Rayleigh peak but their 7 intensities are very different. The vibrational energy transitions that can occur and which give rise to fundamental modes of vibrations are presented in Figure 1.6.
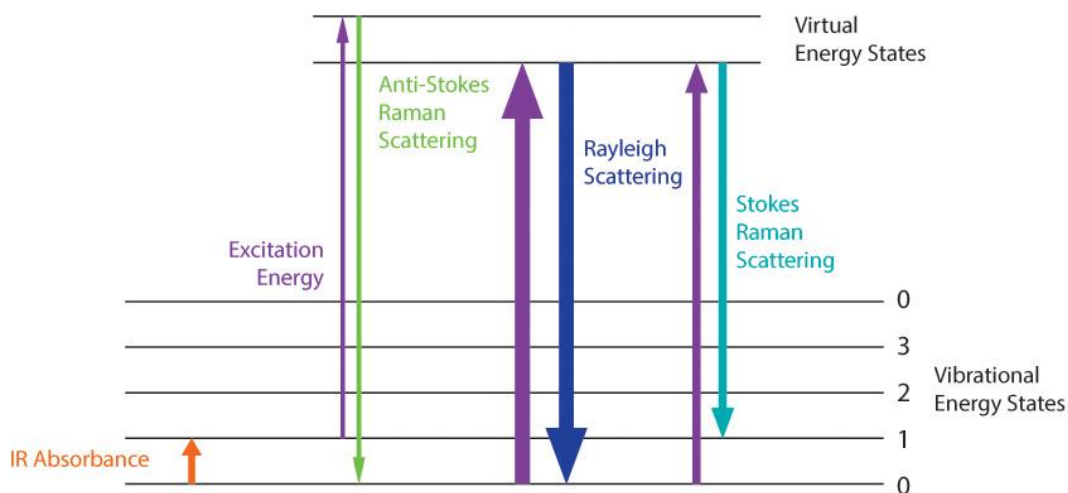
Figure 1.6 An energy level diagram showing transitions equivalent to Raman and Rayleigh
scattering and IR absorption
Reprinted from Heller et al., (2016)

The ratio of Stokes to anti-Stokes intensity is governed by the temperature. According to the Boltzmann distribution, at ambient temperature most molecules are in their vibrational ground state (Caram et al., 2012). The anti-Stokes lines therefore are less intense than the Stokes line because these transitions arise from higher vibrational energy levels that contain fewer molecules. Hence, Stokes Raman scattering is generally used for the Raman spectrum.

1.4.1 Quantitative infrared measurements

The absorption of radiation can be calculated through the Beer-Lambert law (Drake, 2004):

$$A = \log (I_0/I_t) = \varepsilon.c.l$$

where:

A = Absorbance

I_0 = intensity of incident radiation

I_t = intensity of radiation transmitted by the sample

l = path length of the sample

c = molar concentration

ε = molar extinction coefficient/absorption coefficient

The various vibrational modes have different tendencies to absorb with different molar extinction coefficients and therefore they have different intensities in the IR spectrum.

1.5 Data analysis and chemometrics

Geladi (2003), defined chemometrics as a chemical discipline that uses mathematics, statistics and formal logic (a) to design or select optimal experimental procedures; (b) to provide

maximum relevant chemical information by analyzing chemical data; and (c) to obtain knowledge about chemical systems.

1.5.1 Pretreatment of spectra (spectral conversion and manipulation)

Nonhomogeneous distribution of particles in a sample, particle size differences, sample density variations, sample morphology differences (shape and roughness of sample surface), lead to sample-to-sample variations in the overall path-length the photons have to travel before they reach the detector. These small physical differences from sample to sample lead to light scattering effects that influence the measured NIR spectra and result in baseline shifts and scaling variations (intensity variations). If the physical appearance of a sample is not of interest (e.g., as the determination of the composition of a sample), altered spectra can be detrimental to subsequent quantitative analysis as inaccurate results are obtained. The pretreatment of NIR raw data is the first step of model development and optimization. Data pretreatment helps to correct unwanted systematic sample-to-sample variation in measured NIR spectral data. Additionally, pretreatment also helps to correct spectral variations due to intermolecular hydrogen bonds or spectrometer hardware. A suitable data pretreatment depends on the data to be analyzed (many measurements of a particular sample).

Several spectral manipulation methods were employed to the vibrational spectra in this work in order get a more suitable method for our study. Spectral manipulation refers to mathematical transformation of the original spectra by using specific software. All vibrational spectra were imported to the Unscrambler software format (The Unscrambler, Camo AS, Trondheim, Norway Ver. 10.3). Several techniques of spectral manipulation were used; the principal ones being baseline correction, multiplicative scatter correction, smoothing, and second derivative which have been done using the Unscrambler package. Partial least square statistical method was used for these analyses.

1.5.2 Baseline correction

Background in vibrational spectra is common and arises from either luminescence processes (fluorescence), non-laser-induced emissive processes (room lights) or Raman scattering from optics or solvents (Lan et al., 2007). The background can be removed by employing baseline correction, i.e. by selecting multiple points through which a straight line or curve is drawn. The resulting curve is then subtracted from the original spectrum.

1.5.3 Multiplicative scatter correction

Multiplicative scatter correction (MSC) is a transformation method used to compensate for multiplicative and/ or additive scatter effect in the data. MSC is a row oriented transformation method; that is to say the contents of a cell are likely to be influenced by its

horizontal neighbors. The idea behind MSC is that the two effects, amplification (multiplicative, scattering) and offset (additive, chemical), should be removed from the data table to avoid that they dominate the information (signal) in the data table. MSC cannot be performed with non-numeric data or where there are missing data.

1.5.4 Smoothing

'Noise' in a spectrum can be diminished by a smoothing process. After a spectrum is smoothed, it becomes similar to the result of an experiment carried out at a lower spectral resolution. A smoothing function is basically a convolution between the spectrum and a vector whose points are determined by the degree of smoothing applied. The Savitzky-Golay smoothing algorithm was used in this research with 10 smoothing points and a second order polynomial. The algorithm is based on performing a partial least squares linear regression fit of a polynomial around each point in the spectrum to smooth the data.

1.5.5 Derivatives

Differentiation, i.e. computing derivatives of various orders, is a classical technique widely used for spectroscopic application. Some of the "hidden" information in a spectrum may be more easily revealed when working on a first or second derivative. It is a row oriented transformation; that is to say the contents of a cell are likely to be influenced by its horizontal neighbors.

1.5.6 Multivariate calibration methods and validation

Calibration is performed by using appropriate models that describe the relationship between dependent and independent variables. Shao et al. (2010) reported that, in the case where the data is of univariate nature and the data stems from single wavelength measurements. There, the independent variable is the measured absorbance at one single wavelength (X) that is measured for a number of calibration standards whose concentrations (dependent variable, Y) have been determined by a reference analytical method (e.g., by weighing). Provided that the Lambert-Beer law is followed, the relationship $Y = f(X)$ can be expressed by univariate calibration using linear regression according to Ortiz, et al. (2010). The calculated model parameters «slope» and «intercept» of the linear regression model precisely characterize this relationship and are used for the prediction of new samples. Multivariate data require multivariate calibration, which follows the same basic principle as just described for univariate data as stated by (Marill, 2004). The appropriate multivariate calibration model describes the relationship between the dependent and independent parameters. The independent variables are the absorbances at a number of wavelengths and more than one dependent variable (concentration values) can be accounted for. According to (Ortiz et al., 2010), there are a number

of tried and tested regression models that can be used for multivariate calibration like multiple linear regression (MLR) and partial least squares (PLS) regression. Literature also mentions principal component regression (PCR) as another linear calibration technique as well as artificial neural network (ANN) as a nonlinear calibration technique. NIR data analysis using multiple linear regression (MLR) is normally affected by multicollinearity, which leads to a poor prediction performance. To overcome this problem and to obtain a strong and robust predictive model, the number of wavelengths used for the analysis has to be cut down considerably (e.g., only two to ten wavelengths are considered). Su et al. (2012) reported that to analyze only a carefully selected representative part of the recorded spectra, which is not at all or at least less influenced by interferences, can sometimes also lead to a better prediction performance with other regression models. Compared to MLR, the two techniques PCR and PLS allow including more information in the calibration model and are not limited by multicollinearity. PCR is a two-step multivariate calibration method obtained by consecutively applying PCA and MLR. The principal components obtained with PCA are the independent variables in the MLR step as concluded by (Cheon et al., 2015). These principle components were determined solely to account for most of the variance in the measured spectra (X matrix) but did not considering the Y variables at all. Thus, unfortunately, it is possible that these principle components of the PCR model are not always able to predict the component concentrations (Y variables) well as stated by (Cheon et al., 2015).

1.6 Application of NIR spectroscopy in food system

Industries involved with foods and beverages have traditionally used NIR measurements for quality control, blending, and process control (Hari et al., 2011). Developments in computer science and chemometrics have prompted parallel developments in the on/in-line NIR techniques, and have attracted considerable attention from food researchers. For example, this technique was applied for on-line detecting fat, moisture, and protein content during meat processing (Prieto et al., 2009). With respect to grains, some researchers have installed NIRS equipment in the harvester for continuous detection of parameters characterizing grain quality such as protein and moisture content (Montes et al., 2006). These on/in-line applications have established their control capability in food processing.

1.7 Limitations of NIR techniques in food analysis

Although the operating cost of NIRS is low, the instrument itself is highly priced; this limits its practical application. Efforts by researchers and industrial organizations to develop simple and low-cost instruments could revolutionize the use of NIR techniques for on/in-line quality monitoring of foods. Some calibration models based on NIR spectroscopy, especially for

on-line application, are not reliable and stable enough when used practically. Hence, it is imperative for researchers to choose proper chemometrics to build robust models. In some cases, conventional methods may not offer a satisfactory solution to a given problem due to complexity of the data. This also necessitates the development of new chemometrics methods so as to further improve the reliability and accuracy of the calibration models. In addition, there are other limitations of NIR spectroscopy technique. The technique is not sensitive to the mineral content, since there is no absorption of minerals in the NIR spectrum region. An alternative way to solve this problem efficiently is to combine different detection techniques with NIR spectroscopy, such as X-ray fluorescence spectroscopy, UV light, and electronic nose technique. Some papers describing the use of a combination of techniques using different detection methods have been published in recent years (Porep et al., 2015), although more efforts should be made to solve this issue

1.8 Milk production and cow management

1.8.1 What is milk?

Milk is a pale liquid produced by the mammary glands of mammals. It is the primary source of nutrition for infant mammals before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to its young and can reduce the risk of many diseases. It contains many other nutrients including protein and lactose (Pereira, 2014).

1.8.2 Importance of milk

Fresh milk is a rich source of several essential nutrients, including fat, protein, lactose, calcium, phosphorus, vitamin A and vitamin D. The consumption of milk Including three servings of fresh milk in one`s diet each day can help maintain muscle mass, strengthen bones, support a favorable body composition and improve circulatory health.

Consumption of milk and dairy has also been associated with a reduced risk of suffering a heart attack in humans. Dairy helps reduce blood pressure. A diet containing fruit and vegetables, low-fat dairy products and low salt helps reduce blood pressure (Dietary Approaches to Stop Hypertension: DASH diet) by Bazzano et al., (2013).

1.8.3 Japanese dairy farming

The Japanese livestock industry achieved dramatic post-war growth, as higher national income created an increased consumer demand for livestock products. In Japan today, the livestock industry is the most important agricultural sector in terms of gross agricultural output (Figure 1.7). The gross output of the Japanese dairy industry increased rapidly up until 2012, and

slightly declined in year 2013. The livestock sector contributes about 34.7% of the agricultural income in Japan according to MAFF statistics (2016). However, livestock production, including dairying, has been affected by a strong trend towards trade liberalization and structural changes on dairy farms, while consumer needs have become extremely diverse as stated by Japan Dairy Council (JDC) (2016).
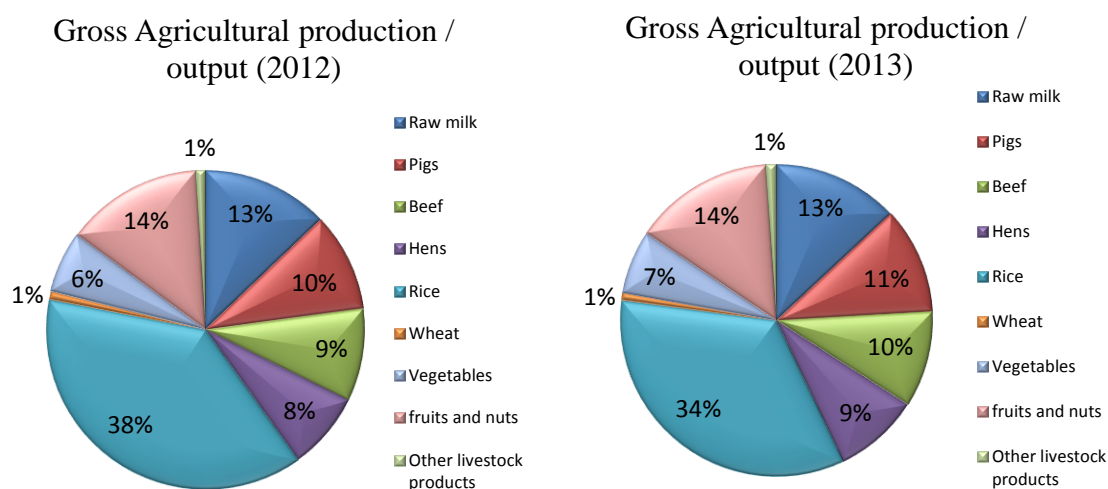
Figure 1.7 Gross agricultural and livestock output in Japan   Unit: Million yen
Source: Self processed from MAFF Statistic, (2016)

1.8.4   Dairy farms in Japan

The number of dairy farms in Japan has declined drastically in recent years (Table 4). The number of farms fell by more than half in about three decades, from 1975 to 2005, and this decline has since remained. The number of dairy cattle, 99% of which were Holstein, increased until 1980 and thereafter remained stable at 2.0 to 2.1 million head. This means that the number of cattle per farm has increased greatly, reaching 38 head in 1992. In 1970, farms with less than ten head constituted 67% of all dairy farms (Table 1.4). These had fallen to only 20% by 1992. On the other hand, farms with more than 30 head increased from 0.5% of all farms in 1970 to 37% in 1992. The most dramatic change in the number of dairy cattle was in these farms with 30 head or more. Their total inventory increased from 340,000 head in 1975 to 1.43 million head in 1992, while the total number and percentage of dairy cattle on farms with less than 30 head declined. A similar trend took place in the United States, where the number of dairy farms fell from 444,000 in 1975 to 182,000 in 1991. A comparison of the dairy industry in Japan with that of the United States shows far more similarities than differences (Simpson, 2005).

Table 1.4 Trends in dairy farming in Japan

Year	No. of Dairy farms	No. of Dairy Cattle	Avg. Herd size	No. of Cows	Total Milk production	Avg. Production per cow (kg)
1975	160.1	1,787	11.2	1,111	5,006	4,464
1990	63.3	2,058	32.5	1,285	8,203	6,383
1995	44.3	1,951	44	1,213	8,467	6,986
1996	41.6	1,927	46.3	1,211	8,659	7,168
1997	39.4	1,899	48.2	1,205	8,629	7,206
1998	37.4	1,860	49.7	1,190	8,549	7,242
1999	35.4	1,816	51.3	1,171	8,513	7,336
2000	33.6	1,764	52.5	1,150	8,415	7,401
2001	32.2	1,725	53.6	1,124	8,312	7,388
2002	31	1,726	55.7	1,126	8380	7,462
2003	29.8	1,719	57.7	1,120	8,405	7,613
2004	28.8	1,690	58.7	1,088	8,285	7,732
2005	27.7	1,655	59.7	1,055	8,293	7,894
2006	26.6	1,636	61.5	1,046	8,091	7,867
2007	25.4	1,592	62.7	1,011	8,024	7988
2008	24.4	1,533	62.8	998	7,945	8,012
2009	23.1	1,500	64.9	985	7,881	8,088
2010	21.9	1,484	67.8	964	7,631	8,046
2011	21	1,467	69.9	933	-	-

Milk production unit: 1,000 tons   No. of dairy farms and cattle in Japan unit: 1,000 head
Source: MAFF Statistics, (2014)

Dairy production across Japan continued to face significant headwinds in 2014. The increasing age of farmers and the lack of successors to continue farming operations in the next generation (problems that are common across Japanese agriculture) were and will continue to be the most significant challenges for the dairy sector. The physical demands of dairying, low profitability relative to the size of required investment in facilities, equipment and machinery, as well as the increased input costs for feed, fuel and electricity were all contributing factors to the continued exit of dairy operators, especially small- and medium-sized dairy farms in 2014. Even in the core dairy production center of Hokkaido, where relatively larger scale operators produce over half of the national fluid milk output, dairy farm operators have been exiting out of production at an average rate of three percent per year in recent years. Elsewhere across Japan, where fluid milk production for drinking is fairly evenly dispersed, the average rate of exit has been five percent per year as reported by Global Agricultural Information Network (2016).

Improved weather conditions in 2015 have eased the impact of the continuing decline of Japan's national dairy herd on total milk production. Despite the steady exit of dairy farmers from the industry, Japanese fluid milk production is forecast to increase modestly in 2015 as output per cow showed marked improvement in the milder summer weather of 2015. On higher

negotiated fluid milk prices and in response to national sentiment following 2014's perceived butter shortage, Japanese dairy product producers have significantly expanded butter and non-fat dry milk (NFDM) production in 2015 alongside Japan's so-called 'additional' imports of both commodities, restoring stock levels to more customary levels. Expansion of butter and NFDM production, however, appears to have come at the cost of lower Japanese production and greater imports of natural cheeses. The market share for suppliers of imported cheeses in 2015 and beyond could be greatly affected by the emergence of greater competition from EU suppliers, following the abolishment of the EU production quota system that had been in place for more than 30 years. The outlook for Japanese milk production in 2016 could be negatively impacted if continued high feeder calf prices restrict the supply of replacement heifers. While the Japanese supply for butter and NFDM could be more stable in 2016 if dairy manufacturers sustain 2015 levels of production, the possibility of 'additional' dairy importation for either commodity cannot be ruled out as stated by (Phillips, 2016).

1.8.5 Milk production

According to FAOSTAT (2019), raw milk with an average milk production per cow of approximately 700 million tons, ranked number one among the livestock commodities produced in Japan within the period of 2009 to 2013 (Figure 1.8).
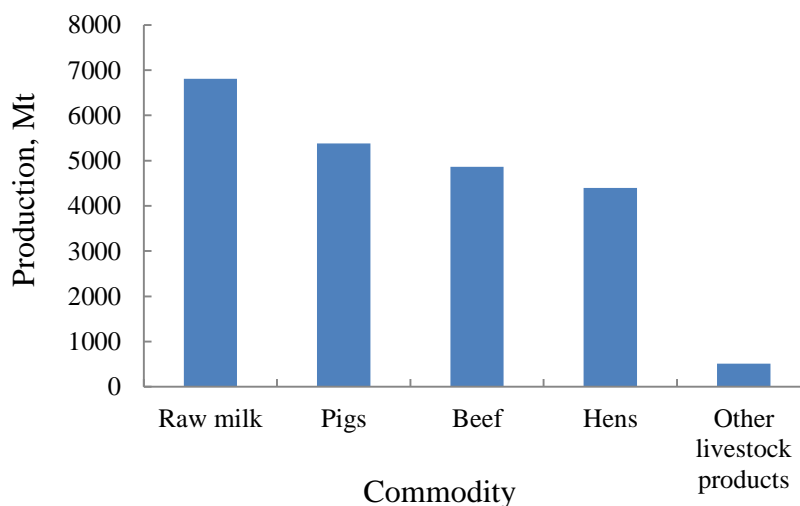
Figure 1.8 Livestock production in Japan from 2009 to 2013
Self-processed data from FAO statistic data
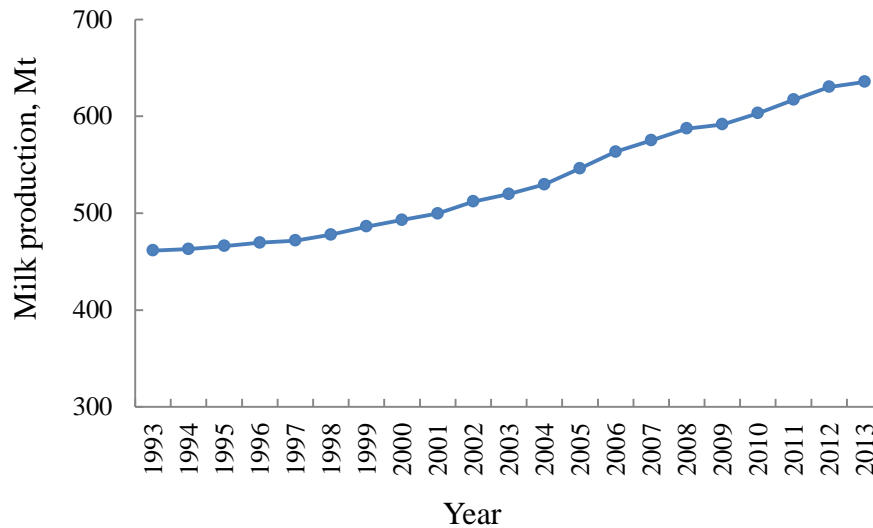
Figure 1.9 The trend of world milk production
Self-processed data from FAO statistic data

Figure 1.9 above shows the trend of the world milk production. The world dairy situation remains little changed from the year 1993 to 2013. According to United States Department of Agriculture (USDA, 2018), milk production during 2015 among major suppliers is estimated to have expanded by 1 percent over the previous year; a sharp correction from the high 4 percent growth registered in 2014.

Figure 2.0 below, shows the trend of milk production in Japan. Over the long term, raw milk production is declining. Increased scale and enhanced productivity led to a steady increase of milk production in the decades following World War II. But production volume peaked at 8.66 million tons in fiscal 1996 (April 1996 to March 1997) and had fallen to 7.33 million tons as of fiscal 2014. Consumption has also dropped over the past decade, due in part to the low birthrate and the aging of the population as reported by the Ministry of Agriculture, Forestry and Fisheries (MAFF Statistic, 2016).

Figure 1.10 below shows the trend of milk production in Nigeria. Milk production in Nigeria increased from 380 thousand tons in 1993 to 407 thousand tons in 1996. It then decline to 350 thousand tons in 1997. The proportion of fresh milk in total milk production increased from 357 thousand tons in 1998 to 468 thousand tons in 2007. The total milk production then declined to 420 in 2008. Since then, the total milk production in Nigeria continued to increase till date. The reason is because of the increase in the number of cattles per family in Nigeria as reported by Nigerian Market research (2015).

Although, Nigeria is the largest producer of cow milk in West Africa and the third in Africa, they country is a net importer of the product and in order to increase the percentage of the livestock sector and local milk production in Nigeria, massive investment is required in the dairy

industry to meet up with the 1.45 billion estimated national milk requirement as reported by Nigeria Market Research (2017).
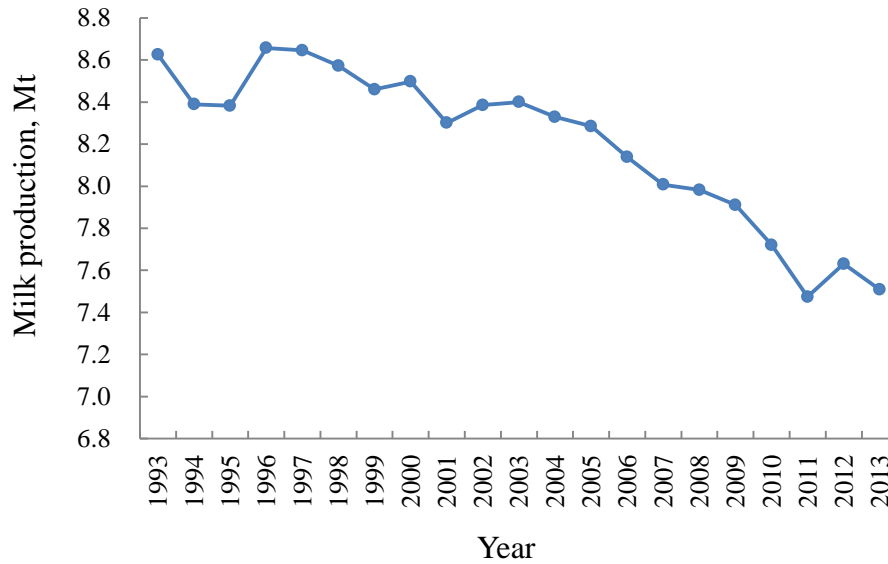
Figure 1.10 The trend of milk production in Japan
Self-processed data from FAO statistic data
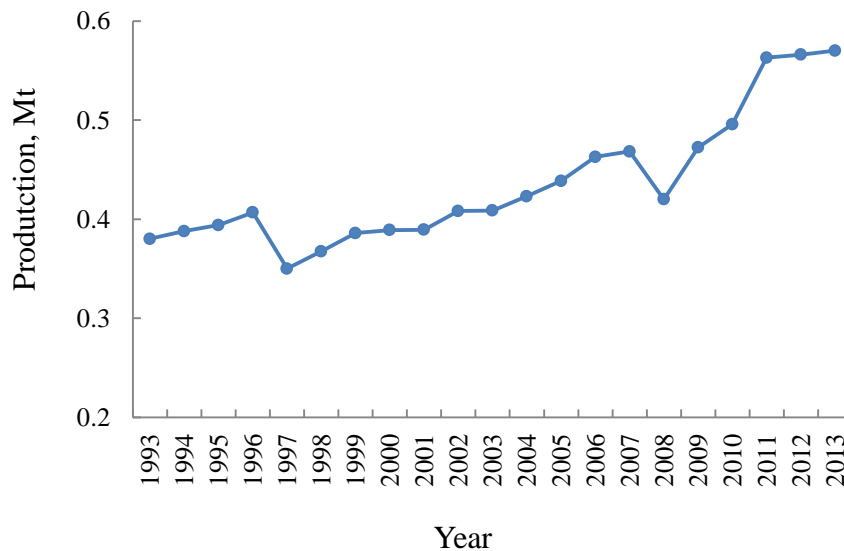
Figure 1.11 The trend of milk production in Nigeria
Self-processed data from FAO statistic data

1.8.6   Milking methods

Milking is the act of removing milk from the mammary glands of an animal, typically cows (cattle), water buffalo, goats, sheep and more rarely camels, horses and donkeys. Milking may be done by hand or by machine, and requires the animal to be currently or recently pregnant. The following are the methods of milking:

1.8.6.1 Hand milking

Hand milking is performed by massaging and pulling down on the teats of the udder, squirting the milk into a bucket. Two main methods are used:

a) The top of the teat is pinched shut between finger and thumb, trapping milk in the lower part, which is then squeezed by the other fingers, squirting the milk out through the hole in the tip of the teat.

b) The top of the teat is pinched shut by the fingers and thumb, which are then slid down the teat, pushing the milk towards the tip.

1.8.6.2 Machine milking

Most milking in the developed world is done using milking machines. The *teat cups* are attached to the cow's teats, then the cups alternate between vacuum and normal air pressure to extract the milk. The milk is filtered and cooled before being added to a large *bulk tank* of milk for storage. Milking machines are used to harvest milk from cows when manual milking becomes inefficient or labor-intensive. One early model was patented in 1907. The milking unit is the portion of a milking machine for removing milk from an udder. It is made up of a claw, four teatcups, (Shells and rubber liners) long milk tube, long pulsation tube, and a pulsator. The claw is an assembly that connects the short pulse tubes and short milk tubes from the teatcups to the long pulse tube and long milk tube. (Cluster assembly) Claws are commonly made of stainless steel or plastic or both. Teatcups are composed of a rigid outer shell (stainless steel or plastic) that holds a soft inner liner or *inflation*. Transparent sections in the shell may allow viewing of liner collapse and milk flow. The annular space between the shell and liner is called the pulse chamber.
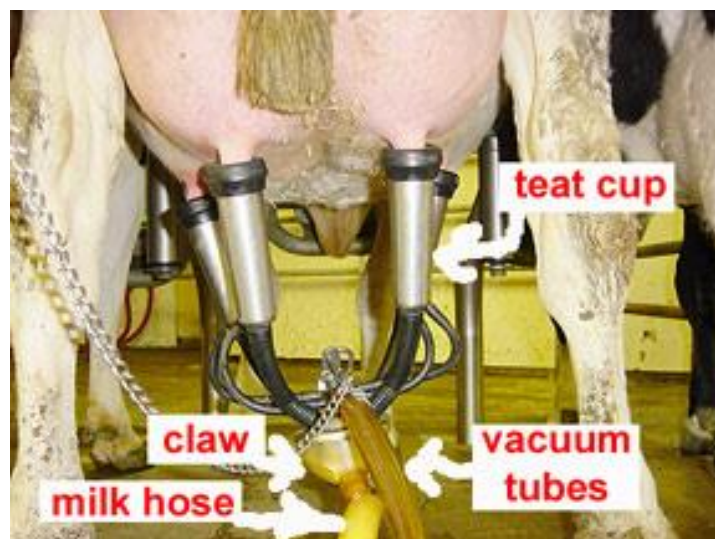
Figure 1.12 Milking machine on a cow indicating machine parts
Reprinted Zimmer, (1996)

Milking machines work in a way that is different from hand milking or calf suckling. Continuous vacuum is applied inside the soft liner to massage milk from the teat by creating a pressure difference across the teat canal (or opening at the end of the teat). Vacuum also helps keep the machine attached to the cow. The vacuum applied to the teat causes congestion of teat tissues (accumulation of blood and other fluids). Atmospheric air is admitted into the pulsation chamber about once per second (the pulsation rate) to allow the liner to collapse around the end of teat and relieve congestion in the teat tissue. The ratio of the time that the liner is open (milking phase) and closed (rest phase) is called the pulsation ratio.

The four streams of milk from the teatcups are usually combined in the claw and transported to the milk line, or the collection bucket (usually sized to the output of one cow) in a single milk hose. Milk is then transported (manually in buckets) or with a combination of airflow and mechanical pump to a central storage vat or bulk tank. Milk is refrigerated on the farm in most countries either by passing through a heat-exchanger or in the bulk tank, or both.

Today, there exist fully automatic milking machines which give a cow the freedom to choose when to be milked, allowing for a larger amount of milk to be obtained more efficiently.

Hand milking system

Pipeline milking system at a
modern dairy facility

A rotary milking parlor at a
modern dairy facility

An automated milking robot at a
modern dairy facility

Figure 1.13 Different milking systems
Source: Reprinted from Dairy Technology. 6 June 2019, http://www.ecoursereonline.aisri.res.in

1.8.7 Importance of milk quality control in dairy management

Milk quality control (QC) is the use of various tests to ensure that milk and milk products are safe, healthy, and meet the standards for chemical composition, purity, and levels of bacteria and other micro-organisms according to Sraïri, et al. (2009). QC has the objective of ensuring the quality and safety of the milk offered to the consumer. The nature and manipulation of raw milk, the hygiene conditions at the farm and the industry, the process to which it is subjected and the conditions of storage change the properties of the product. QC of milk is done at different levels, by the farmer, by the industry and by the government as reported by Belloque et al., (2017). The farmers need to have control on the raw milk in order to improve and maintain the quality of production that is sold to the manufacturing industry. The dairy industry needs to control the raw milk supplied by the farmers and set up controls on the process and/ or the end product in order to ensure the safety and quality of the product going out to the market. The government agencies

control the raw milk, to obtain information of hygiene and safety, and the end product, to monitor the overall manufacturing process and to prevent fraud or mislabeling.

1.8.7.1 Mastitis and milk quality

Mastitis is the inflammation of the mammary gland and udder tissue, and is a major endemic disease of dairy cattle. It usually occurs as an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm, and can also occur as a result of chemical, mechanical, or thermal injury to the cow's udder as stated by Auldist & Hubble, (1998). Bacterial infections are by far the most common cause of mastitis in dairy cattle and much is known about the effects of these bacterial intramammary infections (IMI) on milk composition. Pantoja et al. (2009)state that on the farm the composition of bulked milk is remarkably constant, particularly in lactose, and that it is generally assumed that lactose concentrations cannot vary under physiological conditions and must play a decisive role in controlling the volume of secretion as reported by Auldist, et al. (2007).

1.8.7.2 Pathogenesis of milk composition changes during mastitis

According to Maret al. (2011), there are a number of functions that can be disrupted during intramammary infection and a number of mechanisms that can be disturbed, the outcome are difficult to predict and will depend on the following:

a) Severity of the infection varies from very little effect to completely inhibition of milk secretion depending on the mastitis-causing organism, its virulence and resistance of the host stated by (Pyörälä, 2003).

b) According to (Pyörälä, 2003), extent of the infection, which may be localized to a few alveoli or encompass all alveoli. Quantifying this effect is difficult because in most cases, only one gland is infected; therefore, the effect is diluted when measurements are made on a whole-animal basis. There are considerable amount of variability depending on individual animal variation, breed (in the case of mixed breeds in the same herd), age, and stage of lactation.

c) Alteration of the metabolic activity of milk producing cells, including reduction of milk synthesis, and interference with ion balances, either by a reduced concentration of a galactopoietic hormone or by an increased concentration of an inhibitory hormone or/and an inflammatory mediator.

d) Interference with precursors availability for milk synthesis due to: anorexia, decreased blood flow in the mammary gland or hormonal imbalance.

e) Disruption of epithelial integrity, by opening up paracellular pathway.

f) Decomposition of the milk constituents due to leukocytes' and mastitis-causing organisms' enzymes.

There are many mastitis-causing organisms. The main mastitis-causing organisms in Australia and New Zealand, according to Auldist & Hubble (1998), are from the genus *Streptococcus* and *Staphylococcus*. These organisms enter the mammary gland via the teat canal and multiply in the milk in the teat and mammary cisterns. As part of the cow's defence mechanism, the new intramammary infection is quickly followed by an influx of leucocytes into the milk and an increase of the milk somatic cell counts as reported by Jayarao et al., (2004). The increase in tight junction permeability across endothelial and epithelial layers is due to the products of the inflammatory reaction such as histamine, TNF, IFN-g and acute phase proteins. This increase in permeability may be an important part of the inflammatory process as it allows immune components to reach the infection site according to Kovacova et al., (2015). Muller (2013), state that the enhanced paracellular diapedesis of leukocytes through the epithelial cells causes reduced tight junction integrity and hence exchange of constituents between the blood and the milk through the paracellular pathway. The predominant leucocytes present in milk under such circumstances are polymorphonuclear neutrophils (PMN) as stated by Pyörälä (2003). They are responsible for the high somatic cell counts (SCC) that is characteristic of mastitic milk and are associated with many of the changes to milk composition that occurs during mastitis as reported by Pyörälä (2003).

1.8.7.3 Effects of mastitis on milk production

The reductions in milk production probably are largely due to physical damage to the epithelial cells of the affected mammary gland, and a consequent reduction in the synthetic and secretory capacity of the gland as a whole. Any retardation of the capacity of the mammary gland to synthesize and secrete lactose is of particular importance in this regard, given the key role of lactose as the osmotic regulator of milk volume as concluded by Urashima et al. (2012). However, all of the suppression of milk production associated with mastitis cannot be attributed to the damage of the mammary epithelium. Urashima et al. (2012) reported that the hypogalactia in un-affected quarters is due to systemic effect of mastitis in the affected quarters or by the systemic absorption of an inhibitor of milk production from the affected quarters acting on the un-affected quarters. A number of inflammatory mediators including cytokines and metabolites of arachidonic acid; changes in stimulatory or inhibitory hormone concentrations, and reduced milk precursor availability may play a role. Shuster et al., (1991) explained that the milk production from the affected quarters is more evident than in the un-affected quarters, resulting from the localized inflammation. Other possible explanations for the local suppression of milk production include direct effects of locally produced inflammatory mediators, leukocytosis and localized mammary oedema. The situation is more complex as it is generally accepted that uninfected quarters can increase production and compensate in part for the decrease in

production by the infected quarters according to Ingman et al., (2014). However, this compensation may only occur after the infection is cured. The increased permeability of the blood-milk barrier in the affected quarters leads to a decrease in the volume or milk component concentrations. Not all of the decrease in milk output is due to reduced synthesis; some is the result of escape from the gland into the circulation as stated by Urashima et al. (2012).

1.8.7.4 Effects of mastitis on milk composition

Fat protein and lactose contents

It is generally accepted that during mastitis, there is an increase in milk proteins as reported by Auldist (1995), Auldist and Hubble (1998) that has been attributed to the influx of blood-borne proteins (such as serum albumin, immunoglobulins, the minor serum proteins, transferring, a- macroglobulin into the milk coupled with a decrease in caseins.

According to *Auldist et al*. (1995) and Auldist and Hubble (1998) this increase in proteins of blood serum origin during mastitis is possibly due to a disruption to the integrity of the mammary epithelia by microbial toxins and opening of the tight junctions. Auldist and Hubble (1998) continue that the decrease in casein concentrations during mastitis is largely due to post-secretory degradation of casein by proteinases originating from mastitis-causing organisms, leucocytes or the blood and in part to a reduction in the synthesis and secretion of casein as a result of physical damage to the mammary epithelial cells by microbial toxins during mastitis as concluded by Auldist (1998).

The effect of mastitis on the characteristics of milk fat has not been studied nearly as extensively as milk proteins. There are contradictory results in the literature dealing with this matter. For example, Auldist and Hubble (1998) report a decrease in fat concentration, but the majority of the authors recorded an increase in total fat content of mastitic milk according to Pyorala (2003) and (Ingman et al., 2014).

Urashima et al. (2012) reported that the increase in fat concentration indicates that there is a reduced lactose synthesis and therefore reduced milk volume while the fat synthesis is only slightly depressed. Very similarly Holdaway, 1990 states that over a period of time, the total output of fat from a quarter is likely to be reduced, because of the lower volume of milk. In addition the leakage of lactose from the milk will take with it water and the volume of secretion left in the gland will decrease. The fat droplets however are large relative to the gaps between the cells and are contained within the alveolar and consequently their concentration increases.

In summary, the results of changes in the fat content of milk caused by mastitis are diverse. According to the results of most investigations, the fat content decreases by less than 10%. The fat composition, however, changes considerably, lowering the quality of milk products

as stated by Auldist et al. (2018). The total amount of fatty acids remains unchanged, but the quantity of free fatty acids increases. On the other hand, the amount of phospholipids diminishes due to the decrease in the amount and size of fat globules. The membrane matter of fat globules decreases by approximately 10% and its composition changes in comparison with that of a healthy cow's milk according to Leitner et al. (2004). The composition of fatty acids changes so that the amount of short-chained fatty acids (C4-C12) increases slightly and the amount of the long-chained fatty acids (C16-C18) drops correspondingly.

The quantity of unsaturated long-chained fatty acids is; however, higher in mastitic milk than in normal milk. The changes in the lipid phase increase the lipolytic sensitivity in mastitic milk according to Nguyen et al. (1998) and Shennan et al. (2000). This is intensified by the increased lipase activity. The total quantity of milk proteins does not decrease clearly until the somatic cell count (SCC) exceeds 1,000,000/ml. The ratios between the different proteins, however, change at a much lower SCC. A highly significant negative correlation exists between lactose content and SCC (Seelemann, 1964). The changes of mineral and trace element contents of milk have considerable importance both for processing properties and its nutritive value (Szakály, 2001). The quantity of water soluble vitamins fall by 10-50%. The changes affect bacteriological fermentation process and lower the quality of sour milk products reported Szakály (1982). Mastitis generally increases the enzymatic and biochemical activity in milk. Some of these characteristics have for many years been used to detect mastitis. Increased biochemical activity in milk may in particular cause faulty fermentation of sour milk products and induce various quality problems (Politis; Ma et al., 2000; Szakály, 2001).

1.8.8 Components of feed and milk quality

Plants serve as the major source of feed for livestock. Nutrients required for maintenance and production are provided by various plants and plant derived feeds along with small amounts from non-plant sources. The major constituents of plants are water, carbohydrates, protein, fat, minerals and vitamins. Both plants and animals contain these nutrients, but the relative proportions vary more in plants as reported by Fox & Kelly (2012).

1.8.8.1 Dietary effects on milk fat

The precursors required by the mammary gland to synthesize fat are produced during fermentation of feedstuffs in rumen, diets that change fermentation affect fat content. For instance, a low intake ratio of roughage to carbohydrate will result in decrease in acetate and butyrate, which are the major fat precursors, and increase in propionate, which negatively affect milk fat. Studies have shown that dry matter (DM) basis, the minimum forage to concentrate ratio needed to maintain milk fat content is about 40:60 according to Mosavi et al. (2012) and

Cabrita, et al., (2009). To maintain the fat content above 3.6%, average forage length should be at least 0.65 cm. The utilization of forages that are finely ground result in greater propionate production in the rumen, resulting in reduced in milk fat. To maximize fat percentage as well as yield, a minimum of 28% neutral detergent fiber is recommended in the dietary dry matter. Moreover, only the highest quality roughage should be used, because it not only promotes increase in yield but also results in a high level of acetate production to maintain milk fat. Studies have shown that excess fiber in the ration will reduce dry matter intake, resulting in lowered milk production as stated by Hall et al., (2010).

Type of concentrate employed in the ratio also influence milk fat percentage. Comparing with corn-based concentrates, those using barley may reduce the digestibility of fiber. Processing grains used in concentrates by pelleting, grinding, etc. increases the digestion of starch in the rumen and the production propionate, which subsequently reduce milk fat. Cereal grains as concentrates may be substituted with soluble carbohydrates (lactose, whey, molasses) to alleviate the reduction in fat caused by feeding low-forage rations. These carbohydrates appear to promote the growth of rumen bacteria that produce acetate for fatty acid synthesis as concluded by Wang et al., (2009) and Penner, et al., (2011).

1.8.8.2 Effect of ration on milk protein

According to Gozho & Mutsvangwa (2008), amino acids required for milk protein synthesis are derived from microorganisms in the rumen. Thus, the protein content of milk is affected by factors that regulate microbial growth. In rumen, the production of propionate promotes milk protein synthesis, probably by increasing the availability of certain amino acids, such as glutamate. The amount of propionate is produced is increased by feeding chopped forage (less than the 0.64 cm recommended to maintain milk fat). Similarly, increases in starch digesting microorganisms promote the production of propionate.

The increase in milk yield of dairy cows requires the use of large amounts of concentrates that are rich in energy and crude protein (CP) to meet their nutrients requirements (Cabrita, A. R. et al., 2009). Dietary carbohydrate is composed of neutral detergent fibre (NDF) and non-fibre fractions, which collectively compose 65% to 75% of the diets of lactating dairy cattle. Non-fibre carbohydrates (NFC) may provide 30 to 45% of the diet on a DM basis (Hall et al., 2010). Root crops such as potato have been used in dairy rations, but have been replaced by grains and maize silage because of labour costs (Eriksson et al., 2004). Dairy cow diets usually contain barley, maize and wheat as the main carbohydrate sources because they are cost-effective sources of digestible energy. Starch is the major nutrient providing energy from these cereal grains and potato. These cereal grains and potato differ in their starch content, with wheat containing (DM

basis) 77% starch, maize 72%, barley 58% (Huntington, 1997) and potato 63% (Sylvia & Maria, 2015).

Differences also exist among these starch sources in their rates and extents of ruminal starch degradation, with 32%/h for wheat starch, 2%/h for maize starch, 29%/h for barley starch, and 5%/h for potato starch being digested in the rumen (Wang et al., 2009). Potato starch could not be expected to be superior to grain starch as a readily available energy source, but it could reduce diurnal fluctuations in energy supply and limit the problems caused by the incorporation of a large amount of wheat or barley starch in the diet. In principle, the rate and extent of fermentation of dietary carbohydrates (especially starch) in the rumen are important parameters that determine nutrient supply to the animal (Santra & Karim, 2003; Hall, 2004). Greater dietary concentration of nonstructural carbohydrates increases the utilization of ruminal ammonia-N for microbial protein synthesis. Increasing ruminally available energy concentration of diets for dairy cows has the potential to enhance milk production through increased metabolizable nutrient supply (Gozho & Mutsvangwa, 2008). Most studies on the effect of starch source have been conducted with grains (barley, maize, millet, oat, sorghum or wheat), but rarely with potato. The study of different sources of starch using in sacco technique (Monteils et al., 2002; Wang et al., 2009) showed that the ruminal degradation of potato starch is slower than that of wheat or barley, but faster than maize starch. These observations have been confirmed by enzymatic tests with ruminal fluid (Cone, 1991). Owing to differences in digestion characteristics and fermentation products, the starch source of the diet has the potential to alter feed intake, milk production and milk composition.

1.9 Statement of research problem, objectives and hypothesis

Dairy farming is very demanding and requires lots of tasks such as feeding, milking, manure treatment, feed crop production and livestock management. Extensive dairy farmers manage their livestock in groups a system known as herd management. Fat, protein and lactose are the three major milk constituents and are essential indicators for milk quality. However, a system known as individual cow management is important so as to monitor the milk quality and physiological situation of each cow. This type of cow management is needed to upgrade the production of high-quality milk as stated by Wathes et al. (2008)

Near-infrared spectroscopy (NIRS) has become a popular method for simultaneous chemical analysis and is being studied extensively in a number of different fields such as process monitoring, biotechnology, and pharmaceutical industry because of its potential for on-line, nondestructive and noninvasive instrumentation according to Tran et al., (2004).

The traditional systems for determining the quality of milk and its major components are generally expensive and time-consuming and require great expertise, making them unsuitable for routine milk recording as concluded by Rasmussen,et al. (2002). On the contrary, the NIRS is a suitable alternative to traditional analytical techniques, being simple, fast, non-destructive and requiring minimal or no sample preparation; furthermore, it is inexpensive, non-polluting and safe, because it does not require chemical reagents and particularly trained staff according to Ru and Glatz 2000; Porep et al. 2015. Infrared Spectroscopy (IR) techniques are multi-parametric, and are routinely used to non-destructively determine a number of milk constituents such as fat, protein, and other parameters considered when determining milk price as reported by Kucheryavskiy et al. (2014).

Traditionally, NIRS is relatively a recent technique and has found its widest application area in agriculture and food industry as reported by Miralbes, 2004. NIRS as a nondestructive system for collecting qualitative information on foods and agricultural products has already been utilized for the evaluation of the physiochemical properties of rice and wheat (Kawamura, et al., 2003; Natsuga & Kawamura, 2006). NIRS has also been used to determine milk quality (Sato et al., 1987; Tsenkova et al., 1999; Tsenkova, et al., 2001a). This method was used to measure the contents of various constituents in homogenized milk (Sato et al., 1987; Ru and Glatz 2000). Tsenkova *et al.* (1999, 2000) reported on the analysis of non-homogenized milk samples; these authors determined the positive coefficients for fat, lactose, and total protein. Šustová & Kuchtík (2006) used a fibre optic probe to analyze raw milk. However, ***the problem of the research*** is that, it has been difficult to use NIRS for real-time on-line monitoring of milk quality of each cow during milking.

A new on-line near-infrared (NIR) spectroscopic sensing system has been designed to analyze milk quality. Kawamura et al., (2007) and Iweka et al., (2016, 2017 and 2018) stated that the NIR spectroscopic sensing system can be used for real-time on-line monitoring of fat, lactose, protein, solids not fat, moisture content, milk urea nitrogen and somatic cell count during milking with sufficient precision and accuracy and that this system of milking would improve dairy farm management. According to Kawasaki et al., (2005), robust calibration models can be developed using NIR spectra including factors affecting the performance of calibration models.

Hence, ***the main objectives of the research are:***

1. To test the performance of the NIR spectroscopic sensing system developed in this study and to determine which statistical methods (pretreatments and statistical analysis) are most suitable for this study using data obtained from several cows.

2. To determine and compare the precision and accuracy of cow individuality, different milking seasons and parity.

3. To examine the potentials of the NIR sensing system for the determination of progesterone concentration of each cow during milking and

4. Finally the measurement accuracy of NIR sensing system when installed into an automatic milking. Therefore, *the hypothesis of this research* is that, if the design of the NIR equipment required for milk quality determination during milking is optimized with higher precision and accuracy, a complete study of monitoring the milk constituents' quality of each cow during milking could be achieved.

2.   Chapter II: Evaluation of Seasonal Milk Constituents Trends

2.1 Objective

The objective of this research was to monitor the trends of milk constituents in real time during milking for different milking seasons using reference data.

2.2 Materials and methods

2.2.1   Near-infrared spectroscopic sensing system

An experimental online NIR spectroscopic sensing system was designed for determining milk quality of each cow during milking. The system comprised of an NIR spectrometer, milk flow meter, milk sampler and a laptop computer. The system was fixed between a teatcup cluster and a milk bucket of a milking system. Non-homogenized milk from the teatcup cluster flowed continuously through a bypass into the milk chamber of the NIR spectrum sensor. The remaining raw milk flowed pass the milk flow meter and then released through a line tube into the bucket. The volume of milk sample in the milk chamber was about 30mL. The spectrum sensor acquired absorbance spectra through the milk. The spectra were recorded in the range of 700 nm to 1050 nm at 1 nm interval every 20 seconds during milking. The milk flow rate was recorded simultaneously.

2.2.2   Cow information

Two Holstein cows, numbered 1256 and 1257 owned by Hokkaido University dairy barn were used in this study. These cows were used in the experiment during their different lactation periods. The samples were collected from the period of August, 26 to August 28, 2015 for summer season; October 5, 2015 to October 7, 2015 for autumn season; November 30, 2015 to December 12, 2015 for winter season and April 13, 2016 to April 15, 2016 for spring season. A pipeline milking system was used for milking the cows at Hokkaido University cow barn. Measurements were performed in two successive milkings, i.e., milking in the evening and milking in the following morning for about five weeks during the experiment period. Two cows were milked at the same milking time and each cow was measured for four or five milking times. Milk spectra and milk samples were collected from the sampler every 20 seconds during milking. The experiment was conducted to cover variation in milk spectra caused by cow individuality, calving times, lactation stage and milking time.

2.2.3   Reference analyses

In this study, we measured three major milk constituent (fat, protein and lactose), MUN and SCC of non-homogenized milk as milk quality parameters. The milk constituents and MUN

were determined using a MilkoScan instrument (Foss Electric, Hillerod, Denmark) and SCC was evaluated using Fossomatic instrument (Foss Electric, Hillerod, Denmark).

2.3 Results and discussion

Figure 2.1, 2.3, 2.5 and 2.7 shows the result of milk trend of fat content for three consecutive milking days and milking season (summer, autumn, winter and spring) for cow 1256. While figures 2.2, 2.4, 2.6 and 2.8 shows the result of milk trend of fat content for three consecutive milking days and milking season (autumn, winter and spring) for cow 1257. The above listed figures show milk constituents trend for two consecutive evenings and the following mornings for cow number 1256 and 1257 respectively. Also, the figure 2.9, 2.11, 2.13 and 2.15 reveal the SCC trend for cow 1256 and figure 2.10, 2.12, 2.14 and 2.16 reveal SCC trend for cow 1257 respectively.

2.3.1 Milk constituents trend for summer

The results showed that there was a little difference in milk trends for each cow in summer season. Fat content rapidly increased most especially during morning milking for number 1256 and 1257 respectively (Figure 2.1 and 2.2). The Fat content increased from 5.6% to 7.9% for cow 1256 from the evening of 2015/8/26 to the morning of 2015/8/28 (for three consecutive days) while that of cow 1257 rises from 4.0% to 7.8%. For three successive days, SCC increased from 4.7 log SCC/mL to 5.6 log SCC/mL for cow number 1256 during summer season and the SCC of cow 1257 increased from 5.1 log SCC/mL to 5.5 log SCC/mL respectively.

2.3.2 Milk constituents trend for autumn

The results indicated that the milk trends for each cow in autumn season were slightly similar. Fat content significantly increased from 4.7% to 7.3% for cow 1256 from the evening of 2015/10/5 to the morning of 2015/10/7 (for three consecutive days) while that of cow 1257 rises from 3.7% to 5.9%. For three successive days, SCC increased from 5.0 log SCC/mL to 5.7 log SCC/mL for cow number 1256 during autumn season and the SCC of cow 1257 increased from 4.5 log SCC/mL to 5.3 log SCC/mL respectively.

2.3.3 Milk constituent trend for winter

For winter, the fat content rises from 4.8% to 7.2% and then decline to 5.6% for cow number 1256 whereas for cow number 1257 the fat content rises from 4.1% to 5.3 and gradually decline to 5.1% respectively. In winter season, the SCC for cow 1256 rises from 5 log SCC/mL to 5.5 log SCC/mL but that of cow 1257 was 4.5 log SCC/mL to 5.4 log SCC/mL during morning milking and SCC during evening milking was 5.6 log SCC/mL respectively.

2.3.4   Milk constituent trend for spring

The percentage fat content for spring season increases from 5.1% to 6.5% and then dropped to 5.8% for cow number 1256, while that of cow 1257 increases from 5.0% to 6.5% which gradually dropped to 5.9%. The SCC for spring season rises from 4.9 log SCC/mL to 6.9 log SCC/mL for cow 1256 whereas for cow 1257, there was fluctuation, such that the SCC rises from 5.9 log SCC/mL to 6.0 log SCC/mL and then dropped back to 5.9 log SCC/mL.

There was a huge difference between the fat content of evening milking and that of morning milking from each cow. The difference of SCC was not so much for different season. Seasonal effect on milk yield and constituents are largely attributed to extremes in environmental temperature. The consumption of roughages is reduced during environmental stress, resulting in decreased milk production as well as percentage fat. Similarly, protein and lactose are lower during warm season. The difference in milk constituents between seasons may average 0.4% for fat and 0.2% for protein. MUN concentration is high in warm season compare to cold season which is due to the fact that cows are fed with feed of high protein content in warm season (Shewy et al. 2010; Ferguson et al. 1997). Variation of lactation yield and genetic quality of cows fed diets containing similar level of nutrition, especially of protein, may also affect MUN content. Evaluation of MUN during collection time of milk may give good indication of the protein availability of cows from plane of nutrition that varies in season (Baset et al. 2013). High SCC which is related to seasonal environmental changes showed peak levels during warm season compare to other seasons (Wegner et al. 1972). High SCC is routinely used by dairy farmers to detect mastitis. Therefore, it is important to monitor the quality of milk constituents, MUN and SCC in real time during milking for different seasons as this would ensure proper individual cow management.
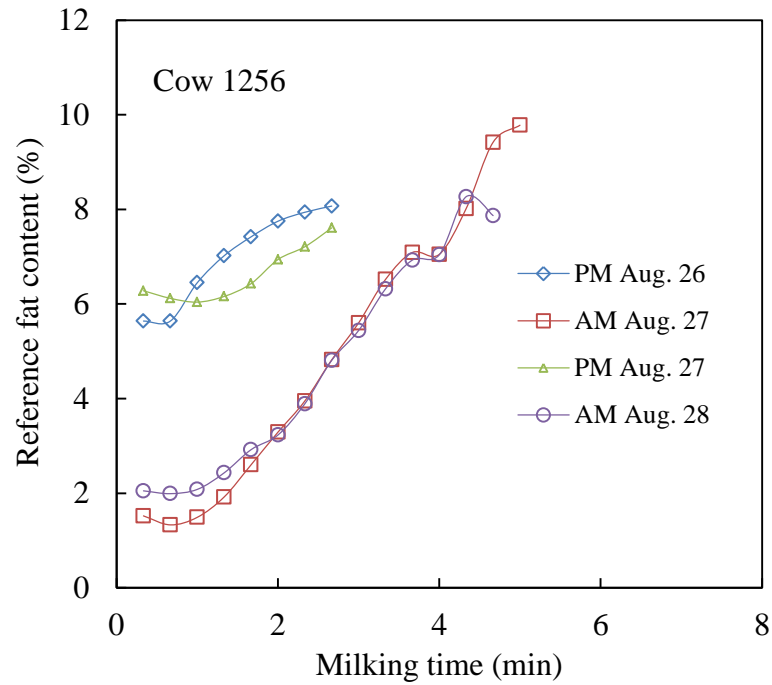
Figure 2.1 Milk fat content trends monitoring during milking from cow number 1256 in summer season
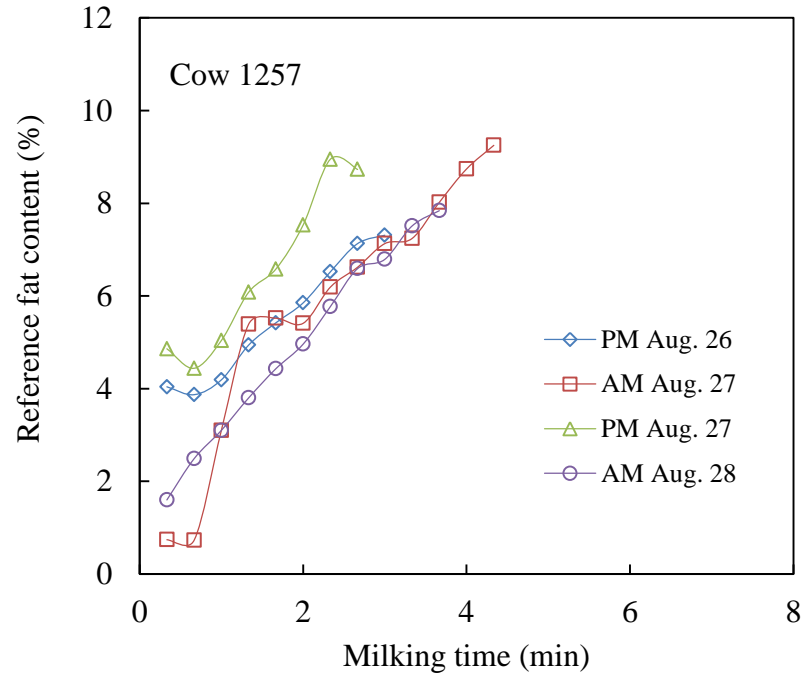
Figure 2.2 Milk fat content trends monitoring during milking from cow number 1257 in summer season
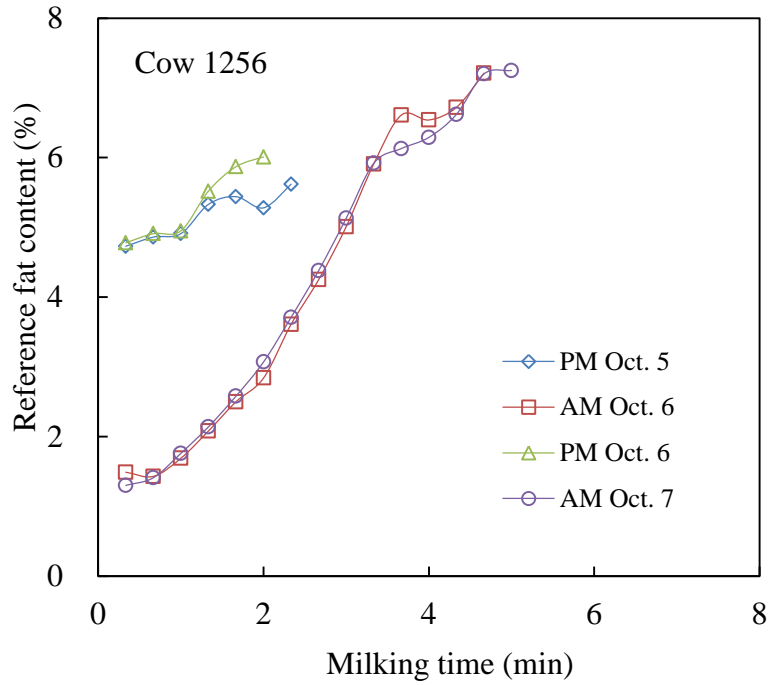
Figure 2.3 Milk fat content trends monitoring during milking from cow number 1256 in autumn season
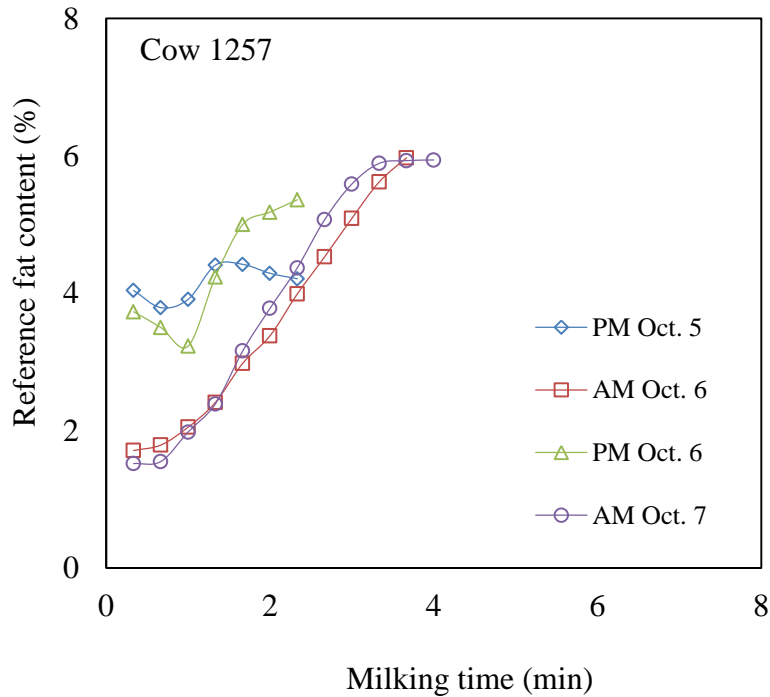
Figure 2.4 Milk fat content trends monitoring during milking from cow number 1257 in autumn season
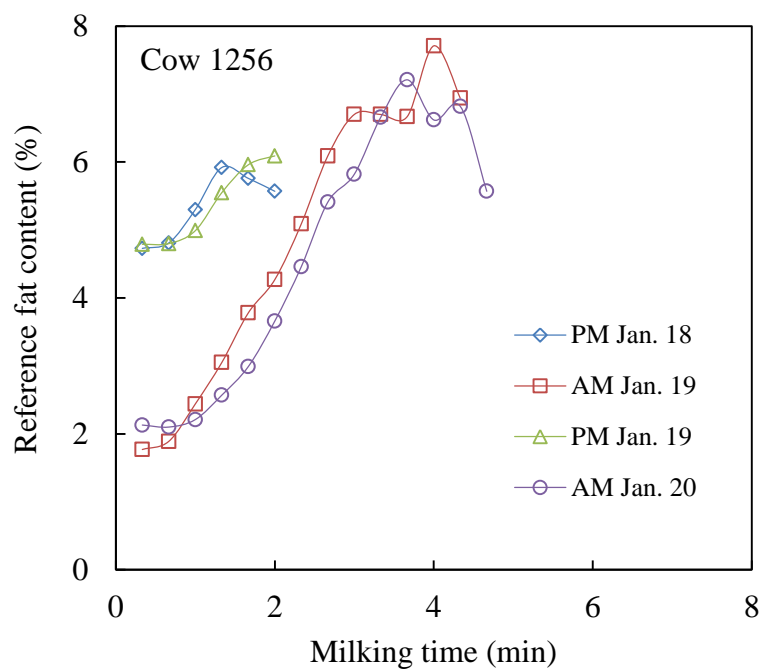
Figure 2.5 Milk fat content trends monitoring during milking from cow number 1256 in winter season
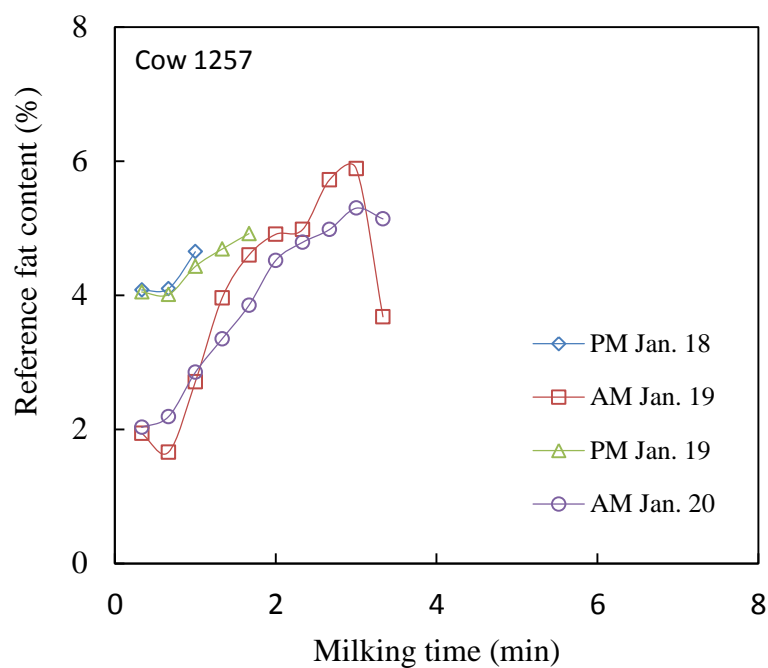
Figure 2.6 Milk fat content trends monitoring during milking from cow number 1257 in winter season
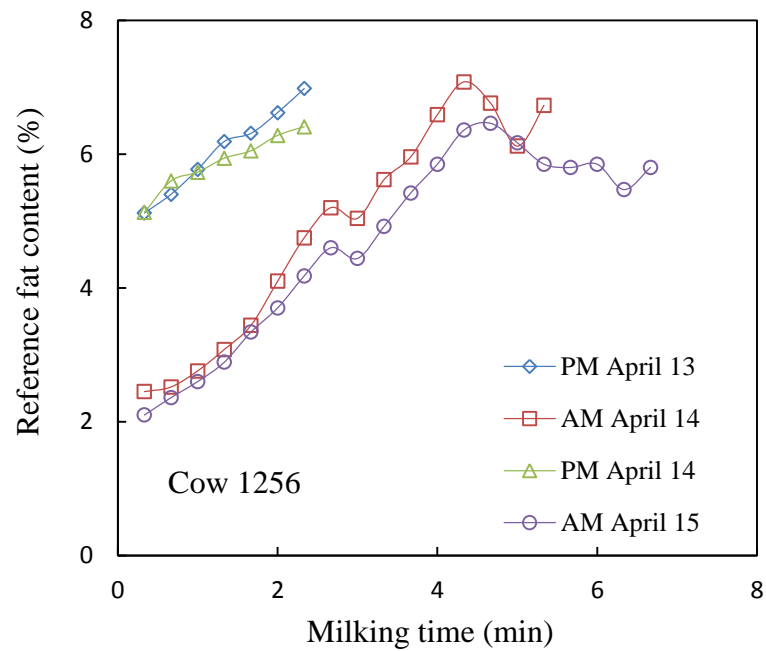
Figure 2.7 Milk fat content trends monitoring during milking from cow number 1256 in spring season
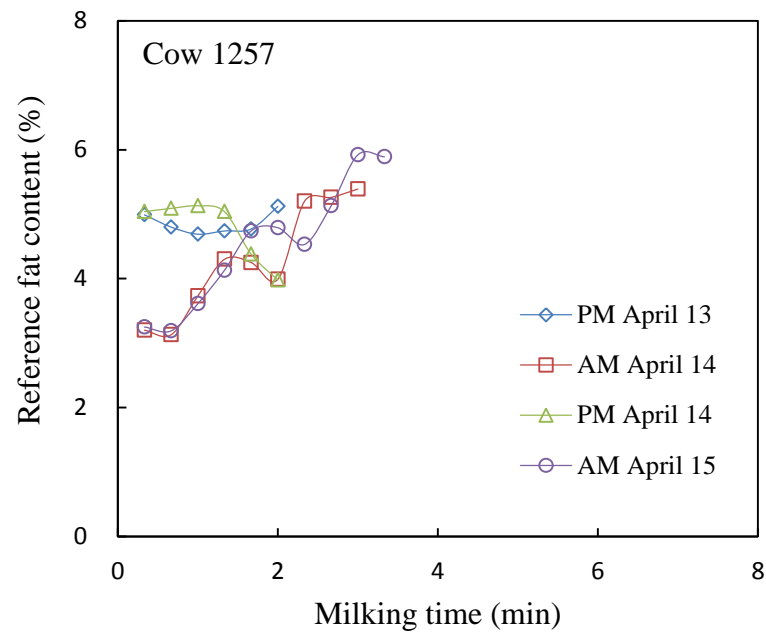
Figure 2.8 Milk fat content trends monitoring during milking from cow number 1257 in spring season

41
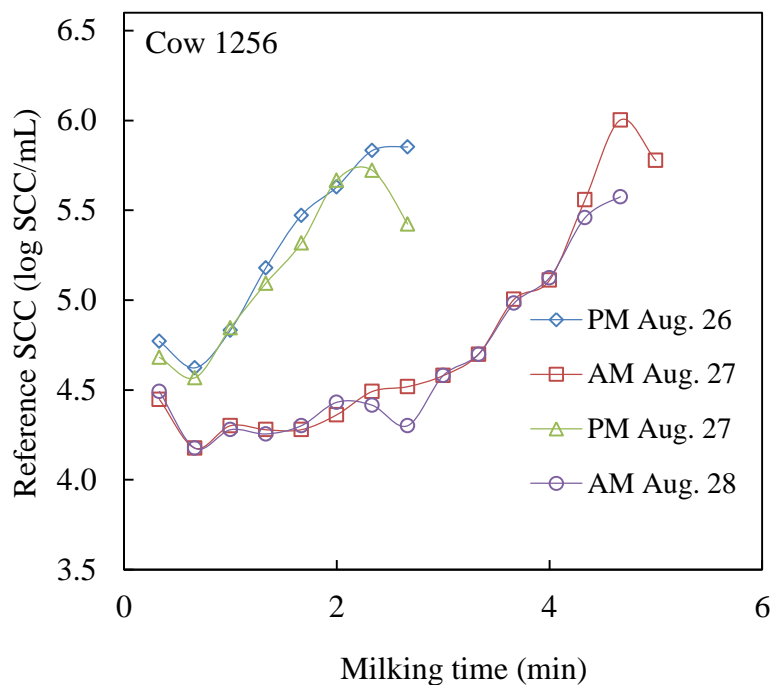
Figure 2.9 Milk SCC trends monitoring during milking from cow number 1256 in summer season
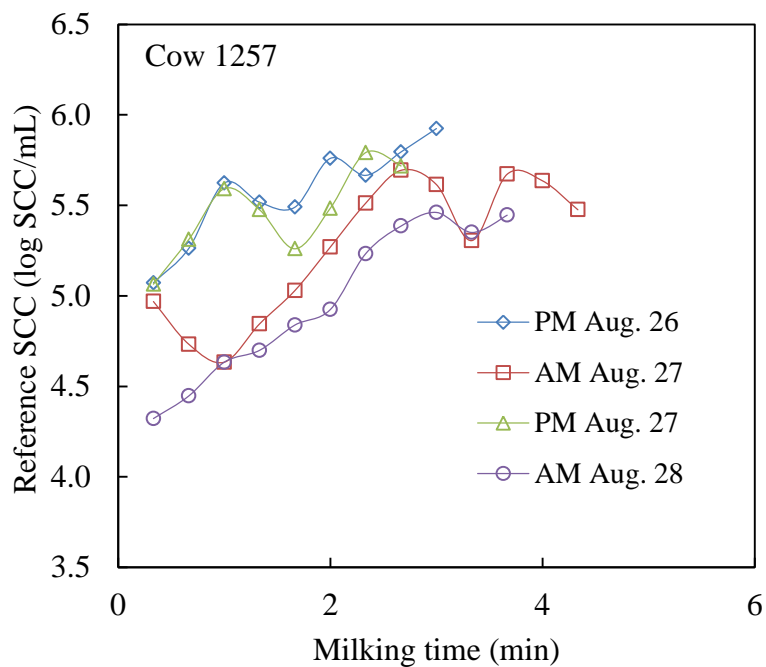
Figure 2.10 Milk SCC trends monitoring during milking from cow number 1257 in summer season
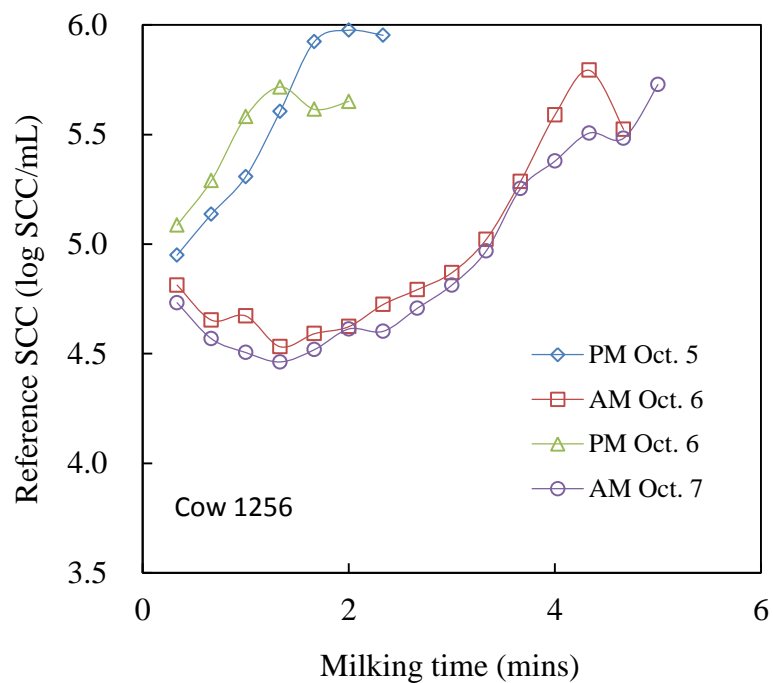
Figure 2.11 Milk SCC trends monitoring during milking from cow number 1256 in autumn season
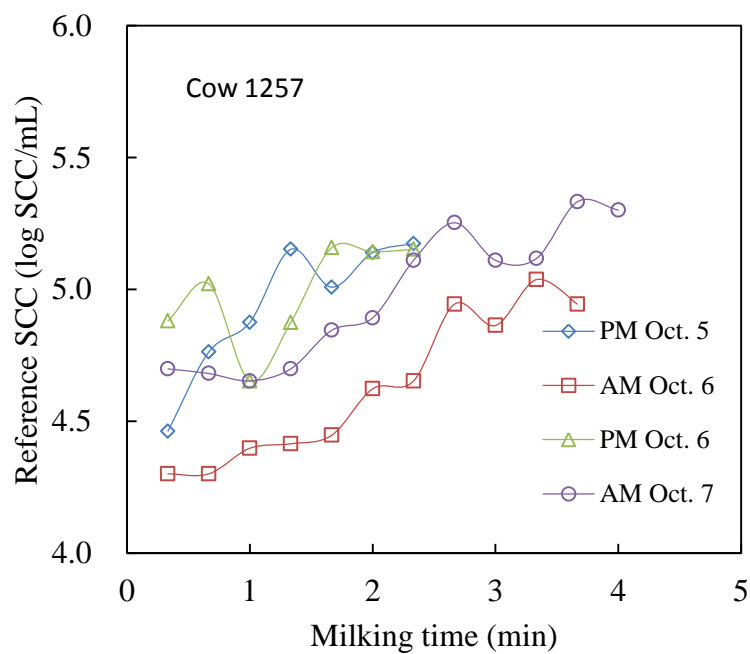
Figure 2.12 Milk SCC trends monitoring during milking from cow number 1257 in autumn season
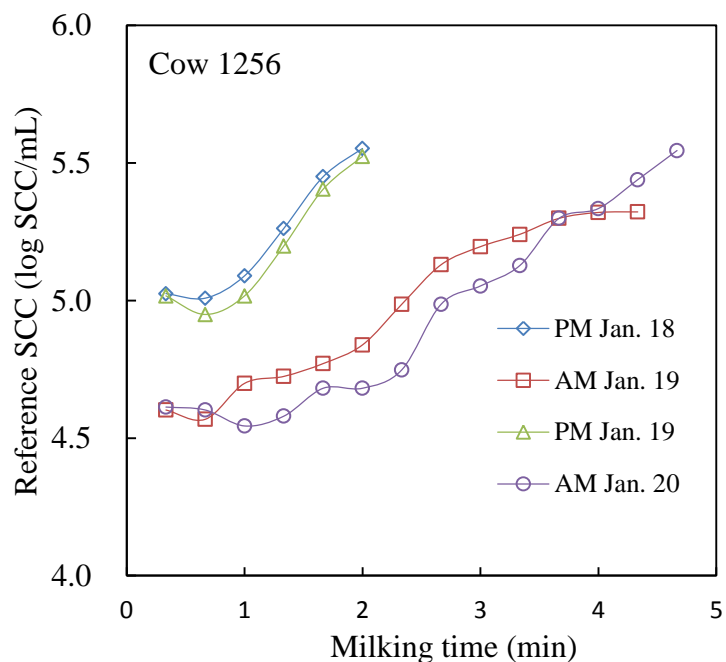
Figure 2.13 Milk SCC trends monitoring during milking from cow number 1256 in winter season
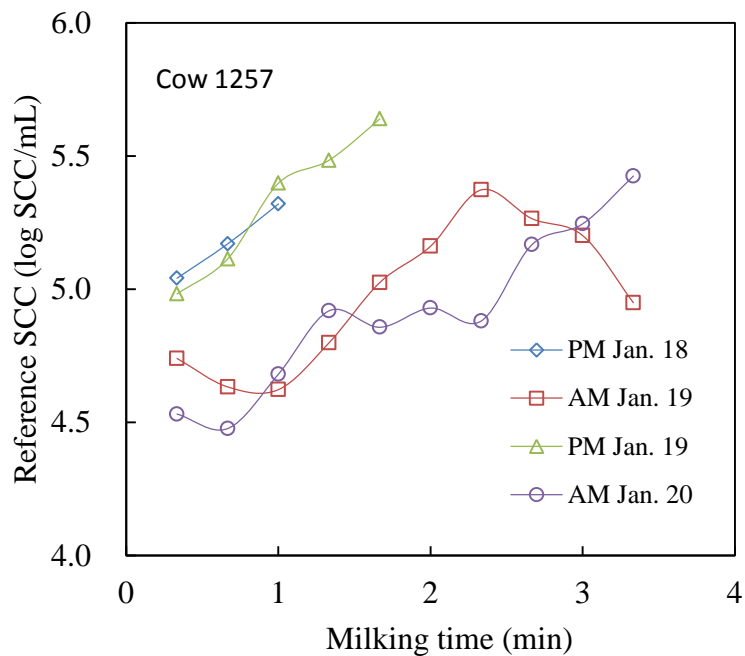
Figure 2.14 Milk SCC trends monitoring during milking from cow number 1257 in winter season
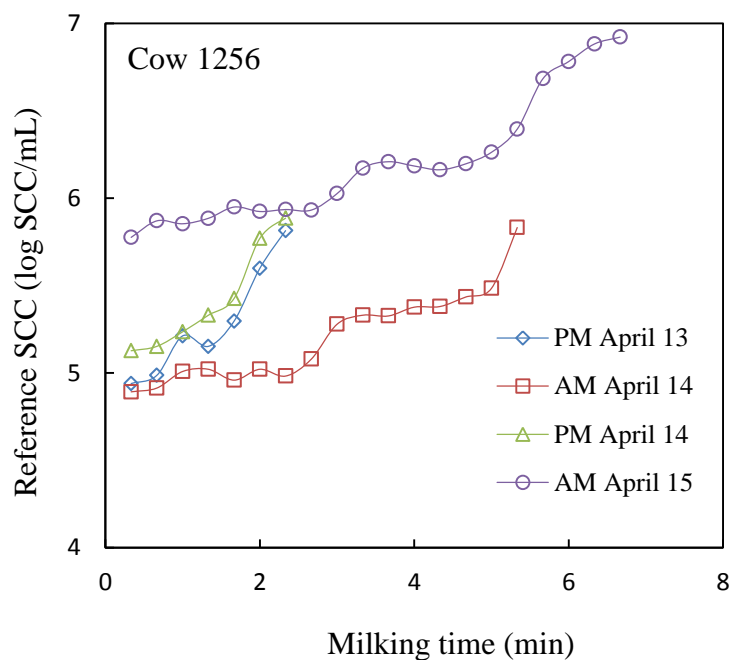
Figure 2.15 Milk SCC trends monitoring during milking from cow number 1256 in spring season
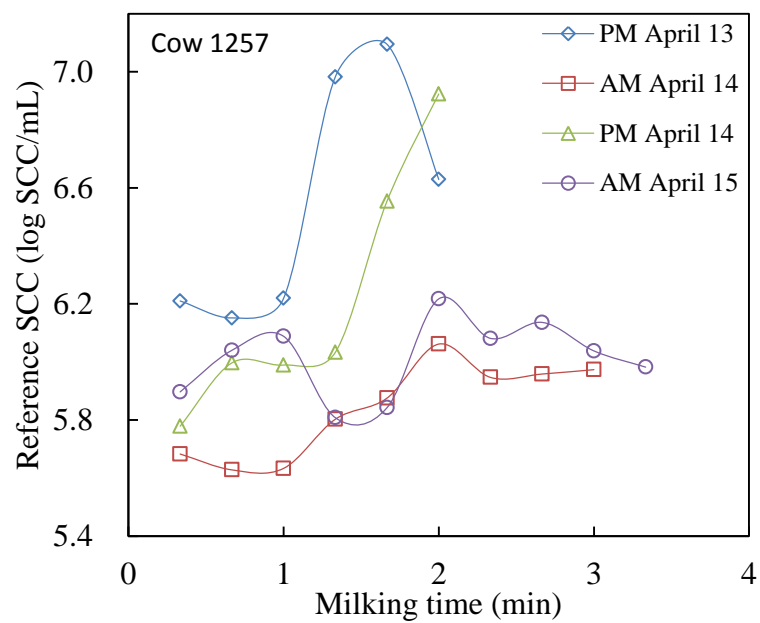
Figure 2.16 Milk SCC trends monitoring during milking from cow number 1257 in spring season

2.4 Conclusion

It is important to understand the effect of different milking season on milk quality. The trend of milk quality is also essential for investigating how cow milk constituents content

45

changes during different seasons with respect to cow health, feeding status and lactation stage. Therefore, it is important to monitor the quality of milk constituents, MUN and SCC in real time during milking for different seasons as this would ensure proper individual cow management.

3.   Chapter III: Design of Near-Infrared Spectroscopic Sensing System

This chapter discusses the milk quality measurement machine used for the collection of the data presented within this research. In this section, the NIR spectroscopic sensing system used for online real-time monitoring of milk quality during milking was described.

3.1 Description of milking machine

The milking machine was designed for milking cows. It is an assembly of different components. The system consists of NIR spectrometer, the cluster (the assembly that is manually attached to the cow), a milk tube, a pulse tube, a vacuum pump, and a milk flow meter that measures milk yield or production, milk sampler and a laptop computer.

The cluster consists of teatcups, a shell and liner device that actually performs the milking action, and a claw that spaces the teatcups and connects them to the milk and pulse tubes. The milk tube carries the milk and air mixture away from the cow's udder and flow continuously through a bypass into the milk chamber of the NIR spectrum sensor. The remaining raw milk flowed pass the milk flow meter and then released through a line tube into the bucket (Figure 3.1). The spectrum sensor acquired absorbance spectra through the milk. The laptop computer then records the spectra data in the wavelength range of 700 nm to 1050 nm at 1 nm interval every 20 seconds during milking and the milk flow rate was recorded simultaneously.

This machine determines milk quality during milking by NIRS and carries out measurement, correction of data and so on by signals from milk flow meter and thermometer.

3.2 Specification of milking machine

This machine determines milk quality during milking by NIRS and carries out measurement, correction of data and so on by signals from milk flow meter and thermometer (Figure 3.2). Liner array is used for the spectroscope and wavelength resolution is 10 nm. This machine calculates transmission and scattering spectra of raw milk from 700 to 1050 nm by ratios of signal intensities of milk to water.
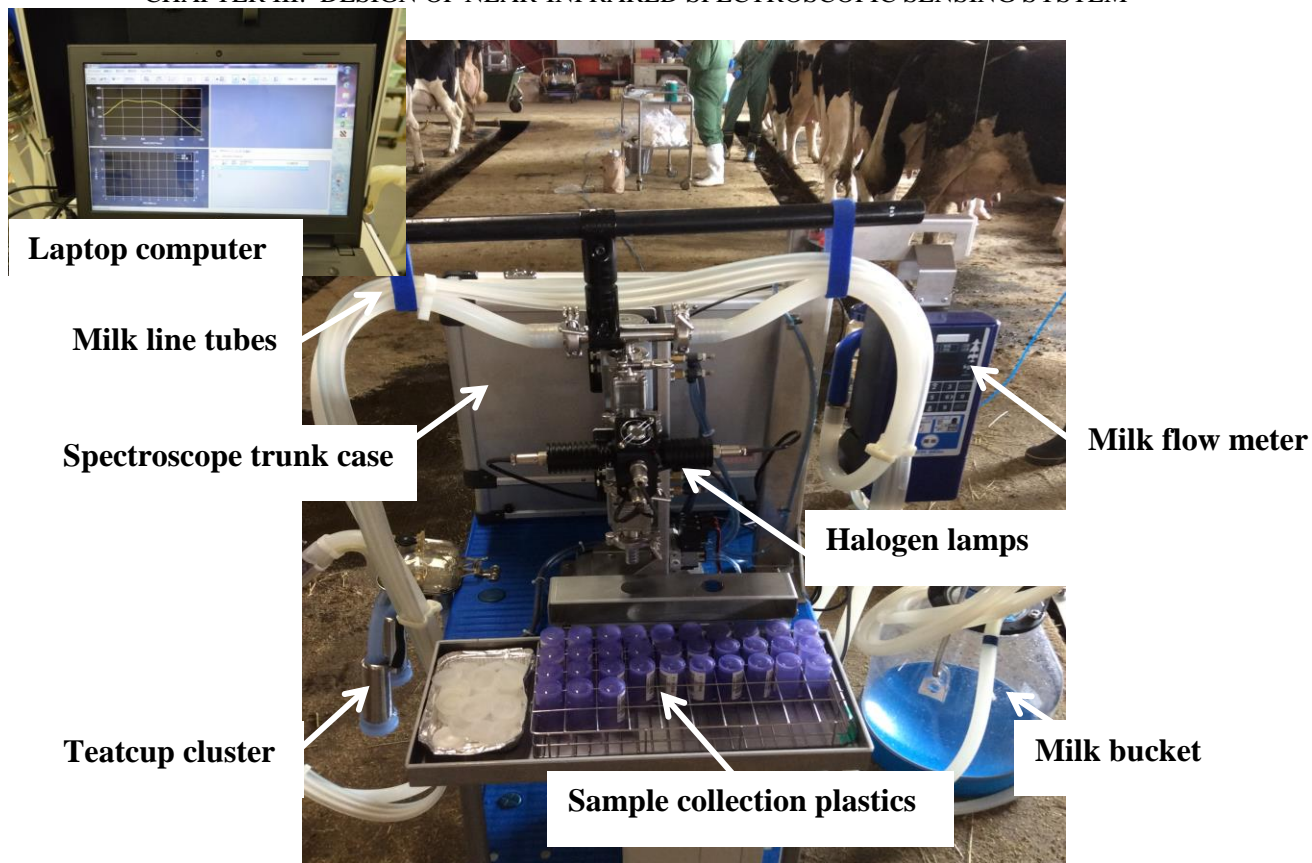
Figure 3.1 Overview of the NIR spectroscopic sensing system
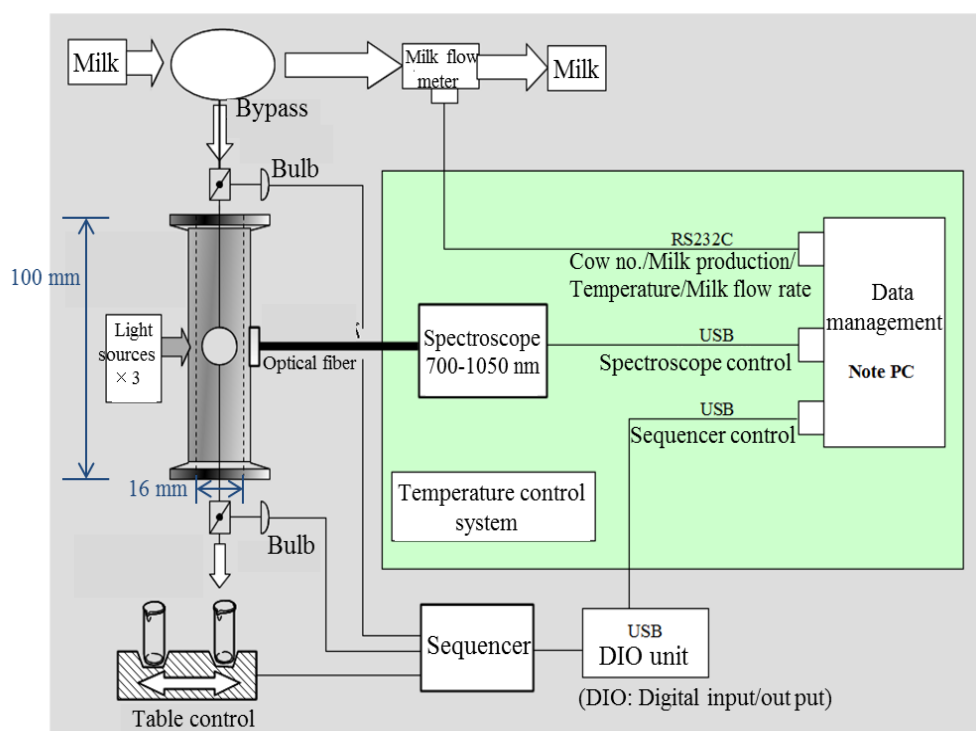
Figure 3.2 Block diagram of the NIR spectroscopic sensing system

3.3 Spectroscope and spectrum measurement

Spectroscope is an instrument used to measure properties of light over a specific portion of the electromagnetic spectrum, typically used in spectroscopic analysis to identify materials. The variable measured is most often the light's intensity and the independent variable is usually the wavelength of the light. Spectroscope consists of a light source, concave mirror, diffraction grating and a linear array detector (Figure 3.3). The linear array detector consist of a linear array of integrating photo sensing pixels which measure incident light over a user-defined exposure time and generate a voltage or digital output which represents the light exposure of each pixel. Output signals from liner array detector are in proportion to quantity of incident light. This detector has ability to add results of multiple measurements. We can get high signal to noise ratio by high signal intensity and repetition time.

Absorbance at a certain wavelength (λ) is calculated by following formula.

$$A(\lambda) = -\log\left(\frac{I_S(\lambda)}{I_R(\lambda)}\right)$$

A (λ): Absorbance

I_S (λ): Signal intensity of milk measurement

I_R (λ): Signal intensity of water measurement

This machine calculates transmission and scattering spectra of raw milk from 700 to 1050 nm by ratios of signal intensities of milk to water at the wavelength resolution of 10 nm. The diffraction grating allowed the light intensity at different wavelengths to be recorded. The NIR sensor comprises of three halogen lamps that irradiate milk samples from three directions. Three halogen light bulbs were used as broadband sources of near-infrared radiation. Light-emitting diodes (LEDs) were also used as they offer greater lifetime and spectral stability and reduced power requirements.
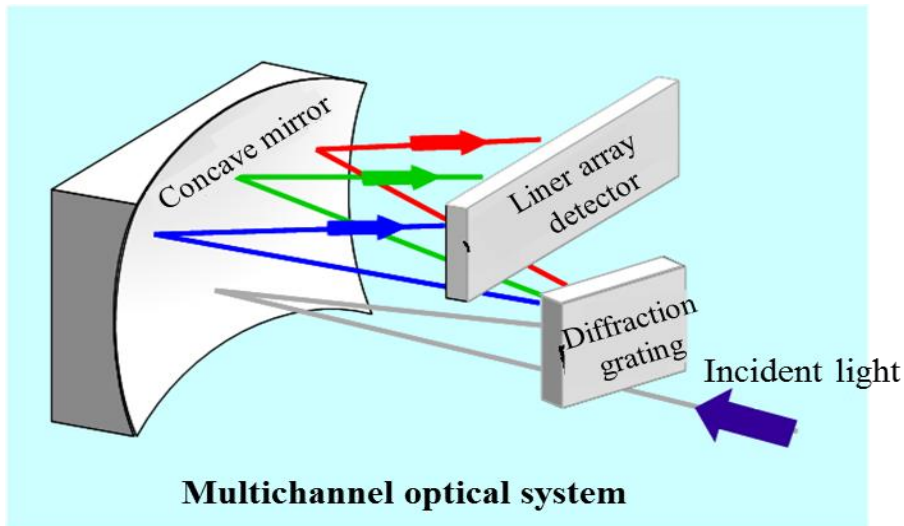
Figure 3.3 Shows a schematic diagram of a spectroscope

3.4 Data management

Data management is the process of controlling the information generated during a research project. A laptop computer was used for the data management section of this instrument (Figure 3.4). The milk spectra data, dark measurement, total milk production and milk flow rate were recorded in the laptop computer (Figure 3.5). The box housing the laptop computer consists of the power switch, temperature control switch and the optical fiber (Figure 3.4). The temperature control switch is used to control temperature change during milking such that it adjusts itself to achieve a desired average temperature.
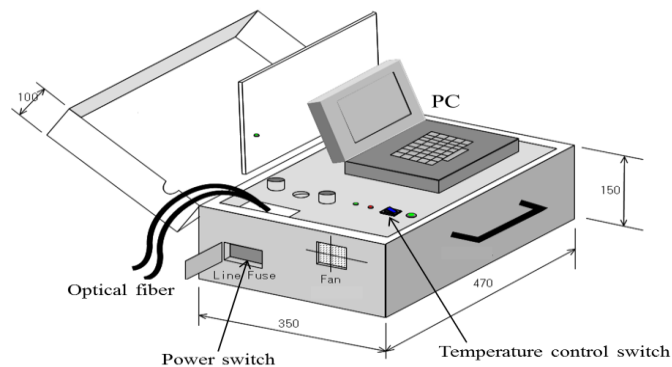
Figure 3.4 Shows a schematic diagram of a spectroscope and a laptop computer
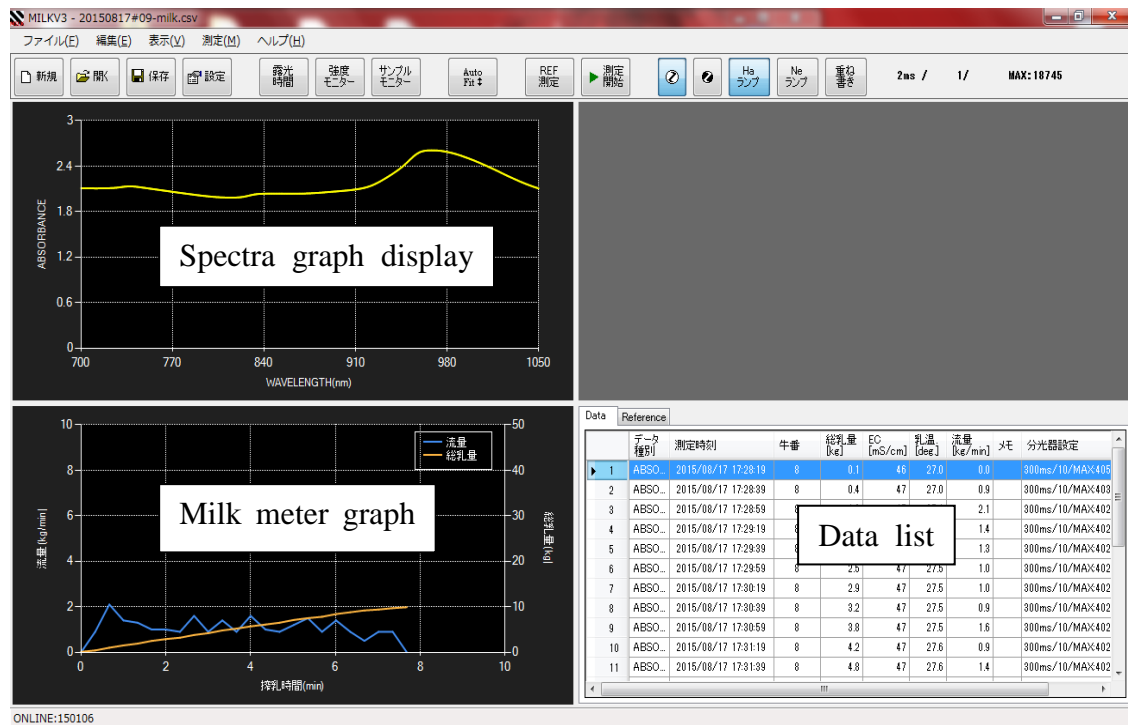
Figure 3.5 An example of data management on a laptop computer interface

3.5 Flow meter and samplers

Flow meter is a device used to measure flow rate of liquid or gas. In this instrument, the flow meter was used to measure the milk flow rate every 20 s (Figure 3.1). That is, it measures the actual volume of milk flow during milking. The milk flow meter output signal is directly related to the volume passing through the meter. This milk flow meter can help to know the total milk yield/production of each cow and for each milking section (AM/PM).

Milk samplers collect milk samples from the milk chamber every 20 s during milking for laboratory analysis of the milk content such as milk fat, protein, lactose, moisture content, milk urea nitrogen and somatic cell count. The volume of milk sample in the milk chamber was about 30 mL and so, for each milk sample collected using the milk sampler (plastic container) was approximately 30 mL.

4.   Chapter IV Measurement Accuracy of Near-Infrared Spectroscopic Sensing System

4.1 Objectives

1. The develop robust calibration models for the evaluation of milk quality indicators using NIR spectrum data obtained from two dairy cows with sufficient precision and accuracy.

2. To investigate the utilization of NIR absorbance, using PLSR method with different pretreatment techniques to develop predictive models for three major milk quality indicators such as (fat, protein, lactose), somatic cell count (SCC) and milk urea nitrogen (MUN).

3. To validate the precision and accuracy of the developed NIR spectroscopic sensing system and to determine the measurement accuracy of NIR spectroscopic sensing system for monitoring the trend of milk constituents during milking.

4.2 Materials and methods

4.2.1   Near-infrared spectroscopic sensing system

An empirical online NIR spectroscopic sensing system was designed for analyzing milk quality of each cow during milking. The system consisted of an NIR spectrum sensor, NIR spectrometer, milk flow meter, milk sampler and a laptop computer (Figure 4.1). The system was fixed between a teatcup cluster and a milk bucket of the milking system. Non-homogenized milk from the teatcup cluster flowed continuously across a bypass into the milk chamber of the NIR spectrum sensor. Excess raw milk flowed past the milk flow meter and was then released through a line tube into the bucket. The volume of milk sample in the milk chamber was about 30 mL. The optical axes of halogen lamps A and B and the optical fiber were set at the same level, but the optical axis for halogen lamp C was set at 5 mm higher the optical fiber (Figure 4.2). The spectrum sensor acquired absorbance spectra through the milk. Spectra were obtained in the range of 700 nm to 1050 nm at 1-nm intervals every 20 seconds during milking (Table 4.1). The milk flow rate was simultaneously recorded.
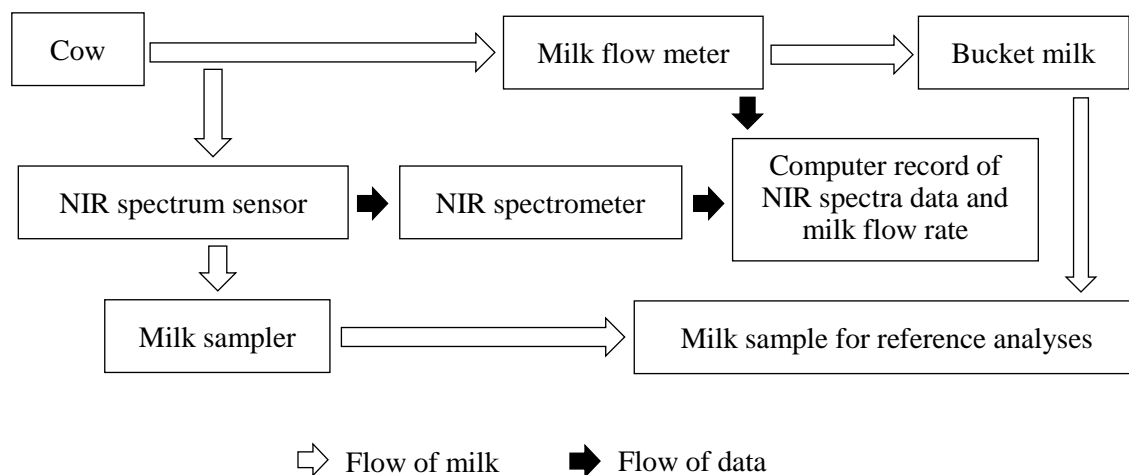
Figure 4.1 Flow chart of the on-line near-infrared spectroscopic sensing system for assessing milk quality during milking
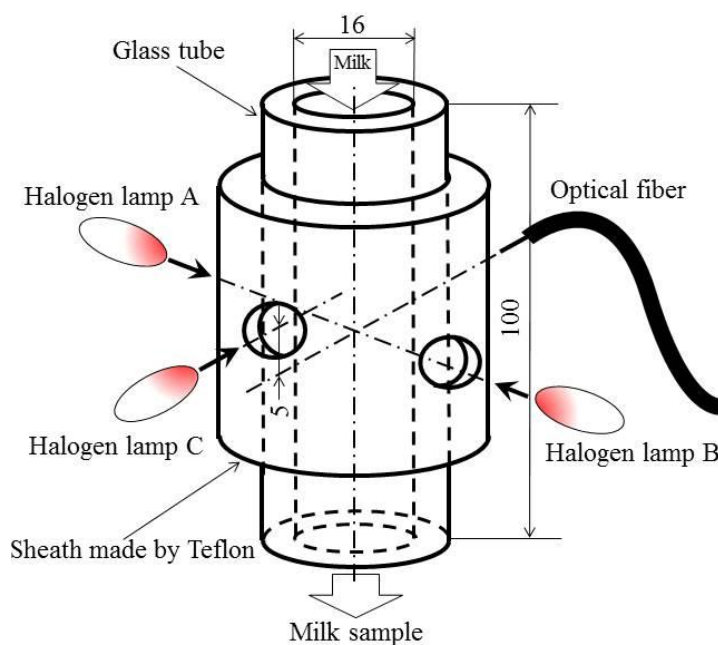
Figure 4.2 Schematic of the optical system of milk chamber of the near-infrared spectrum sensor

Table 4.1 Specification of the near-infrared spectroscopic sensing system

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 and milk level	55 mm
NIR spectrometer	Diffraction grating spectrometer
Optical density	Absorbance
Wavelength range	700–1050 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

4.2.2 Reference analyses

In this work, we measured three major milk constituent, SCC and MUN of non-homogenized milk as milk quality indicators. The milk constituents and MUN were determined using a MilkoScan[TM]FC+ (Foss Electric, Hillerod, Denmark). SCC was determined using Fossomatic[TM]FC (Foss Electric, Hillerod, Denmark).

4.2.3 Cow information

Two Holstein cows, numbered 1256 and 1257 belonging to Hokkaido University were used in this study. These cows were used in the experiment during their early lactation period. This study was conducted from August 24, 2015 to September 4, 2015. Measurements were performed in two successive milkings, i.e., milking in the evening and milking the following morning for about two weeks during the experiment period. A pipeline milking system was utilized for milking the cows at Hokkaido University cow barn. Milk samples were collected from the sampler every 20 seconds during milking. The experiment was conducted to cover variation in milk spectra caused by cow individuality, calving times, lactation stage, milking time and environmental temperature.

4.2.4 Chemometric analyses

Chemometric analyses were performed to develop calibration models for each quality indicator and to validate the precision and accuracy of the models. Spectra data analyses software (The Unscrambler ver. 10.3 Camo AS, Trondheim Norway) was used for the analyses. All the reference samples were used for the development of calibration models and full cross validation was used to validate the calibration models. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. Pretreatment of the spectra such as smoothing and second derivative were performed.

4.3 Results and discussion

NIR spectra

Figure 4.3 and 4.4 shows an example of original and pretreated NIR spectra set of non-homogenized milk from a cow respectively. The strong peak of the spectra in the wavelength of about 960 nm in Figure 3.12 indicates second-overtone absorption by water molecules. The two spectra peaks at around 740 nm and 840 nm indicate overtone absorption by C-H strings and C-C strings. Also, for the pretreated spectra, the two spectra troughs around 740 nm and 840 nm indicate overtone absorption by C-H strings and C-C strings which are typically related to the absorption of fat globules in raw milk while the spectra trough in the wavelength of about 960 nm in Figure 4.4 indicates second-overtone absorption by water molecules.
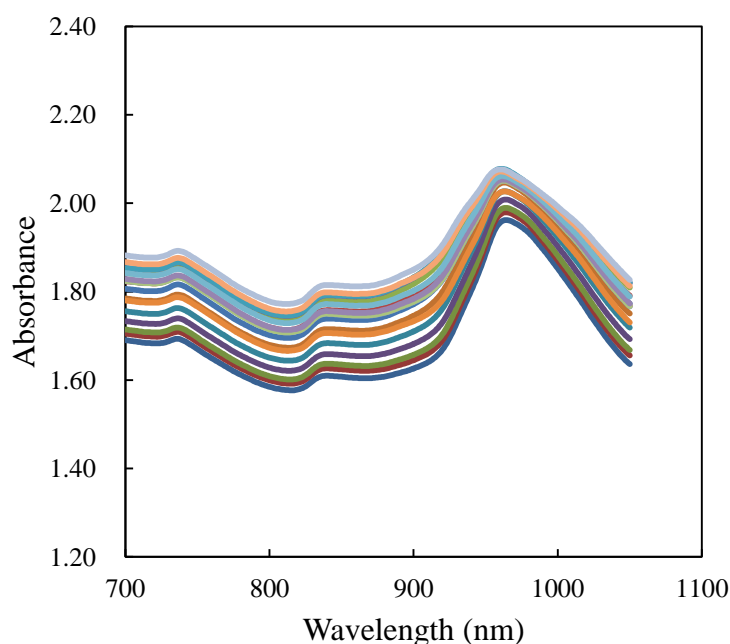
Figure 4.3 Original spectra of non-homogenized milk from cow number 1256 during milking on August 25, 2015
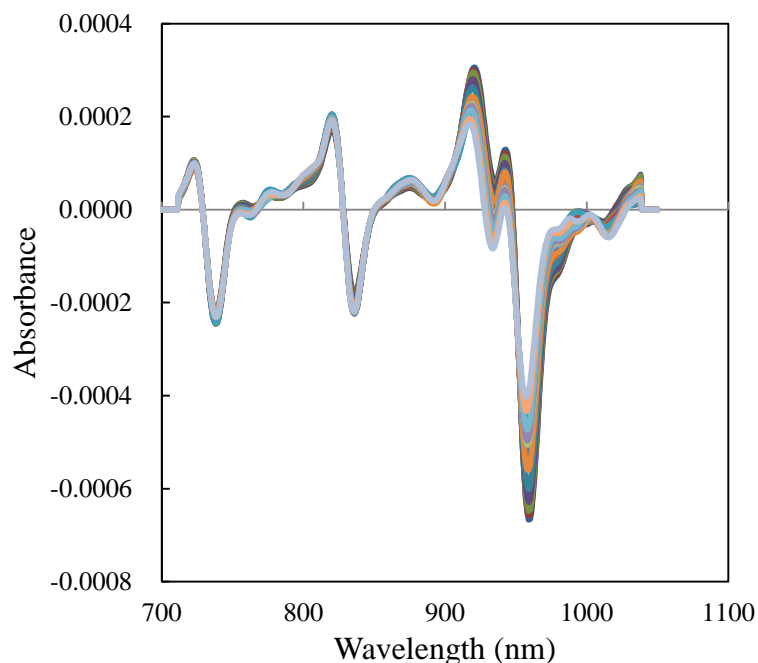
Figure 4.4 Pretreated spectra of non-homogenized milk from cow number 1256 during milking
on August 25, 2015

4.3.1 Validation of measurement accuracy using full cross validation

The validation statistics of the NIR sensing system for determination of milk quality are summarized in Table 4.2. The coefficient of determination (r^2), standard error of prediction (SEP) and bias of the validation set for fat were 0.98, 0.30% and 0.00% respectively. The values of r^2, SEP and bias were 0.93, 0.06% and -0.00% for lactose and 0.92, 0.03% and 3.59% for protein respectively. Sufficient levels of precision and accuracy for predicting the three main milk constituents were revealed by high values of r^2 and small values of SEP compared with range of each constituent and by the negligible values of bias. The performance of the calibration model for fat was outstanding and this was due to the fact that milk spectra had much emphasis on fat content from scattering of light by fat globules and absorption by C-H strings and C-C strings of triacylglycerol. The results show that the NIR spectroscopic sensing system designed for this study can be utilized for real-time online analysis of milk constituent during milking. This method can help to monitor milk constituents during every day milking of cows and consequently check mate the health status of each cow and their feedstuff as they affect milk quality.

MUN is an index used to monitor the efficiency of crude protein utilization in dairy cows (Kauffman & St-Pierre, 2001; Nousiainen et al., 2004). When MUN is very low, milk production becomes poor while very high MUN, environmental nitrogen emission is increased by urine and fecal output from the cow (Frank & Swensson 2002; Hansen et al., 2014) and infertility of the cow increases (Rajala-Schultz et al., 2001; Walsh et al., 2011) The values of r^2, SEP and bias for

MUN prediction were 0.88, 0.55 mg/dL, and -0.01 mg/dL respectively. The performance of the calibration model was very good. Calibration model with these levels of precision and accuracy could be used for monitoring the nutritional status of each cow.

SCC is a recognized indicator of cow's health and milk quality and it is used as a global standard for mastitis diagnosis. A cow produces milk containing less than 100,000 somatic cells per mL (4 log SCC/mL) is healthy, whereas a cow that produces milk containing more than 200,000 somatic cells per mL (5 log SCC/mL) may have subclinical mastitis (Satu, 2003). The values of r^2, SEP and bias for SCC prediction were 0.92, 0.14 log SCC/mL and 0.00 log SCC/mL respectively. The high values of r^2 and SEP for SCC indicated that the calibration model could be used to investigate subclinical mastitis.

The results of the validation of the calibration models in this study point out that the NIR spectroscopic sensing system could be used to evaluate milk quality during milking.

Table 4.2 Validation statistic of the NIR sensing system for determination of milk quality

Quality indicators	n	Range	Average	r^2	Bias	SEP	RPD	Regression line
Fat (%)	128	0.6-8.3	5.34	0.97	-0.00	0.34	6.24	y= 0.97x +0.15
Protein (%)	128	2.6-3.1	2.92	0.94	0.00	0.03	4.23	y = 0.95x +0.16
Lactose (%)	128	3.5-5.0	4.55	0.80	0.00	0.12	2.22	y = 0.84x +0.73
MUN (mg/dL)	128	4.8-16.1	10.77	0.15	-0.01	1.77	1.08	y = 0.17x +8.95
SCC (log SCC/mL)	128	4.2-5.8	5.07	0.93	0.00	0.13	3.68	y = 0.93x +0.34

n: number of validation samples. r: coefficient of determination SEP: standard error of prediction. RPD: ratio of SEP to standard error of prediction.
Regression line: Regression line from predicted value (x) to reference value (y).

1. Cow information

Two Holstein cows, numbered 1256 and 1257 belonging to Hokkaido University were used in this study. These cows were used in the experiment during their early lactation period. We collected milk samples from the period of August 24, 2015 to September 4, 2015. Measurements were performed in two successive milkings, i.e., milking in the evening and milking the following morning for about two weeks during the experimental period. A pipeline milking system was utilized for milking the cows at Hokkaido University cow barn. Milk samples were collected from the sampler every 20 seconds during milking. The experiment was conducted to cover variation in milk spectra caused by cow individuality, calving times, lactation stage, milking time and environmental temperature.

2. Chemometric analyses

Chemometric analyses were performed to develop calibration models for each milk quality indicator and to validate the precision and accuracy of the models. Spectra data analyses software (The Unscrambler ver. 10.3 Camo AS, Trondheim Norway) was used for the analyses. All the reference samples were used for calibration and validation. The statistical method of PLS

and PCA were used to develop calibration models from the absorbance spectra and reference data. Pretreatment of the spectra such as MSC, SG25 and second derivatives were performed

4.3.2 Validation of measurement accuracy using pretreatment techniques

Table 3.5 summarizes the results obtained using PLSR and PCR statistical methods. Using PLSR statistical method, the coefficient of determination (r^2) and standard error of prediction (SEP) of the validation set for fat were 0.98 and 0.28% respectively. The values of r^2 and SEP for protein were 0.89 and 0.05%. Those for lactose were 0.75 and 0.13%, MUN were 0.72 and 1.04mg/dL and those for SCC were 0.66 and 0.29 log SCC/mL respectively. By using the PCR statistical method, the values of r^2 and SEP of the validation set for fat were 0.96 and 0.40% respectively. The values of r^2 and SEP for protein were 0.66 and 0.09%, those for lactose were 0.61 and 0.16%, MUN were 0.52 and 1.35mg/dL and those for SCC were 0.59 and 0.31 log SCC/mL respectively. These results indicated that PLSR is more efficient than PCR statistical method for the development of robust calibration models for measurement of milk quality indicators using NIR spectrum data obtained from two Holstein cows. An early comparison of PLSR and PCR can be found in the work by (Mevik et al., 2004; Abdi, 2010). In both PLSR and PCR, the regression factors are linear combinations of the NIR wavelengths, estimated from NIR spectra, but their estimation varies. Yeniay & Goktas, (2002) reported that in PCR, the data compression stage is performed independently of calibration regression in such a way that the principal components accounts for the maximum total variation in NIR data alone whereas the resulting PLS factors describe important variations in the NIR data themselves and thus leads to more efficient data compression which results to a better calibration. Hence, by comparing the results obtained in our study, one can conclude that PLSR modelling is better choice than PCR since it displaces random noise contribution by the samples and then improve the precision and accuracy of the calibration models. These results indicated that PLSR is better than PCR statistical method for the development of robust calibration models for the measurement of milk quality indicators using NIR spectra data.

Table 4.3 below summarizes the results obtained from pretreated spectra data and non-pretreated spectra data. Using PLSR statistical method, the values of r^2 and SEP of the validation set for fat were 0.98 and 0.28% respectively. The values of r^2 and SEP for protein were 0.89 and 0.05%, those for lactose were 0.75 and 0.13%, those for MUN were 0.72 and 1.04mg/dL and those for SCC were 0.66 and 0.29 log SCC/mL and respectively. MSC is one of the data pretreatment techniques used for the transformation of spectroscopic measurements data. When spectra are measured on a set of samples containing several components, it is usually necessary to pretreat the data before they are subjected to multivariate data analysis. Savitzky-Golay

smoothing filters are typically used to "smooth out" noisy signal whose frequency span is large. Using MSC, the r² and SEP of the validation set for fat were 0.94 and 0.49% respectively. The values of r² and SEP for protein were 0.82 and 0.07%, those for lactose were 0.70 and 0.14%, those for MUN were 0.64 and 1.17mg/dL and those for SCC were 0.61 and 0.31 log SCC/mL respectively. For SG25, the r² and SEP of the validation set for fat were 0.98 and 0.32% respectively. The values of r² and SEP for protein were 0.78 and 0.69%, those for lactose were 0.49 and 0.18%, those for MUN were 0.757 and 1.30mg/dL and those for SCC were 0.61 and 0.31 log SCC/mL respectively. The use of original spectra data without pretreatments produced high values of r^2 and small values of SEP compared with range of each constituent and by the negligible values of bias (Table 4.4). This result using non-pretreated spectra data for the development of calibration models, revealed sufficient levels of precision and accuracy for predicting the three major milk constituents. The performance of the calibration model for fat was outstanding and this was due to the fact that milk spectra had much emphasis on fat content from scattering of light by fat globules and absorption by C-H strings and C-C strings of triacylglycerol. Comparing these results obtained from two different pretreatment techniques used for NIR spectra data transformation with the results obtained from non-pretreated spectra data, it was clear that the use of these types of pretreatments would not be adequate for the development of robust calibration models in our study. The results showed that the NIR spectroscopic sensing system designed in this study had high precision and accuracy and can be utilized for real-time online analysis of milk constituent during milking. This NIR spectroscopic sensing system can as well aid the monitoring of milk constituents during every day milking of each cow and consequently check mate the health status of cows and their feedstuff as this affect milk quality.

Table 4.3 Validation statistics of the –infrared sensing system for determination of milk quality using PLSR and PCR

Quality indicators	Statistical methods	n	Range	r^2	SEP	Bias	nF/nPC	Regression Line
Fat (%)	PLSR	310	0.7-9.4	0.98	0.28	-0.00	5	y = 0.98x + 0.12
	PCR	310	0.7-9.4	0.96	0.40	0.00	4	y = 0.96x + 0.19
Protein (%)	PLSR	310	2.5-3.3	0.89	0.05	-0.00	13	y = 0.92x + 0.23
	PCR	310	2.5-3.3	0.66	0.09	0.00	7	y = 0.67 x + 0.93
Lactose (%)	PLSR	310	3.5-4.9	0.75	0.13	-0.00	12	y = 0.80x +0.92
	PCR	310	3.5-4.9	0.61	0.16	0.00	15	y = 0.64x + 1.63
MUN (mg/dL)	PLSR	310	6.0-16.1	0.72	1.04	-0.02	16	y = 0.78x +2.66
	PCR	310	6.0-16.1	0.52	1.35	-0.00	15	y = 0.55x + 5.36
SCC (log SCC/mL)	PLSR	310	4.0-6.0	0.66	0.29	-0.00	11	y = 0.73x + 1.38
	PCR	310	4.0-6.0	0.59	0.31	0.00	8	y = 0.61x + 1.97

n: number of validation samples. r^2: coefficient of determination. SEP: standard error of prediction

nF: number of PLS factor. nPC: number of Principal components. Regression line: Regression line from predicted value (x) to reference value (y)

Table 4.4 Validation statistics of the near-infrared sensing system for the determination of milk quality using multiplicative scatter correction (MSC) and Savitzky-Golay (SG15) methods

Quality indicators	Pretreatment	Statistical method	n	Range	r^2	SEP	Bias	nF	Regression Line
Fat (%)	No pretreatment	PLS	310	0.7-9.4	0.98	0.28	-0.00	5	y = 0.98x + 0.12
	MSC	PLS	310	0.7-9.4	0.94	0.49	0.00	2	y = 0.95x + 0.27
	SG25	PLS	310	0.7-9.4	0.98	0.32	0.00	3	y = 0.97x +0.13
Protein (%)	No pretreatment	PLS	310	2.5-3.3	0.89	0.05	0.00	13	y = 0.92x + 0.23
	MSC	PLS	310	2.5-3.3	0.82	0.07	0.00	13	y = 0.87x +0.38
	SG25	PLS	310	2.5-3.3	0.78	0.69	0.00	9	y = 0.84 + 0.47
Lactose (%)	No pretreatment	PLS	310	3.5-4.9	0.75	0.13	-0.00	12	y = 0.80x +0.92
	MSC	PLS	310	3.5-4.9	0.7	0.14	0.00	13	y = 0.75x + 1.12
	SG25	PLS	310	3.5-4.9	0.49	0.18	0.00	7	y = 0.55x + 2.05
MUN (mg/dL)	No pretreatment	PLS	310	6.0-16.1	0.72	1.04	-0.02	16	y = 0.78x +2.66
	MSC	PLS	310	6.0-16.1	0.64	1.17	-0.04	14	y = 0.70x + 3.59
	SG25	PLS	310	6.0-16.1	0.57	1.30	-0.01	10	y = 0.65x + 4.08
SCC (log SCC/mL)	No pretreatment	PLS	310	4.0-6.0	0.66	0.29	0.00	11	y = 0.73x + 1.38
	MSC	PLS	310	4.0-6.0	0.61	0.31	-0.01	12	y = 0.68x + 1.63
	SG25	PLS	310	4.0-6.0	0.61	0.31	-0.00	7	y = 0.66x + 1.72

n: number of validation samples. r^2: coefficient of determination. SEP: standard error of prediction
nF: number of PLS factor. Regression line: Regression line from predicted value (x) to reference value (y)

5. Cow information

Four Holstein cows belonging to Hokkaido University were used in this study. The cows were used in the experiment during different lactation periods. This experiment was carried out

from the period of October 16, 2015 to November 2, 2015. Measurements were performed in two consecutive milkings, milking in the evening and milking the following morning, for about two weeks during the experiment. A pipeline milking system was used for milking the cows at the Hokkaido University cow barn. Two cows were milked at the same time and each was measured for about four milking times. Milk spectra data were recorded and then milk samples were collected from the milk sampler every 20 seconds during milking. The experiment was conducted to cover variation in milk spectra caused by cow individuality, calving times and lactation stage (Table 3.7).

Table 4.5 Information of cows used in the experiment

Cow number	Date of birth	Date of latest calving	Calving times
1221	22-Oct-2006	16-Dec-2014	6
1230	01-Nov-2010	22-Oct-2015	3
1236	11-Jul-2011	28-Jan-2015	2
1237	24-Jul-2011	15-Feb-2015	2

5. Chemometric analyses

Chemometric analyses were carried out to develop calibration models for each milk quality indicator and to validate the precision and accuracy of the models. One data set was obtained from the experiment conducted using four cows. The data set from the four cows was used to develop calibration models and the same data set from the four cows was used for validation of the calibration models. Full cross validation was used to validate the calibration models. Spectra data analysis software (The Unscrambler ver. 10.3, Camo AS, Trondheim, Norway) was used for the analyses. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. Pretreatment of the spectra such as multiplicative scatter correction and smoothing were not performed.

4.3.3 Validation of measurement accuracy of the near-infrared spectroscopic sensing system

Near-infrared spectra

Figure 4.5 shows an example of an original spectra set for non-homogenized milk from a cow. 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 raw milk. The peak of the absorbance spectrum in the wavelength of about 960 nm indicates the second-overtone absorption by water molecules.
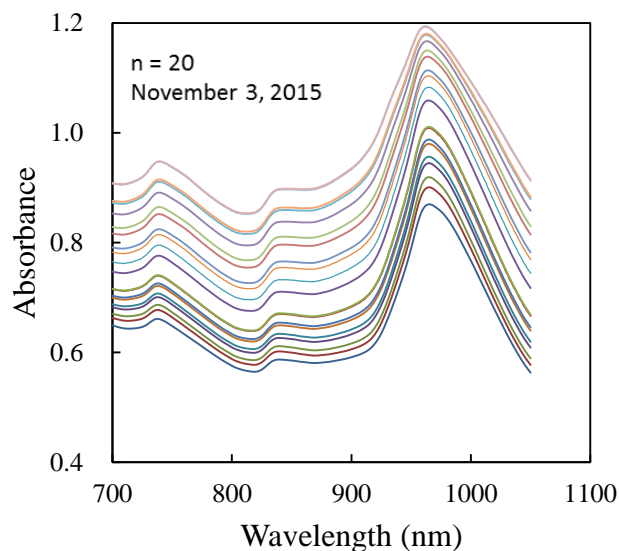
Figure 4.5 Original spectra of non-homogenized milk from cow number 1230 during milking on November 3, 2015

Precision and accuracy of calibration models

The validation statistics of the NIR sensing system for determination of milk quality are summarized in Table 4.6.

The correlations between reference and NIRS-predicted values of fat, lactose, protein, MUN and SCC are shown in Figures 3.15 to 3.19 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. Every day checking of milk constituents while milking can be utilized for individual cow management and nutritional intake of the cow. The r^2, SEP and bias of the validation set for fat were 0.99, 0.17% and 0.00% respectively. The values of r^2, SEP and bias were 0.95, 0.06% and -0.00% for protein, 0.91, 0.06% and 0.00% for lactose, 0.94, 0.88 mg/dL and -0.00 mg/dL for MUN, and 0.91, 0.09 log SCC/mL and -0.00 log SCC/mL for SCC, respectively. Sufficient levels of precision and accuracy for predicting the three major milk constituents were indicated by the high values of r^2 and small values of SEP compare with the range of each constituent and by the negligible values of the bias (almost zero).

These results (Table 4.6) showed higher levels of accuracy which was thought to be due to the fact that the NIR sensing system used our study, had higher signal-to-noise ratio and can thus be utilized for online real-time milk quality evaluation. The higher levels of accuracy are thought to be due to the improvement of the NIR spectroscopic sensing system which consists of three halogen lamps and were used as near-infrared light sources. The milk samples were irradiated from three directions with a longer exposure time of 200ms. It was observed that the three

halogen lamps accurately captured the near-infrared light by fat content unlike the one halogen lamp in the previous study. Increased repetition times (ten repetition times) were thought to have contributed to the improvement of calibration models. Repetition times are the multiple milk measurements taken in the same experimental run. The increase in repetition times reduced the noise data and improved the precision of calibration models. Thus, high signal intensity was generated, which consequently increased the signal-to-noise ratio. Exposure time is the length of time the NIR sensor is exposed to infrared light. The long exposure time ensured that the important bright part of the captured spectra is not lost and thus reduced various random noise and fixed pattern noise, consequently improving the performance of calibration models. The results obtained indicated that the NIR spectroscopic sensing system designed in this study can be utilized for online real-time monitoring of milk constituents while milking.

Table 4.6 Validation statistics of the new near-infrared sensing system for determination of milk quality

Milk quality indicators	n	Range	r^2	SEP	Bias	RPD	Regression line
Fat (%)	218	1.1 - 9.0	0.99	0.17	0.00	9.53	y = 0.99 x + 0.04
Protein (%)	218	3.1 - 4.3	0.95	0.06	-0.00	4.31	y = 0.95 x + 0.18
Lactose (%)	218	3.7 - 4.6	0.91	0.06	0.00	3.36	y = 0.92 x + 0.34
MUN (mg/dL)	218	5.7 - 16.9	0.94	0.88	-0.00	3.92	y = 0.94 x + 0.72
SCC (log SCC/mL)	218	4.3 - 5.7	0.91	0.09	-0.00	3.41	y = 0.92 x + 0.40

n: number of validation samples. r^2: coefficient of determination. SEP: standard error of prediction RPD: Residual predictive deviation. Regression line: Regression line from predicted value (x) to reference value (y)
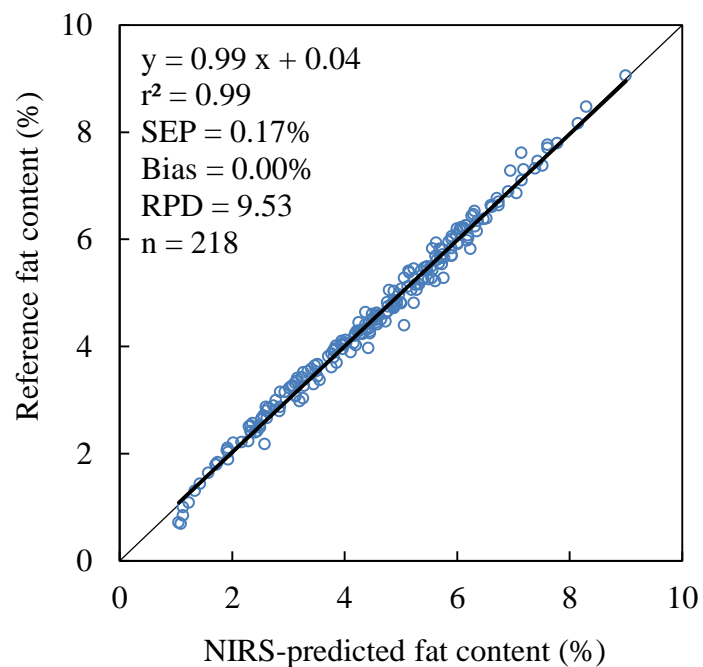
Figure 4.6 Correlation between reference fat content and NIRS-predicted fat content
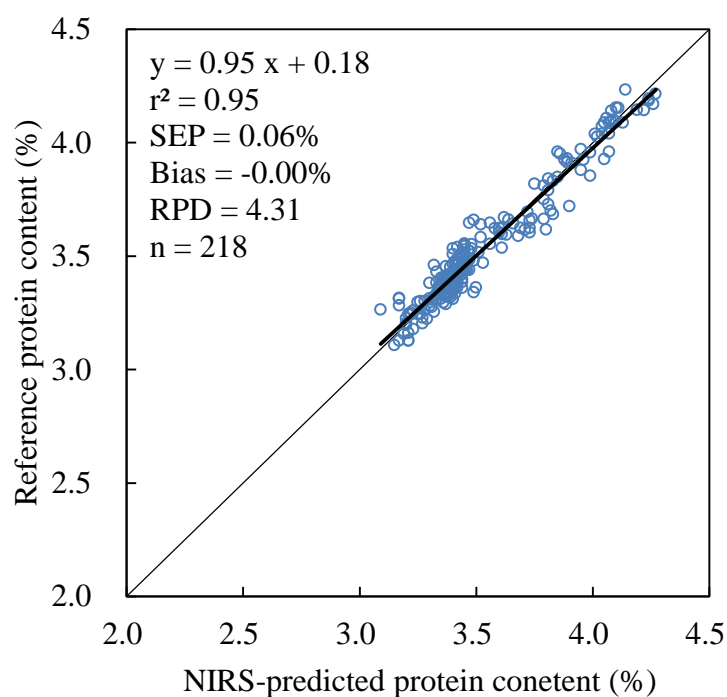
Figure 4.7 Correlation between reference fat content and NIRS-predicted protein content

y = 0.92 x + 0.34
r² = 0.91
SEP = 0.06%
Bias = 0.00%
RPD = 3.36
n = 218
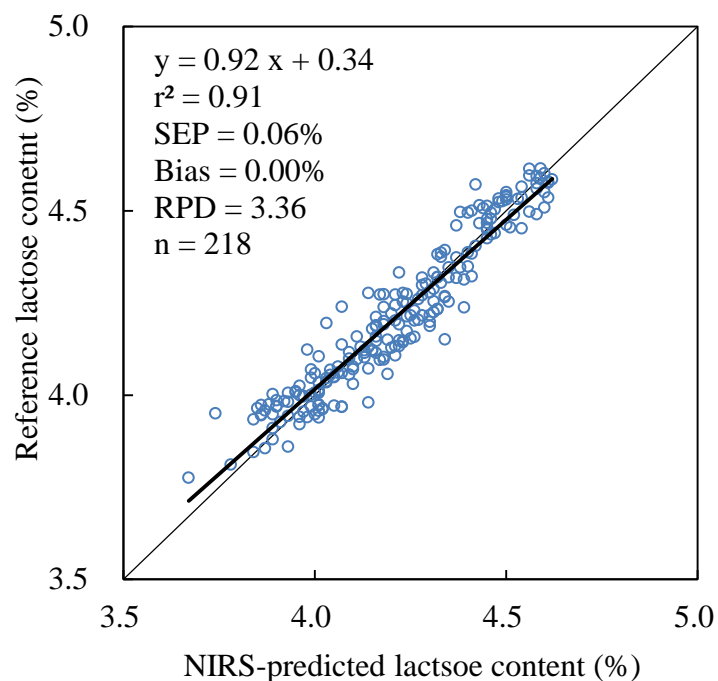
Figure 4.8 Correlation between reference fat content and NIRS-predicted lactose content

y = 0.94 x + 0.72
r² = 0.94
SEP = 0.88 mg/dL
Bias = -0.00 mg/dL
RPD = 3.92
n = 218
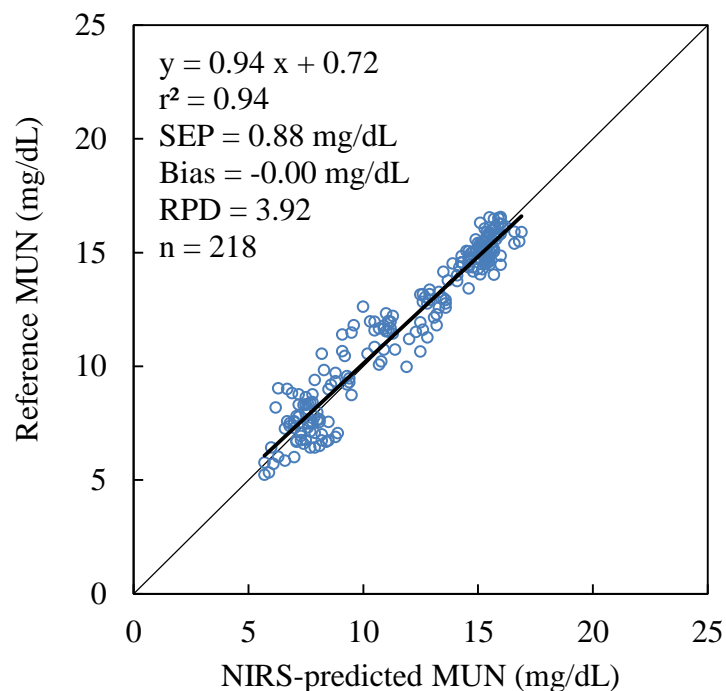
Figure 4.9 Correlation between reference fat content and NIRS-predicted MUN
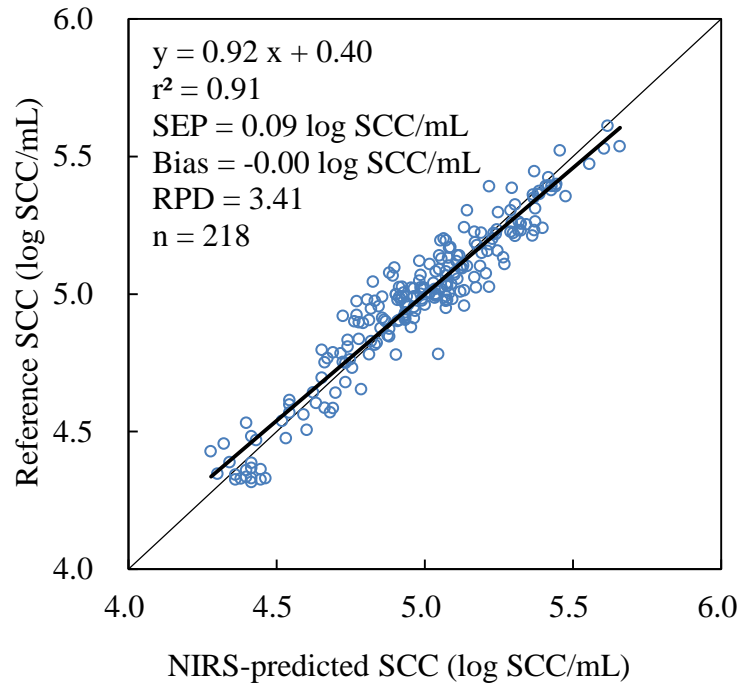
Figure 4.10 Correlation between reference fat content and NIRS-predicted MUN

Monitoring of milk trend using NIR spectroscopic sensing system

The results obtained for fat content and SCC in monitoring cow 1221 and 1226 using reference data and NIR predicted data during evening (PM) and morning (AM) milking on October 14, 2015 and October 15, 2015 for cow number 1221 are shown in Figure 4.11 to 4.18 respectively Also, for cow number 1226, the results obtained for fat content and SCC during PM and AM milking on October 6, 2015 and October 7, 2015 are shown in Figure 3.24 to 3.27 respectively. For fat content, there was a gradual increase and steady fluctuation from 2.6 to 4.7% using reference data and from 2.6 to 4.6% using NIR predicted data during AM milking for cow number 1221 respectively. Fat content for cow number 1226 rapidly increase from 1.8 to 5.2% using reference data and from 1.3 to 5.4% using NIR predicted data. Then, it decreased to 2.7% and later increase to 4.7% for reference data and decreased to 2.8% and later increased to 4.7% using NIR predicted data respectively during AM milking. There was different between the fat content for PM and AM milking of cow number 1221 and 1226. Also, the quantity of milk produced in the PM is usually lower than that of AM. The mammary udder of a cow produces raw milk that flows down to the mammary gland through the mammary duct. Milk fat which serves as a source energy for the cow is stored in the mammary gland of cows until when the cow is milked. Milk fat stays at the top of the alveolus of mammary gland. Usually, fat content is very low at the beginning of milking and increases during milking. The remaining milk fat content in the cow's udder after milking is usually very high and this is the reason why the milk

fat content of PM milking is high. In this study, the milking time gap between the AM and PM was about 6 hours. SCC during AM milking increased from 4.9 to 5.3 log SCC/mL using reference data and from 4.9 to 5.2 log SCC/mL using NIR predicted data for cow 1221 respectively while SCC for PM milking was about 5.2 and 5.3 log SCC/mL using reference and NIR predicted data. For cow 1226, during AM milking, SCC increased from 4.0 to 4.3 log SCC/mL using reference data and 4.5 to 5.0 log SCC/mL using NIR predicted data respectively while the SCC during evening milking was around 4.6 and 4.9 log SCC/mL using reference data and NIR predicted data respectively. There was a slight difference between the reference and NIR predicted results obtained for SCC for cow number 1226. However, the results obtained showed very close trends using reference and NIR predicted data. These results suggested that it is possible to use the NIR spectroscopic sensing system developed in our study to monitor the milking trends of each milk indicator during milking.
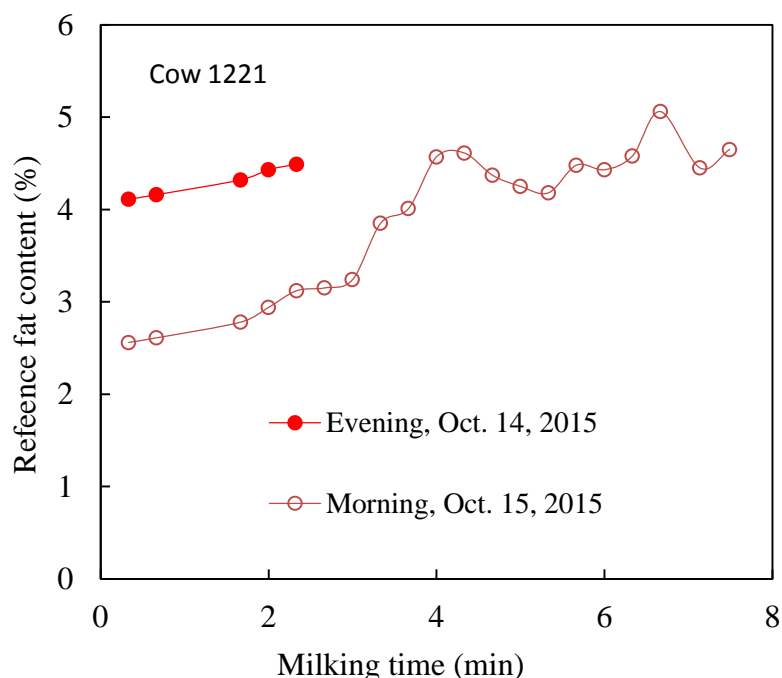
Figure 4.11 Monitoring of fat content during milking from cow 1221 using reference data
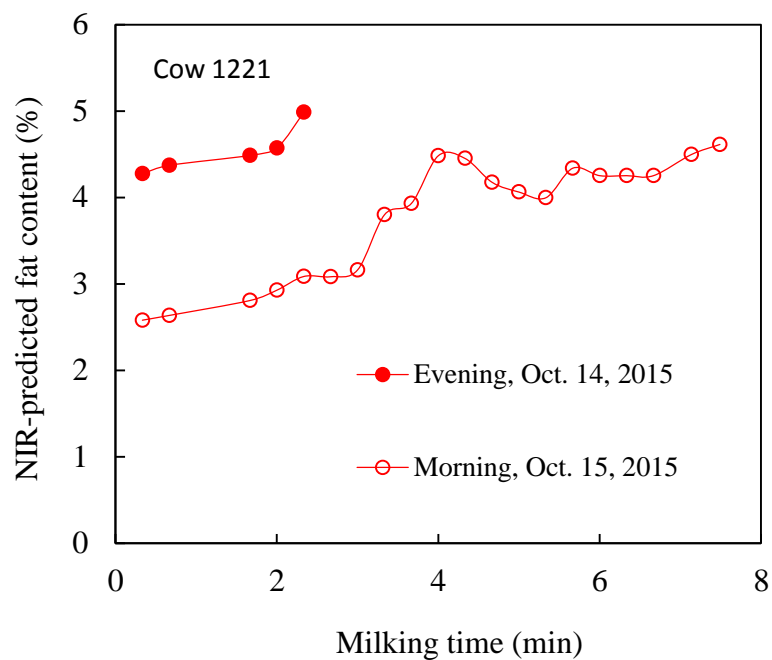
Figure 4.12 Monitoring of fat content during milking from cow 1221 using NIR predicted data
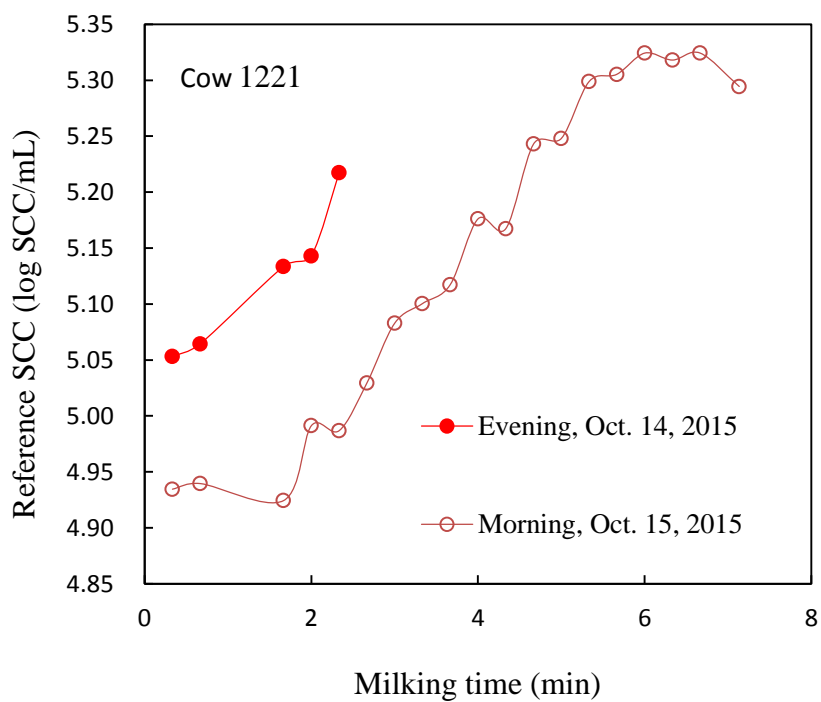
Figure 4.13 Monitoring of SCC during milking from cow 1221 using reference data
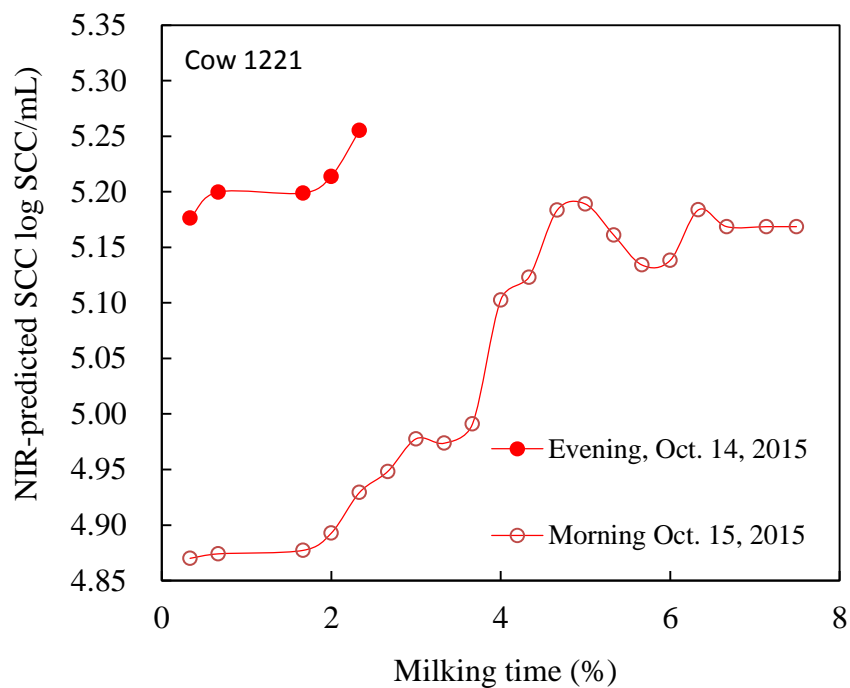
Figure 4.14 Monitoring of SCC during milking from cow 1221 using NIR predicted data
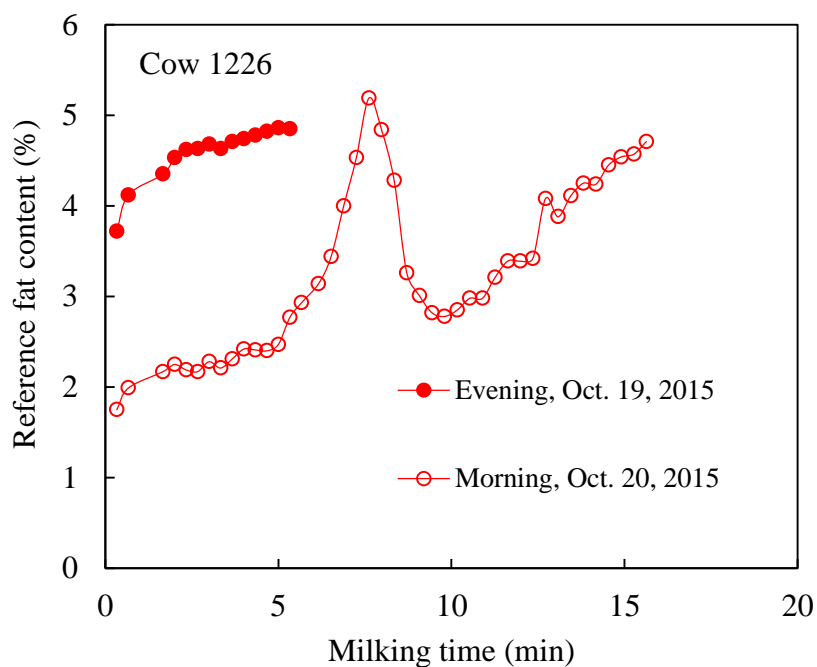
Figure 4.15 Monitoring of fat content during milking from cow 1226 using reference data
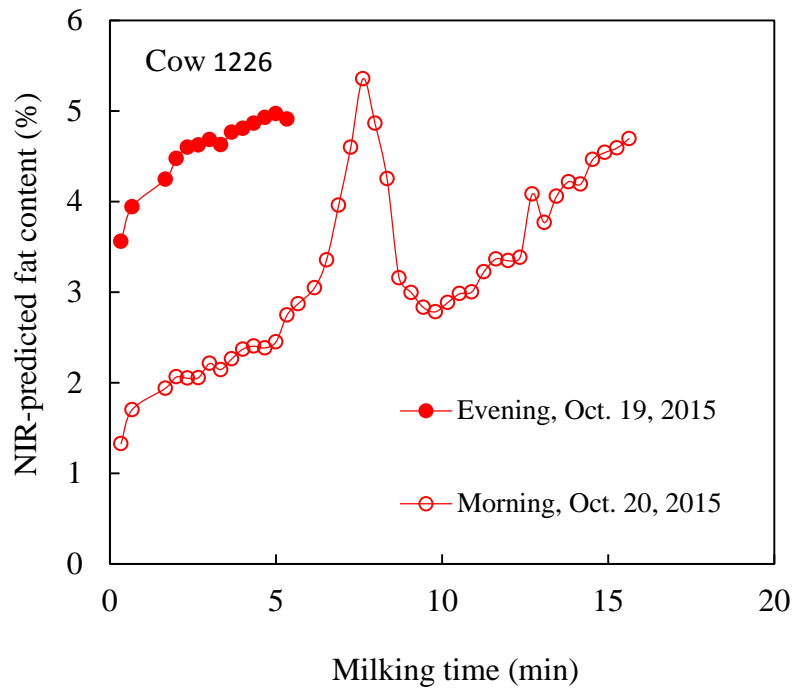
Figure 4.16 Monitoring of fat content during milking from cow 1226 using NIR predicted data
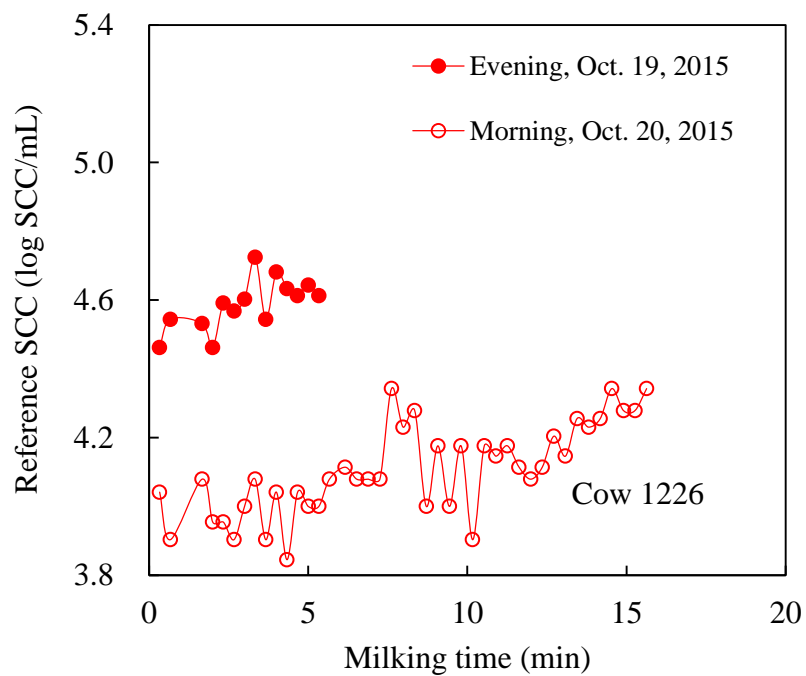
Figure 4.17 Monitoring of SCC during milking from cow 1226 using reference data
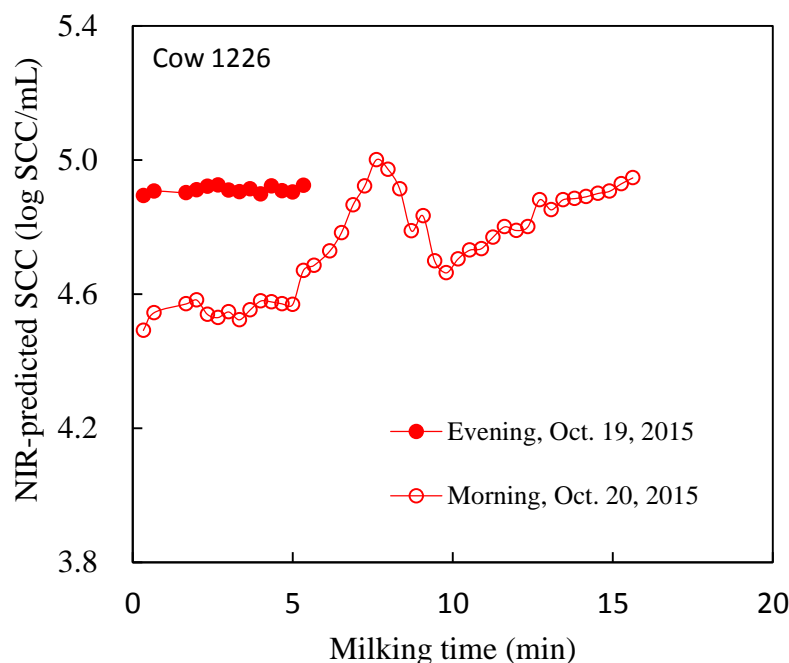
Figure 4.18 Monitoring of SCC during milking from cow 1226 using NIR predicted data

4.4 Conclusions

1. In this study, the on-line NIR Spectroscopy sensing system developed can be used for real-time on-line monitoring of milk constituents, MUN and SCC during milking with sufficient precision and accuracy. This system can be very useful to dairy farmers by giving them information about milk quality and the health condition of each cow and thus, enable them to upgrade dairy farm management.

2. The utilization of original spectra data without pretreatments could be advantageous for the development of robust calibration models. Partial least squares statistical method could help to improve calibration models unlike Principal component analysis.

3. Milk constituents, 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 farmers with important information on milk quality, diagnosis of mastitis and assessing the physiological condition of each cow with sufficient precision and accuracy while milking. The system will also give livestock farmers feedback control for upgrading dairy farm management and consequently enable them to produce high-quality milk. Thus, the system will enable realization of precision dairy farming.

5. Chapter V: Factors Affecting the Accuracy of Calibration Models Using Near-Infrared Spectroscopic Sensing System for Milk Quality Determination during Milking

5.1 Objectives

1. The objective of this study was to examine the effect of cow individuality on the accuracy of calibration models using near-infrared spectroscopic sensing system for determining milk constituents during milking.

2. To examine the effect of milking season (summer, autumn and winter season) on the precision and accuracy of calibration models developed using near-infrared spectroscopic sensing system.

3. To examine the effect of parity on the performance of calibration models for each milk quality indicator using near-infrared spectroscopic sensing system.

4. To investigate the effect of number of PLS factors on the measurement accuracy of milk constituents, MUN and SCC.

5.2 Materials and methods

5.2.1 Near-infrared spectroscopic sensing system

An experimental online NIR spectroscopic sensing system was designed for determining milk quality of each cow during milking. The system comprised of an NIR spectrometer, milk flow meter, milk sampler and a laptop computer. The system was fixed between a teatcup cluster and a milk bucket of a milking system. Non-homogenized milk from the teatcup cluster flowed continuously through a bypass into the milk chamber of the NIR spectrum sensor. The remaining raw milk flowed pass the milk flow meter and then released through a line tube into the bucket. The volume of milk sample in the chamber was about 30mL. The spectrum sensor acquired absorbance spectra through the milk. The spectra were recorded in the wavelength range of 700 nm to 1050 nm at 1 nm interval every 20 seconds during milking. The milk flow rate was recorded simultaneously.

5.2.2 Reference analyses

In this study, we measured three major milk constituent (fat, protein and lactose), MUN and SCC of non-homogenized milk as milk quality parameters. The milk constituents and MUN were determined using a MilkoScan instrument (Foss Electric, Hillerod, Denmark) and SCC was evaluated using Fossomatic instrument (Foss Electric, Hillerod, Denmark).

5.2.3 Cow information

1. Cow information

Four Holstein cows belonging to Hokkaido University were used in this study. These cows were used in the experiment during their different lactation periods. The samples were collected from the period of July 12, 2017 to August 9, 2017. A pipeline milking system was utilized for milking the cows at Hokkaido University cow barn. Measurements were performed in two successive milkings, i.e., milking in the evening and milking in the following morning for about four weeks during the experiment period. Two cows were milked at the same milking time and each cow was measured for twenty eight milking times. Milk spectra and milk samples were collected from the sampler every 20 s during milking.

Table 5.1 Information of cows used in the experiment

Cow number	Date of birth	Date of latest calving	Calving times
1234	2010/12/07	2016/03/04	3
1246	2012/02/19	2015/04/15	2
1252	2012/11/16	2015/03/05	2
1263	2014/03/02	2016/05/08	1

5.2.4 Chemometric analyses

1. Chemometric analysis

Chemometric analyses were used to develop calibration models for each milk quality indicator and to validate the precision and accuracy of the models. Four data sets were obtained from the experiment conducted using four cows. The data analyses methods that were performed are as follow:

1. Calibration models for each cow and for each milk quality indicator were developed using full cross validation method.

2. Calibration models for each cow and for each milk quality indicator were developed using the two-third of the randomly divided data set and validation using the remaining one-third of the data set.

3. Calibration models for each cow and for each milk quality indicator using the first three weeks data set and validation using the last one week data set.

4. Calibration models for each milk quality indicator were developed using three cows and validation using one cow data set. This validation method was repeated for each cow.

Also, spectra data analyses software (The Unscrambler, Camo AS, Trondheim, Norway) was used for the analyses. The statistical method of partial least squares (PLS) was used to

develop calibration models from the absorbance spectra and reference data. Pretreatment of the spectra such as multiplicative scatter correction and second derivatives were not performed.

5.3 Results and discussion

1. Results and discussion

5.3.1 Cow individuality

5.3.1.1 Calibration using full cross validation method

The validation statistics of the NIR sensing system for determination of milk quality for the first data analysis method are summarized in Table 5.2.

The precision and accuracy levels of calibration models developed for each cow and for each milk quality indicator from the full cross validation method were sufficiently high. The r^2 values for fat and moisture content were very high and the r^2 values for protein, lactose, solids not fat (SNF), milk urea nitrogen (MUN) and somatic cell count (SCC) were sufficiently high. The standard error of prediction (SEP) values for each milk quality indicator were low. The SEP values are values that were used in this study to determine how accurate each calibration models are such that the closer it is to zero the better and the farther it is from zero, the worse it becomes. Thus, the differences in the SEP values by >0.1 was considered to be a big difference. The SEP values for the fat content of each cow were 0.18%, 0.16%, 0.30% and 0.22% for cow number 1234, 1246, 1252, and 1263 respectively. The SEP results for fat content of cow number 1252 and 1263 were similar but were different from that of cow number 1234 and 1246. The SEP values for the moisture content of each cow were 0.15%, 0.17%, 0.14% and 0.27% for cow 1234, 1246, 1252 and 1263 respectively. The SEP value of moisture content for cow number 1263 was different from the other three cows. There were differences in all the MUN SEP values obtained for each cow which were 1.18 mg/dL, 1.45 mg/dL, 0.62 mg/dL and 3.14 mg/dL for cow 1234, 1246, 1252 and 1263 respectively. The SEP values for were SCC 0.26 log SCC/mL, 0.29 log SCC/mL, 0.18 log SCC/mL and 0.19 log SCC/mL for cow number 1234, 1246, 1252 and 1263 respectively. The SEP results for SCC for the first two cows were similar but difference from the last two cows whose results were also similar. These results show that there were differences in the precision and accuracy for determining fat content, moisture content, MUN and SCC of each cow. The SEP results obtained when the data set of all cows were combined were good but SCC result was not so good. However the SEP values for protein, lactose and SNF content for each cow were all similar. The bias values obtained for each cow and for each milk quality indicator were all zero.

5.3.1.2 Calibration using two-third of data set and validation using the remaining one-third of the data set of each cow

The validation statistics of the NIR sensing system for determination of milk quality for the second data analysis method are summarized in Table 5.3.

The precision and accuracy levels of calibration models developed for each cow and for each milk quality indicator were sufficiently high. The r^2 values for fat and moisture content were very high and the r^2 values for protein, lactose, SNF, MUN and SCC were sufficiently high. The SEP values for each milk quality indicator were low and all the bias values were negligible to zero. The SEP values for the fat content of each cow were 0.18%, 0.14%, 0.29% and 0.30% for cow number 1234, 1246, 1252, and 1263 respectively. Just like the first method above, the SEP results for fat content of cow number 1252 and 1263 were similar but were different from that of cow number 1234 and 1246. The SEP values for the moisture content of each cow were 0.07%, 0.10%, 0.06% and 0.06% for cow 1234, 1246, 1252 and 1263 respectively. The SEP value of moisture content for cow number 1263 was different from the other three cows. The MUN SEP value (3.02 mg/dL) for cow number 1263 was different from the SEP values (1.11 mg/dL, 1.53 mg/dL and 0.61 mg/dL) of other three cows; cow number 1234, 1246 and 1252 respectively. These results show that there were differences in the precision and accuracy for determining fat content, moisture content and MUN of each cow. However the SEP values for protein, lactose, SNF content and SCC for each cow were all similar. The SEP results obtained when the data set of all cows were combined were good except for MUN and SCC.

5.3.1.3 Calibration using first three weeks data set and validation using the last one week data set of each cow

The validation statistics of the NIR sensing system for determination of milk quality for the third data analysis method are summarized in Table 5.4.

The precision and accuracy levels of calibration models developed when the first three weeks data set of each cow was used to validate the last one week data set of the same cow were sufficiently high. The r^2 values for fat and moisture content were very high and the r^2 values for protein, lactose, SNF, MUN and SCC were sufficiently high. The SEP values for each milk quality indicator were low except for MUN and all the bias values were negligible to zero. The SEP values for the fat, protein, lactose, SNF, moisture content and SCC were all similar for each cow. The MUN SEP results obtained were 0.86 mg/dL, 1.35 mg/dL, 0.84 mg/dL and 5.68 mg/dL for cow number 1234, 1246, 1252 and 1263 respectively. The MUN SEP results obtained for number 1234 and 1252 were similar whereas, the SEP values obtained for the other two cows

were different most especially cow number 1263 with a bigger SEP value. These particular results show that there were no differences in the precision and accuracy for determining fat, protein, lactose, moisture content and SCC except for MUN. The SEP results obtained when the data set of all cows were combined were all good since the SEP results was similar to the results obtained for each cow and for each milk quality indicator.

5.3.1.4 Calibration using three cows' data set and validation using one week data set

The validation statistics of the NIR sensing system for determination of milk quality for the fourth data analysis method are summarized in Table 5.5.

The precision and accuracy levels of calibration models developed when each cow was used to validate other three cows were sufficiently high. The r^2 values for fat and moisture content were very high and the r^2 values for protein, lactose, SNF, MUN and SCC were sufficiently high. The SEP values for each milk quality indicator were low except for MUN and SCC. All the bias values were negligible to zero. The MUN SEP results were 2.00 mg/dL, 2.16 mg/dL, 1.27 mg/dL and 5.39 mg/dL for cow number 1234, 1246, 1252 and 1263 respectively. The SEP results of MUN for each was totally different. The SCC SEP results were 0.53 log SCC/mL, 0.38 log SCC/mL, 0.25 log SCC/mL and 0.24 log SCC/mL for cow number 1234, 1246, 1252 and 1263 respectively. The SEP results of SCC for the first two cows were similar and that of the remaining two cows were similar but different from the first two cows. These results also show that there are differences in the precision and accuracy results of each cow.

Milk fat content and somatic cell count are very important milk quality indicators. The percentage of milk fat content is very essential such that can determine milk payment scheme. Knowing the percentage or quality of milk fat present in raw milk can give information about dietary fiber content, quality and cow fiber intake (Victoria, 2017). More so, the determination of SCC in raw milk plays a vital role in the diagnosis of subclinical mastitis, which is a very serious disease of milking cow.

According to the results (Table 4.2 and 4.3) obtained, these results (Table 4.3) were better than the results obtained using other three data analysis methods. The better accuracy was because; the cow information contained in the calibration data set was also contained in the validation data set such that it covers the differences that occur between individual cows. This is significant for milk quality determination in its practical application.

On the other hand, these results (Table 5.4 and 5.5) showed that the precision and accuracy level of the model for fat and moisture content was sufficiently high, whereas the performance of the calibration models for other quality indicators was worse than that of Table

4.3 due to cow individuality. The decrease in the accuracy of the validation statistics (Table 4.4 and 4.5) were due to the difference in cow information, that is, the cow information contained in the calibration data set is different from the cow information contained in the validation data set. This is the case of future collection of milk sample data from a different set of cows such that when the data set from a different set of cows is used to develop calibration models and another cows` data set is used for validation, the accuracy of the calibration models tends to decrease. The findings of this study indicated that cow individuality can affect the accuracy of calibration models.

Thus, ensuring the collection of a wide range of NIR spectra data from all cows is important which would consequently cover the differences between cows and then improve the accuracy of calibration models.

Table 5.2 Validation statistics of the near-infrared sensing system for the determination of
milk quality using full cross validation method

Analytical method	Milk quality items	n	Range	r^2	SEP	Bias	RPD	Regression line
Cow 1234 Full CV	Fat (%)	363	1.09-5.73	0.96	0.18	0.00	4.84	y = 1.00 x + 0.01
	Protein (%)	363	2.91-3.38	0.48	0.07	0.00	1.38	y = 0.96 x + 0.13
	Lactose (%)	363	3.28-4.31	0.56	0.10	0.00	1.51	y = 0.96 x + 0.15
	SNF (%)	363	7.31-8.66	0.67	0.11	0.00	1.73	y = 0.98 x + 0.17
	Moisture (%)	363	86.28-90.64	0.96	0.15	0.00	5.32	y = 1.00 x + 0.19
	MUN (mg/dL)	363	14.40-23.10	0.47	1.18	0.00	1.37	y = 0.95 x + 0.83
	SCC (log SCC/mL)	363	4.00-6.56	0.73	0.26	0.00	1.92	
Cow 1246 Full CV	Fat (%)	255	0.83-7.70	0.99	0.16	0.00	9.10	y = 1.00 x + 0.00
	Protein (%)	255	2.56-3.47	0.35	0.11	0.00	1.24	y = 0.90 x + 0.31
	Lactose (%)	255	3.99-4.86	0.73	0.09	0.00	1.92	y = 0.98 x + 0.09
	SNF (%)	255	8.10-8.91	0.60	0.11	0.00	1.58	y = 0.95 x + 0.41
	Moisture (%)	255	83.98-90.72	0.98	0.17	0.00	7.58	y = 1.00 x + 0.08
	MUN (mg/dL)	255	13.90-23.90	0.47	1.45	0.00	1.37	y = 0.94 x + 1.10
	SCC (log SCC/mL)	255	3.85-5.58	0.49	0.29	0.00	1.4	y = 0.93 x + 0.32
Cow 1252 Full CV	Fat (%)	426	0.45-7.82	0.98	0.30	0.00	6.40	y = 1.00 x + 0.00
	Protein (%)	426	2.39-2.97	0.67	0.06	0.00	1.73	y = 0.99 x + 0.03
	Lactose (%)	426	3.72-4.65	0.86	0.06	0.00	2.66	y = 1.00 x + 0.02
	SNF (%)	426	7.13-8.34	0.77	0.09	0.00	2.07	y = 0.99 x + 0.08
	Moisture (%)	426	84.67-91.86	0.99	0.14	0.00	13.12	y = 1.00 x - 0.01
	MUN (mg/dL)	426	18.50-25.20	0.49	0.62	0.00	1.40	y = 0.96 x + 0.86
	SCC (log SCC/mL)	426	3.00-5.10	0.81	0.18	0.00	2.29	y = 0.99 x + 0.03
Cow 1263 Full CV	Fat (%)	520	0.32-11.23	0.99	0.22	0.00	9.72	y = 1.00 x - 0.00
	Protein (%)	520	2.57-3.18	0.62	0.07	0.00	1.62	y = 0.97 x + 0.07
	Lactose (%)	520	4.03-4.73	0.78	0.07	0.00	2.14	y = 0.99 x + 0.06
	SNF (%)	520	7.65-8.80	0.74	0.12	0.00	1.96	y = 0.99 x + 0.08
	Moisture (%)	520	81.12-91.61	0.98	0.27	0.00	7.27	y = 1.00 x - 0.03
	MUN (mg/dL)	520	6.10-25.80	0.62	3.14	0.00	1.63	y = 0.99 x + 0.20
	SCC (log SCC/mL)	520	3.00-4.88	0.77	0.19	0.00	2.07	y = 0.99 x + 0.03
All 4 cows Full CV	Fat (%)	1564	0.32-10.37	0.98	0.24	0.00	7.18	y = 1.00 x + 0.00
	Protein (%)	1564	2.39-3.47	0.53	0.15	0.00	1.46	y = 0.99 x + 0.03
	Lactose (%)	1564	3.28-4.86	0.43	0.18	0.00	1.32	y = 0.98 x + 0.07
	SNF (%)	1564	7.13-8.91	0.44	0.20	0.00	1.34	y = 0.99 x + 0.11
	Moisture (%)	1564	81.12-91.86	0.96	0.31	0.00	5.33	y = 1.00 x + 0.01
	MUN (mg/dL)	1564	6.10-25.80	0.51	3.16	0.00	1.43	y = 0.99 x + 0.18
	SCC (log SCC/mL)	1564	2.98-5.40	0.63	0.39	0.00	1.32	y = 0.63 x + 1.55

n: number of validation samples r^2: coefficient of determination SEP: standard error of prediction
Regression line: Regression line from predicted value (x) to reference value (y). CV: Cross validation

Table 5.3 Validation statistics of the near-infrared sensing system for the determination of milk quality using two-third of all data sets to validate one-third of the remaining data sets

Analytical method	Milk quality items	n1	n2	Range	r^2	SEP	Bias	RPD	Regression line
	Fat (%)	243	121	1.09-5.73	0.96	0.18	0.02	5.05	y = 0.99 x + 0.01
	Protein (%)	243	121	2.95-3.38	0.51	0.07	-0.01	1.41	y = 0.86 x + 0.43
Cow 1234	Lactose (%)	243	121	3.28-4.31	0.59	0.10	0.00	1.56	y = 1.01 x - 0.04
Randomly	SNF (%)	243	121	7.31-8.60	0.69	0.11	-0.01	1.80	y = 0.97 x + 0.23
divided data set	Moisture (%)	243	121	86.28-90.6	0.95	0.18	0.00	4.57	y = 1.00 x + 0.40
	MUN (mg/dL)	243	121	14.40-22.60	0.53	1.11	-0.01	1.46	y = 0.98 x + 0.40
	SCC (log SCC/mL)	243	121	4.08-6.56	0.76	0.25	0.00	2.02	y = 0.99 x + 0.07
	Fat (%)	171	85	0.83-7.7	0.99	0.14	0.00	10.97	y = 1.00 x + 0.01
	Protein (%)	171	85	2.63-3.47	0.45	0.10	0.01	1.35	y = 1.06 x - 0.18
Cow 1246	Lactose (%)	171	85	4.03-4.86	0.72	0.09	-0.01	1.86	y = 0.92 x + 0.34
Randomly	SNF (%)	171	85	8.17-8.91	0.50	0.12	0.00	1.39	y = 0.84 x + 1.38
divided data set	Moisture (%)	171	85	83.98-90.72	0.99	0.16	0.00	9.00	y = 1.01 x - 0.59
	MUN (mg/dL)	171	85	14.10-23.90	0.44	1.53	0.02	1.33	y = 0.93 x + 1.25
	SCC (log SCC/mL)	171	85	3.85-5.58	0.54	0.29	0.01	1.47	y = 1.02 x - 0.09
	Fat (%)	285	142	0.45-7.76	0.98	0.29	-0.01	6.66	y = 1.00 x - 0.01
	Protein (%)	285	142	2.4-2.96	0.65	0.06	0.00	1.70	y = 1.02 x - 0.05
Cow 1252	Lactose (%)	285	142	3.72-4.65	0.87	0.06	0.00	2.73	y = 1.05 x - 0.20
Randomly	SNF (%)	285	142	7.13-8.34	0.77	0.10	0.00	2.08	y = 1.08 x - 0.63
divided data set	Moisture (%)	285	142	84.67-91.86	0.99	0.14	0.00	13.11	y = 1.00 x - 0.02
	MUN (mg/dL)	285	142	20.00-24.70	0.51	0.61	-0.03	1.42	y = 0.93 x + 1.54
	SCC (log SCC/mL)	285	142	3.00-4.97	0.81	0.19	0.03	2.26	y = 1.05 x - 0.22
	Fat (%)	347	173	0.41-11.23	0.98	0.30	0.00	7.24	y = 1.01 x - 0.01
	Protein (%)	347	173	2.58-3.15	0.64	0.06	0.00	1.65	y = 0.96 x + 0.12
Cow 1263	Lactose (%)	347	173	4.03-4.73	0.83	0.06	0.00	2.40	y = 0.99 x + 0.04
Randomly	SNF (%)	347	173	7.65-8.79	0.77	0.11	0.00	2.06	y = 0.98 x + 0.14
divided data set	Moisture (%)	347	173	81.12-91.18	0.97	0.33	0.00	6.21	y = 1.01 x - 0.58
	MUN (mg/dL)	347	173	6.10-25.80	0.65	3.02	0.03	1.68	y = 0.98 x + 0.20
	SCC (log SCC/mL)	347	173	3.00-4.88	0.78	0.19	0.01	2.12	y = 1.02 x - 0.08
	Fat (%)	1045	522	0.41-11.23	0.98	0.25	0.00	6.91	y = 1.01 x - 0.03
	Protein (%)	1045	522	2.39-3.42	0.53	0.15	0.00	1.46	y = 0.98 x + 0.05
All 4 cows	Lactose (%)	1045	522	3.28-4.86	0.43	0.18	-0.01	1.32	y = 1.02 x - 0.09
Randomly	SNF (%)	1045	522	7.31-8.91	0.50	0.20	-0.01	1.41	y = 1.07 x - 0.58
divided data set	Moisture (%)	1045	522	81.12-91.33	0.96	0.32	0.00	5.22	y = 1.00 x + 0.06
	MUN (mg/dL)	1045	522	6.10-25.80	0.52	3.13	0.00	1.44	y = 1.00 x + 0.06
	SCC (log SCC/mL)	1045	522	3.00-6.56	0.62	0.39	0.00	1.63	y = 0.99 x + 0.04

n1: number of calibration samples. n2: number of validation set. r^2: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).

Table 5.4 Validation statistics of the near-infrared sensing system for the determination of milk quality using first three weeks data set as calibration set and last one week data set as validation set

Analytical method	Milk quality items	n1	n2	Range	r²	SEP	Bias	RPD	Regression line
Cow 1234 1st 3weeks vs last 1 week data set	Fat (%)	275	89	1.60-5.69	0.95	0.18	0.10	4.45	y = 0.97 x - 0.00
	Protein (%)	275	89	2.97-3.15	0.02	0.06	0.04	0.64	y = 0.10 x + 2.77
	Lactose (%)	275	89	3.85-4.27	0.35	0.08	-0.04	1.22	y = 0.80 x + 0.85
	SNF (%)	275	89	7.87-8.4	0.56	0.08	-0.03	1.49	y = 0.93 x + 0.62
	Moisture (%)	275	89	86.29-90	0.96	0.15	-0.07	4.75	y = 0.98 x + 1.44
	MUN (mg/dL)	275	89	16.80-21.50	0.42	0.86	-0.64	1.27	y = 1.40 x - 6.64
	SCC (log SCC/mL)	275	89	4.11-4.97	0.26	0.23	0.24	0.85	y = 0.38 x + 2.76
Cow 1246 1st 3weeks vs last 1 week data set	Fat (%)	201	55	1.08-5.10	0.96	0.21	0.18	5.12	y = 0.95 x + 0.00
	Protein (%)	201	55	2.93-3.29	0.17	0.07	-0.06	1.10	y = 1.21 x - 0.60
	Lactose (%)	201	55	4.18-4.59	0.48	0.08	0.01	1.34	y = 0.79 x + 0.93
	SNF (%)	201	55	8.22-8.71	0.41	0.10	0.05	1.22	y = 0.69 x + 2.58
	Moisture (%)	201	55	86.48-90.70	0.96	0.20	-0.24	5.30	y = 0.99 x + 1.08
	MUN (mg/dL)	201	55	13.90-18.80	0.00	1.35	2.97	0.94	y = -0.07 x + 17.38
	SCC (log SCC/mL)	201	55	4.32-5.48	0.53	0.20	-0.12	1.46	y = 1.04 x - 0.05
Cow 1252 1st 3weeks vs last 1 week data set	Fat (%)	313	114	0.46-7.76	0.99	0.20	-0.07	10.20	y = 1.03 x - 0.04
	Protein (%)	313	114	2.41-2.77	0.42	0.06	-0.02	1.30	y = 1.22 x - 0.57
	Lactose (%)	313	114	3.72-4.63	0.80	0.08	-0.02	2.11	y = 1.19 x - 0.83
	SNF (%)	313	114	7.13-8.34	0.80	0.12	-0.04	1.90	y = 1.45 x - 3.52
	Moisture (%)	313	114	84.67-91.60	0.99	0.21	0.12	9.28	y = 1.01 x - 0.90
	MUN (mg/dL)	313	114	20.00-24.80	0.37	0.84	-0.10	1.21	y = 1.60 x - 13.28
	SCC (log SCC/mL)	313	114	3.30-4.98	0.79	0.20	-0.21	2.2	y = 1.00 x + 0.21
Cow 1263 1st 3weeks vs last 1 week data set	Fat (%)	399	121	0.57-9.06	0.98	0.28	0.02	6.95	y = 0.96 x + 0.09
	Protein (%)	399	121	2.64-3.03	0.30	0.06	0.01	1.20	y = 1.00 x - 0.01
	Lactose (%)	399	121	4.25-4.71	0.75	0.06	-0.04	1.99	y = 1.06 x - 0.21
	SNF (%)	399	121	7.95-8.66	0.79	0.08	-0.05	2.19	y = 1.01 x - 0.00
	Moisture (%)	399	121	82.96-90.86	0.98	0.26	0.02	6.93	y = 0.95 x + 4.30
	MUN (mg/dL)	399	121	8.30-25.80	0.25	5.68	-0.23	1.12	y = 1.83 x - 12.02
	SCC (log SCC/mL)	399	121	3.00-4.46	0.73	0.19	0.01	1.93	y = 1.00 x - 0.02
All 4 cows 1st 3weeks vs last 1 week data set	Fat (%)	1231	336	0.57-9.06	0.98	0.27	0.07	6.25	y = 0.98 x + 0.02
	Protein (%)	1231	336	2.41-3.29	0.53	0.13	0.00	1.45	y = 1.12 x - 0.36
	Lactose (%)	1231	336	3.72-4.71	0.56	0.15	-0.01	1.48	y = 1.22 x - 0.96
	SNF (%)	1231	336	7.13-8.71	0.73	0.14	0.00	1.93	y = 1.00 x - 0.00
	Moisture (%)	1231	336	82.96-91.33	0.96	0.34	-0.05	4.55	y = 0.94 x + 5.14
	MUN (mg/dL)	1231	336	8.30-25.80	0.23	4.42	0.02	1.13	y = 0.82 x + 3.26
	SCC (log SCC/mL)	1231	336	3.00-5.48	0.67	0.32	-0.10	1.72	y = 0.92 x + 0.44

n1: number of calibration samples. n2: number of validation set. r²: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).

Table 5.5 Validation statistics of the near-infrared sensing system for the determination of milk quality using three cows data set as calibration set and one cow data set as validation set

Analytical method	Milk quality items	n1	n2	Range	r^2	SEP	Bias	RPD	Regression line
	Fat (%)	1203	364	1.09-5.73	0.95	0.20	-0.02	4.31	y = 1.00 x + 0.03
3 cows vs	Protein (%)	1203	364	2.91-3.38	0.19	0.11	-0.17	0.85	y = 0.37 x + 2.06
Cow 1234	Lactose (%)	1203	364	3.28-4.31	0.22	0.13	0.39	1.11	y = 1.68 x - 3.41
	SNF (%)	1203	364	7.31-8.66	0.18	0.17	0.05	1.10	y = 1.31 x - 2.58
	Moisture (%)	1203	364	86.28-90.64	0.89	0.28	-0.24	2.89	y = 0.89 x + 9.82
	MUN (mg/dL)	1203	364	14.40-23.10	0.02	2.00	0.37	0.81	y = -0.26 x + 23.38
	SCC (log SCC/mL)	1203	364	4.00-6.56	0.02	0.53	-0.45	0.95	y = 0.29 x + 3.60
	Fat (%)	1311	256	0.83-7.70	0.96	0.28	-0.03	5.10	y = 1.01 x - 0.02
3 cows vs	Protein (%)	1311	256	2.56-3.47	0.00	0.14	-0.19	0.99	y = 0.29 x + 2.23
Cow 1246	Lactose (%)	1311	256	3.99-4.86	0.28	0.15	-0.25	1.17	y = 1.12 x - 0.25
	SNF (%)	1311	256	8.10-8.91	0.34	0.14	-0.44	1.23	y = 0.92 x + 1.11
	Moisture (%)	1311	256	83.98-90.72	0.96	0.28	0.47	4.67	y = 1.03 x - 3.44
	MUN (mg/dL)	1311	256	13.90-23.90	0.04	2.16	1.06	0.92	y = 0.30 x + 12.46
	SCC (log SCC/mL)	1311	256	3.85-5.58	0.14	0.38	-0.26	1.07	y = 0.74 x + 1.41
	Fat (%)	1140	427	0.45-7.82	1.00	-0.30	0.06	-6.45	y = 0.9 x + 0.3
3 cows vs	Protein (%)	1140	427	2.39-2.97	0.00	0.10	0.40	0.95	y = -0.07 x + 2.85
Cow 1252	Lactose (%)	1140	427	3.72-4.65	0.44	0.12	-0.14	1.34	y = 1.10 x - 0.27
	SNF (%)	1140	427	7.13-8.34	0.35	0.16	0.26	1.20	y = 1.57 x - 4.98
	Moisture (%)	1140	427	84.67-91.86	0.98	0.33	-0.39	5.39	y = 0.88 x + 11.20
	MUN (mg/dL)	1140	427	18.50-25.20	0.05	1.27	-2.88	0.70	y = 0.17 x + 19.19
	SCC (log SCC/mL)	1140	427	3.00-5.10	0.64	0.25	0.82	1.67	y = 0.94 x - 0.56
	Fat (%)	1047	520	0.32-11.23	0.99	0.27	-0.34	7.98	y = 1.03 x + 0.26
3 cows vs	Protein (%)	1047	520	2.57-3.18	0.18	0.10	0.13	1.05	y = 0.58 x + 1.13
Cow 1263	Lactose (%)	1047	520	4.03-4.73	0.54	0.10	-0.09	1.46	y = 1.19 x - 0.73
	SNF (%)	1047	520	7.65-8.80	0.43	0.18	0.04	1.32	y = 0.92 x + 0.62
	Moisture (%)	1047	520	81.12-91.61	0.98	0.31	0.40	6.36	y = 1.06 x - 5.88
	MUN (mg/dL)	1047	520	6.10-25.80	0.02	5.39	2.68	0.95	y = -0.75 x + 27.10
	SCC (log SCC/mL)	1047	520	3.00-4.88	0.65	0.24	0.28	1.67	y = 1.11 x - 0.75

n1: number of calibration samples. n2: number of validation set. r^2: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).

2. Cow information

Two Holstein cows, numbered 1256 and 1257 belonging to Hokkaido University were used in this study. The cows were used in the experiment during different lactation periods. Measurement period was from October 5, 2015 to October 7, 2015 and November 5, 2015 for autumn season, November 30, 2015 to December 12, 2015 for winter season and April 18, 2016 to April 21, 2016 for spring season. Measurements were performed in two consecutive milkings, milking in the evening and milking the following morning, for about two weeks during the experiment. A pipeline milking system was used for milking the cows at the Hokkaido University cow barn. Two of the cows were milked at the same time and each was measured for

about four milking times. Milk spectra data were recorded and then milk samples were collected from the milk sampler every 20 s during milking.

2. Chemometric analyses

Chemometric analyses were carried out to develop calibration models for each milk quality indicator and to validate the precision and accuracy of the models. One data set was obtained from the experiment conducted using the two cows. The data set from the two cows was used to develop calibration models and the same data set was used for validation of the calibration models. Full cross validation was used to validate the calibration models. Spectra data analysis software (The Unscrambler ver. 10.3, Camo AS, Trondheim, Norway) was used for the analyses. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. Pretreatment of the spectra such as multiplicative scatter correction and smoothing were not performed.

2. Results and discussion

5.3.2 Milking season

Precision and accuracy of calibration models

The validation statistics for the determination of measurement accuracy for the three major milk constituents, SNF, moisture MUN and SCC during different milking season (autumn, winter and spring) are shown in Table 5.6.

For the autumn season, the r^2 and SEP values were 0.99 and 0.15% for fat, 0.87 and 0.07% for protein, 0.79 and 0.16% for lactose, 0.88 and 0.15% for SNF, 0.96 and 0.29% for moisture, 0.68 and 1.40 mg/dL for MUN, and 0.81 and 0.18 log SCC/mL for SCC, respectively. For winter season, the r^2 and SEP were 0.99 and 0.11% for fat, 0.91 and 0.03% for protein, 0.67 and 0.13% for lactose, 0.73 and 0.17% for SNF, 0.94 and 0.24% for moisture, 0.76 and 0.76 mg/dL for MUN, and 0.90 and 0.11 log SCC/mL for SCC, respectively. Also, for spring season, the r^2 and SEP values were 0.98 and 0.12% for fat, 0.73 and 0.08% for protein, 0.48 and 0.22% for lactose, 0.33 and 0.24%, 0.93 and 0.21%, 0.81 and 0.84mg/dL for MUN, and 0.59 and 0.20 log SCC/mL for SCC, respectively. The r^2 results showed that the precision and accuracy to determine milk fat and moisture content was very high for each season and was sufficiently high for other milk quality indicators. However, the SEP values as shown in Figure 5.6 indicated that the precision and accuracy of each milk quality indicator were different for each milking season. Lactation stage of the two cows was throughout the autumn, winter and spring season. The lactation stage affected the constituent contents of the milk and amount of milk production. Feed for the two cows were varied in each season. They were fed hay, silage and concentrated feed,

and no fresh pasture grass in winter. The feed also affected the constituent contents of the milk and amount of milk production. Environmental temperature was different in each season. The temperature may have affected NIR spectra. Many factors such as lactation stage, feed and temperature could affect the accuracy of the calibration models. Further study is recommended to improve the accuracy of the calibration models using NIR sensing system during milking.

Table 5.6 Validation statistics of the near-infrared spectroscopic sensing system for determination of milk quality during autumn, winter and spring

Milking season	Milk quality indicators	n	Range	r^2	SEP	Bias	RPD	Regression line
Autumn (October 2015)	Fat (%)	89	1.30-7.25	0.99	0.15	-0.00	10.47	y = 1.00 x + 0.00
	Protein (%)	89	2.72-3.50	0.87	0.07	-0.00	2.80	y = 0.95 x + 0.14
	Lactose (%)	89	3.25-4.84	0.79	0.16	0.00	2.17	y = 0.98 x + 0.07
	SNF (%)	89	7.20-9.24	0.88	0.15	0.00	2.93	y = 1.00 x - 0.03
	Moisture (%)	89	85.07-90.27	0.96	0.29	0.00	4.76	y = 0.99 x + 0.53
	MUN (mgdL^{-1})	89	6.30-14.20	0.68	1.40	0.05	1.75	y = 0.92 x + 0.81
	SCC (log SCCmL^{-1})	89	4.30-5.42	0.81	0.18	-0.00	2.29	y = 0.98 x + 0.11
Winter (December 2015)	Fat (%)	77	1.90-6.62	0.99	0.11	0.00	9.92	y = 1.00 x + 0.00
	Protein (%)	77	3.08-3.60	0.91	0.03	-0.00	3.36	y = 0.98 x + 0.07
	Lactose (%)	77	3.79-4.78	0.67	0.13	0.00	1.74	y = 0.97 x + 0.14
	SNF (%)	77	7.93-9.28	0.73	0.17	0.00	1.92	y = 0.97 x + 0.25
	Moisture (%)	77	84.85-88.85	0.94	0.24	0.00	3.96	y = 1.00 x + 0.15
	MUN (mgdL^{-1})	77	6.80-13.20	0.76	0.76	0.01	2.03	y = 0.94 x + 0.57
	SCC (log SCCmL^{-1})	77	4.30-5.74	0.90	0.11	0.11	3.23	y = 0.99 x + 0.07
Spring (April 2016)	Fat (%)	68	2.50-6.28	0.98	0.12	0.00	7.43	y = 1.00 x + 0.02
	Protein (%)	68	3.49-4.11	0.73	0.08	-0.00	1.91	y = 0.94 x + 0.24
	Lactose (%)	68	3.38-4.84	0.48	0.22	-0.00	1.36	y = 0.82 x + 0.79
	SNF (%)	68	8.45-9.62	0.33	0.24	0.00	1.19	y = 0.74 x + 2.32
	Moisture (%)	68	84.10-88.90	0.93	0.21	0.00	3.74	y = 0.99 x + 0.95
	MUN (mgdL^{-1})	68	4.00-10.70	0.81	0.84	-0.00	2.27	y = 0.96 x + 0.33
	SCC (log SCCmL^{-1})	68	5.06-6.31	0.59	0.20	-0.00	1.54	y = 0.89 x + 0.63

n: number of validation samples r^2: coefficient of determination SEP: standard error of prediction Regression line: Regression line from predicted value (x) to reference value (y).
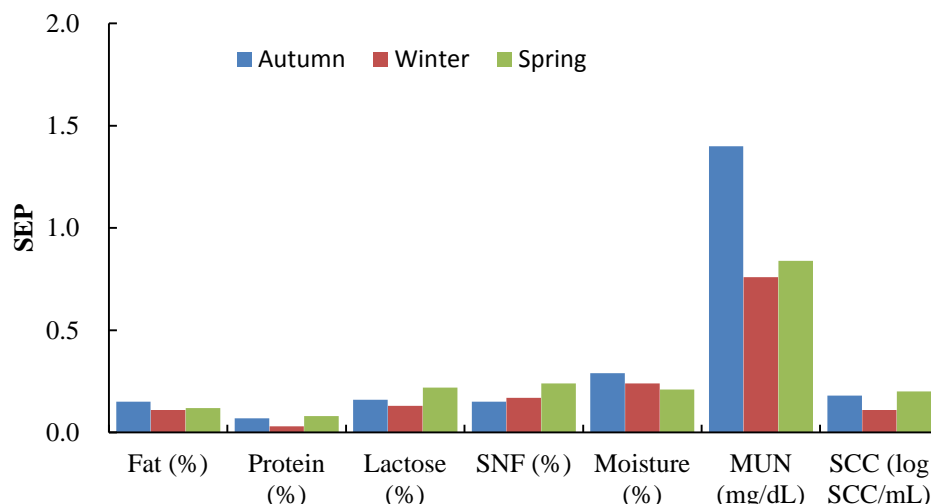
Figure 5.1 Comparison of the standard error of prediction of milk quality indicators for autumn, winter and spring

3. Cow information

Two Holstein cows (cow number 1256 and 1257) belonging to Hokkaido University were used in this study. Data collected from one experimental period of parity (first second and third parity) was used in this study. These cows were used in the experiment during their different lactation periods. The experimental period for the first, second and third parity for both cows was from 18/04/2016 to 27/04/2016, 13/02/2017 to 24/02/2017 and 07/05/2018 to 18/05/2018 respectively. The information about the calving date for cow number 1256 and 1257 were obtained from the records available in the cow barn. The first calving date was 26/07/2015 and 31/07/2015 for cow number 1256 and 1257, the second calving date was 13/06/2016 and 27/08/2016 for cow number 1256 and 1257 and the third calving date was 01/08/ 2017 and 04/10/2017 for cow number 1256 and 1257 respectively. Measurements were performed in two successive milkings, i.e., milking in the evening and milking the following morning. A pipeline milking system was utilized for milking the cows at Hokkaido University cow barn. Milk samples were collected from the sampler every 20 s during milking.

3. Chemometric analyses

Chemometric analyses were carried out to develop calibration models for each milk quality indicator and to validate the precision and accuracy of the models. Spectral data analyses software (The Unscrambler ver. 10.3,Camo AS, Trondheim, Norway) was used for the analyses. The total reference samples of each cow and of each calving time were randomly divided into two sample sets such that two-third of the total sample sets were used as calibration set and the

remaining (one-third) sample set as validation set. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. Pre-treatment of the spectra such as smoothing or second derivatives was not performed.

3. Results and discussion

5.3.3 Parity

Precision and accuracy of calibration models

The validation statistics for the determination of measurement accuracy for the three major milk constituents, SNF, moisture MUN and SCC during different parity (first, second and third) are shown in Table 5.7.

For the first parity, the r^2 and SEP values were 0.99 and 0.09% for fat, 0.60 and 0.11% for protein, 0.43 and 0.26% for lactose, 0.35 and 0.27% for SNF, 0.91 and 0.26% for moisture, 0.50 and 2.44 mg/dL for MUN, and 0.43 and 0.30 log SCC/mL for SCC, respectively. For the second parity, the r^2 and SEP were 0.96 and 0.28% for fat, 0.84 and 0.06% for protein, 0.22 and 0.24% for lactose, 0.63 and 0.17% for SNF, 0.97 and 0.22% for moisture, 0.87 and 1.18 mg/dL for MUN, and 0.78 and 0.25 log SCC/mL for SCC, respectively. Also, for the third parity, the r^2 and SEP values were 0.98 and 0.14% for fat, 0.94 and 0.09% for protein, 0.80 and 0.21% for lactose, 0.89 and 0.22%, 0.95 and 0.21%, 0.32 and 1.25 mg/dL for MUN, and 0.56 and 0.19 log SCC/mL for SCC, respectively. From the r^2 results obtained for each parity and for each milk quality indicator, it showed that the precision and accuracy to determine milk fat and moisture content was very high for each parity. The r^2 results was sufficiently high for protein and MUN but was low for lactose, SNF and SCC for the first parity, sufficiently high for protein, SNF, MUN and SCC but low for lactose for the second parity. For the third calving time, the r^2 results for protein was very high, sufficiently high for lactose, SNF and SCC but was low for MUN. Also, the SEP values indicated that the precision and accuracy of each milk quality indicator were different for each milking season. The difference in calving time of the two cows may have affected the constituent contents of the milk and amount of milk production. Feed for the two cows were varied in each calving time. As at the period of the first and second parity, they were fed hay, silage and concentrated feed, no fresh pasture grass whereas fresh grass was inclusive as at the third calving time. The feed would have affected the constituent contents of the milk and amount of milk production. Other factor such as environmental temperature and lactation stage was different in each parity. The temperature may have affected NIR spectra. Thus, calving time could have affected the performance of the calibration models.

CHAPTER V: FACTORS AFFECTING THE ACCURACY OF CALIBRATION MODELS USING
NEAR-INFRARED SPECTROSCOPIC SENSING SYSTEM FOR MILK QUALITY
DETERMINATION DURING MILKING

The three major milk constituent content, SNF, moisture content, MUN and SCC are very important milk quality indicators. For example; the percentage of milk fat content is very essential such that can determine milk payment scheme. Knowing the percentage or quality of milk fat present in raw milk can give information about dietary fiber content, quality and cow fiber intake (Victoria, 2017). More so, the determination of SCC in raw milk plays a vital role in the diagnosis of subclinical mastitis, which is a very serious disease of milking cow.

According to the results obtained from the first, second and third parity of the two cows, it was suggested that the difference in parity could affect the accuracy of the predicted milk quality indicators most especially milk lactose SNF MUN and SCC. However, the findings of this study clearly show that the NIRS sensing system developed in this study can be used to determine milk quality in real-time during milking. The trend monitoring of SCC at every milking time can reveal subclinical mastitis to dairy farmers and veterinarians.

Table 5.7 Validation statistics of the near-infrared spectroscopic sensing system for determination of milk quality first, second and third calving time

Calving times	Milk quality items	n1	n2	Range	r^2	SEP	Bias	RPD	Regression line
1st Parity	Fat (%)	106	52	2.67-6.66	0.99	0.09	0.00	9.88	y = 0.99 x + 0.02
	Protein (%)	106	52	3.46-4.19	0.60	0.11	0.01	1.57	y = 0.97 x + 0.11
	Lactose (%)	106	52	2.92-4.75	0.43	0.26	0.01	1.33	y = 0.94 x + 0.26
	SNF (%)	106	52	8.11-9.59	0.35	0.27	0.02	1.22	y = 0.81 x + 1.73
	Moisture (%)	106	52	84.21-88.55	0.91	0.26	-0.02	3.26	y = 0.98 x + 1.99
	MUN (mg/dL)	106	52	4.00-18.60	0.50	2.44	0.25	1.42	y = 0.97 x + 0.08
	SCC (log SCC/mL)	106	52	4.97-6.68	0.43	0.30	0.01	1.33	y = 1.07 x - 0.42
2nd Parity	Fat (%)	194	96	2.20-7.43	0.96	0.28	0.02	4.80	y = 1.01 x - 0.08
	Protein (%)	194	96	3.34-4.05	0.84	0.06	0.00	2.49	y = 0.98 x + 0.07
	Lactose (%)	194	96	3.50-5.70	0.22	0.24	-0.03	1.13	y = 0.98 x + 0.13
	SNF (%)	194	96	8.28-9.72	0.63	0.17	-0.02	1.64	y = 1.01 x - 0.07
	Moisture (%)	194	96	83.67-88.81	0.97	0.22	-0.01	5.67	y = 0.99 x + 0.60
	MUN (mg/dL)	194	96	4.00-13.80	0.87	1.18	-0.08	2.76	y = 0.98 x + 0.22
	SCC (log SCC/mL)	194	96	4.04-6.49	0.78	0.25	0.01	2.12	y = 0.96 x + 0.23
3rd Parity	Fat (%)	160	79	2.60-6.69	0.98	0.14	0.00	6.42	y = 1.01 x - 0.06
	Protein (%)	160	79	2.96-4.18	0.94	0.09	0.00	3.98	y = 1.03 x - 0.10
	Lactose (%)	160	79	2.83-4.63	0.80	0.21	-0.02	2.21	y = 0.91 x + 0.38
	SNF (%)	160	79	6.79-9.70	0.89	0.22	-0.02	2.97	y = 0.95 x + 0.47
	Moisture (%)	160	79	85.09-88.73	0.95	0.21	0.01	4.52	y = 1.00 x + 0.38
	MUN (mg/dL)	160	79	6.80-14.50	0.32	1.25	-0.12	1.21	y = 0.88 x + 1.51
	SCC (log SCC/mL)	160	79	5.06-6.37	0.56	0.19	0.02	1.50	y = 0.90 x + 0.57

n1: number of calibration samples. n2: number of validation set. r^2: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).

4. Cow information

Two Holstein cows owned by Hokkaido University dairy barn were used in this study. Two of the cows (cow number 1256 and 1257) were used for this study. These cows were used in the experiment during their different lactation periods. The sample collection period was from the period of April 18, 2016 to April 21, 2016. A pipeline milking system was utilized for milking the cows at Hokkaido University cow barn. Measurements were performed in two successive milkings, i.e., milking in the evening and milking in the following morning for about five weeks during the experiment period. Two cows were milked at the same milking time and each cow was measured for about four milking times. Milk samples were collected from the sampler every 20 seconds during milking and thereafter, the milk spectra were recorded.

4. Chemometric analyses

Chemometric analyses were carried out to develop calibration models for each milk quality indicator and to validate the precision and accuracy of the calibration models. One data set was obtained from the experiment conducted using two cows. The data set from the two cows was used to develop calibration models and the same data set from the two cows was used for validation of the calibration models. Full cross validation was used to validate the calibration models. Spectra data analysis software (The Unscrambler ver. 10.3, Camo AS, Trondheim, Norway) was used for the analyses. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. Pretreatment of the spectra such as multiplicative scatter correction and smoothing were not performed.

3. Results and discussion

5.3.4   Number of PLS factors

Multivariate calibration in PLS consists of estimating models constants, which includes number of factors to use in the model. Each of these PLS factors can be used to generate optimal linear PLS estimations. When very few PLS factors are used, it can lead to not modeling of important NIR spectra whereas, using too many factors generates too much measurement noise from NIR spectra in to the calibration models (Burns & Ciurczak, 2009). The optimal number of PLS for milk quality determination was estimated as the number of factors after which explained variance no longer increase significantly. Figure 5.2 shows the plot of explained variance (EV) against the number of PLS factors (NPF) for the determination of milk quality. The EV for milk quality determination is as follows: EV = 99% for fat; 73% for protein; 47% for lactose; 59% for MUN and 81% for SCC. The explained variances obtained in this study were sufficiently high for the determination of each milk quality indicator.

Analysis of loading weights, explained variance and PLS factors

The loading weight for the first three PLS factors to predict milk fat, protein, and lactose content, MUN and SCC are shown in Figure 5.3 to 5.7 respectively. PLS factor 1 had about 60% contribution to the explained variance of fat, PLS factor 2 had about 39% and PLS factor 3 had 5% (Figure 5.3). PLS 2 had the lowest loading weights which were found around the wavelength range of 700 to 760 nm. The wavelength around 960 nm was influenced by the absorption of water molecules. This result showed that PLS factor 2 explained fat variance including the influence of water molecules. However, PLS factors 1 and 3 explained fat variance including the effect of water which was due to the positive loading weight of PLS factors 1 and 3 around 920-960 nm.

For protein content, PLS factor 1 had the lowest contribution of 0% to the explained variance of protein while PLS factor 2 had the highest contribution which is 39% and PLS factor 3 had about 5% contribution to explaining the variation that occurs in protein content (Figure 5.4). PLS factor 1, 2 and 3 contributed about 12%, 27% and 4% to the explained variance of lactose (Figure 5.5) respectively while PLS factor 1, 2 and 3 contributed about 18%, 26% and 0% to the explained variance of MUN (Figure 5.6) respectively.

As for SCC, PLS factor 1 had the highest contribution of 31% to the explained variance of SCC, PLS factor 2 had 2% and PLS factor 3 had 3% (Figure 5.7). PLS factor 2, had the lowest loading weights which were found around the wavelength range of 700-760 nm. However, factor 2 explained the variance of SCC including the influence of water molecules because of the positive loading weight of PLS 2 around 960 nm. PLS factor 1 and 3 explained SCC variance excluding the influence of water molecules.
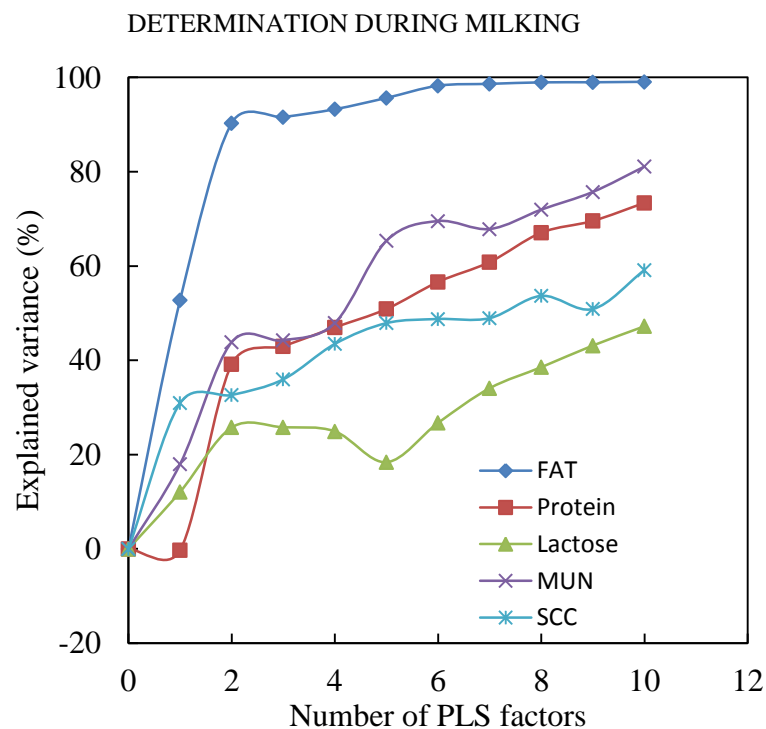
Figure 5.2 Explained variance for determination of milk quality against the number of PLS factors
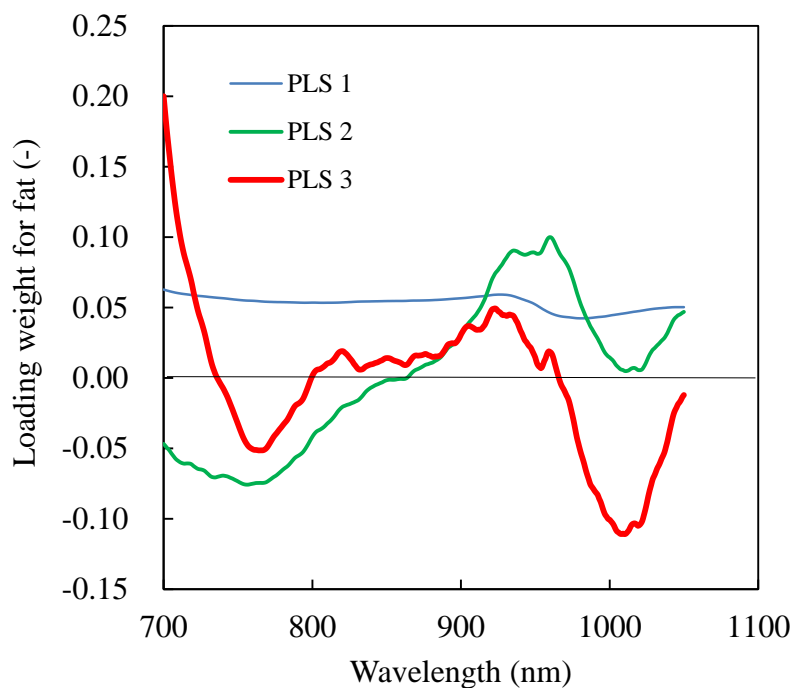
Figure 5.3 Loading weight for the first three PLS factors to predict fat content
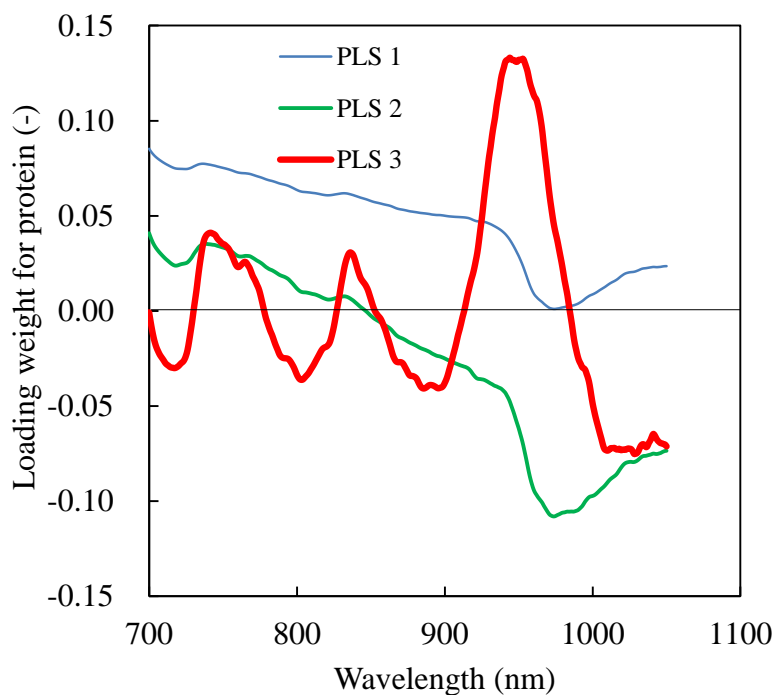
Figure 5.4 Loading weight for the first three PLS factors to predict protein content
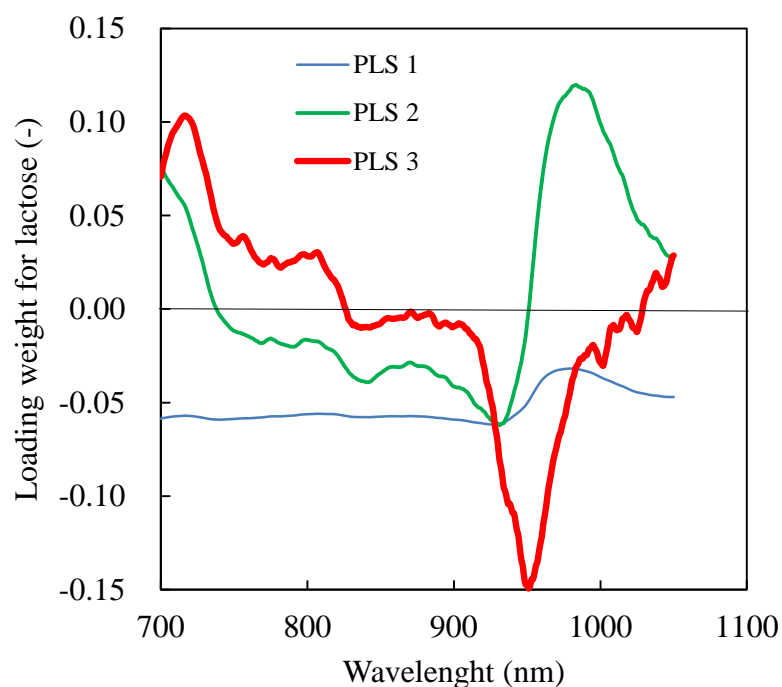
Figure 5.5 Loading weight for the first three PLS factors to predict lactose content
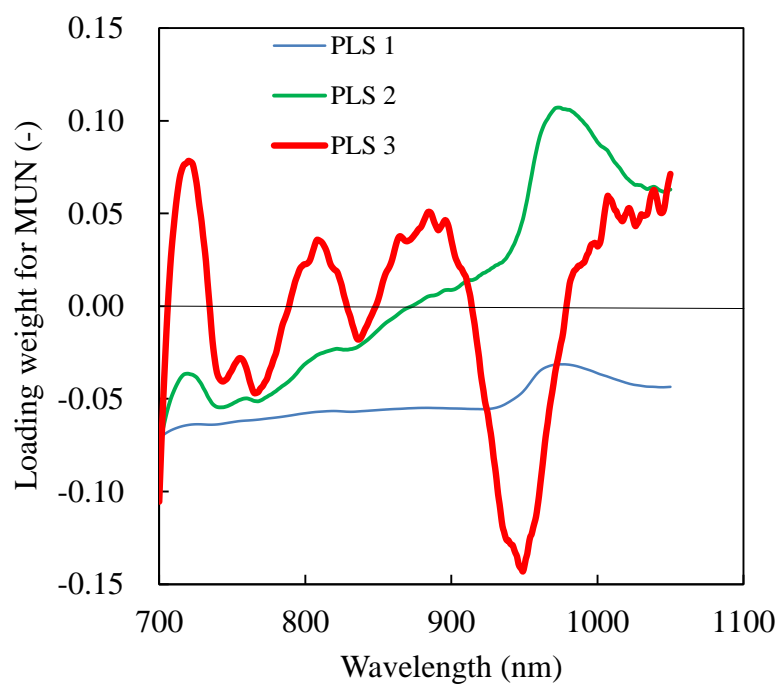
Figure 5.6 Loading weight for the first three PLS factors to predict MUN
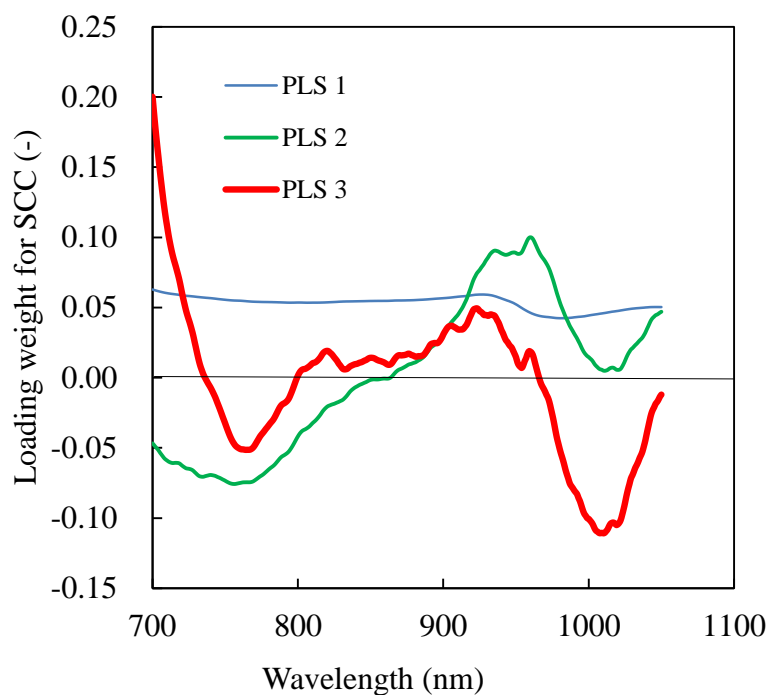
Figure 5.7 Loading weight for the first three PLS factors to predict SCC

5.4 Conclusions

1. The findings of this study indicated that cow individuality can affect the accuracy of calibration models. Thus, ensuring the collection of a wide range of NIR spectra data from all cows is important which would consequently cover the differences between cows and then improve the accuracy of calibration models.

2. It can be concluded that milking season could affect the accuracy of calibration models because, the statistical results obtained showed differences for each milking season and for each milk constituent content, SNF, moisture, MUN and SCC. Milk quality changes because of lactation stage, feeding and stage and calving times. These are contributory factors affecting the accuracy of calibration models.

3. It is very important to determine the contribution of PLS factor to explained variance of each milk quality indicator. This is because, it would help to know the optimal PLS factor for each milk constituent, SNF, moisture content, MUN and SCC. This would as well enable us to understand the influence of each milk constituent's content on milk spectra and it would assist in detecting over fitting of spectra data using chemometric analysis.

4. However, the online real-time NIRS sensing system developed in this study could be used for milk quality determination during milking. The system can provide dairy farmers with information on milk quality and physiological condition of an individual cow and, therefore, give them feedback control for optimizing dairy farm management. By using the system, dairy farmers will be able to produce high-quality milk and precision dairy farming will be realized.

Recommendation

It is necessary to make further studies on how to reduce the effect of these limiting factors and improve the calibration models. Further study is recommended to improve the accuracy of the calibration models developed putting in consideration these factors.

6. Chapter VI: Determination of Milk Progesterone Concentration Using Near-Infrared Spectroscopic Sensing System

Summary

This chapter is focused on improving cow reproductive and health status using near-infrared spectroscopy.

As stated in previous chapters, dairy farming is labor intensive and so in the current dairy industry, there has been an intensive demand for estrus detection and early diagnosis of pregnancy has been increasing. Progesterone is a steroid hormone that is secreted from corpus luteum into bovine blood and milk, and has a role of maintenance of estrus cycle and pregnancy. Therefore, progesterone concentration in bovine milk is used as an important indicator of estrus detection and early diagnosis of pregnancy. Current method for milk progesterone determination requires a hormone extraction procedure that is time consuming, various types of instruments, reagents management, and various assay methods that are destructive in nature. In contrast, near-infrared spectroscopy (NIRS) is a time saving and non-destructive analytical method that can be used for online real-time determination of milk constituents content such as milk fat, protein, lactose, milk urea nitrogen and somatic cell count. However, there has been limited study on using NIRS for online real-time determination of progesterone concentration in milk during milking. Thus, the objective of this study was to develop an online real-time NIR spectroscopic sensing system for milk progesterone determination during milking by using a specific enzyme immunosorbent assay as a reference (chemical) method.

Introduction

Progesterone is a steroid hormone that is secreted in milk by the female mammals. This kind of hormone is produced in the corpus luteum and placenta and its plays an important physiological role in the luteal phase of the menstrual cycle and in the maintenance of pregnancy. Its concentration in milk has a characteristic variation along the estrus cycle and so progesterone is accepted as an ideal indicator to control cow reproductive status, to detect the animal heat and for the diagnosis of cows' pregnancy (Käppel et al., 2007; Posthuma-Trumpie et al., 2009). Usually, the onset of the heat is indicated by a rapid fall in the concentration of progesterone in milk to below 2-5 ng/mL, hence, once the cow is pregnant the progesterone concentration remains high and constant (Posthuma-Trumpie et al., 2009). Accurate estrus detection is crucial for timed and successful artificial insemination and early detection of the pregnancy. An early detection of a failed insemination is critical for maximizing reproductive efficiency, as it could allow a meaningful elapsed time delay before a new repeated insemination.

Furthermore, the poor ability to detect female animals in heat results in longer calving intervals and lowers milk production (Posthuma-Trumpie et al., 2009). Due to those reasons, reliable analytical methods to detect timely and accurately the occurrence of estrus cycle and other reproductive states are needed (Friggens and Chagunda, 2005). Several methods, including measurement of milk temperature and radio-telemetric measurement of vaginal temperature have been used for estrus prediction. However, the most effective and reliable method for the purpose is the direct determination of the level of progesterone in plasma or bovine milk (Simersky et al., 2007; Tolleson et al., 2003). Different methods have been developed for accurate determination of progesterone in milk, including strategies based on thin layer, gas or liquid chromatography coupled to mass spectrometry detection (Díaz-Cruz et al., 2003) but such techniques are limited by several drawbacks including substantial equipment costs and/or extensive and time-consuming sample pre-treatments, rendering progesterone routine determination an expensive analysis. Alternatively, immunochemical assays are the most popular approach nowadays for the determination of progesterone (Gillis et al., 2006).

Similarly, near-infrared spectroscopy (NIRS) methods have been historically very successful at evaluating the quality of agricultural commodities, especially food such as rice, wheat and milk (Sato et al., 1987; Tsenkova et al., 2001; Natsuga et al., 2006; Kawamura et al., 2007; Kawasaki et al., 2008; Iweka et al., 2016). These NIRS approaches are most preferable for analysis of food components because they are rapid, non-destructive, usually require little or no sample preparation, pretreatment free, have the potential to run multiple tests on a single sample and good for on-line analysis (Nawrocka and Lamorsk, 2013). Garnsworthy and Mann (2001) have reported the novel approach to online milk progesterone level using NIRS. NIRS has also been used for early pregnancy detection and gender of hair sheep in the tropics using near-infrared (NIR) reflectance spectroscopy in feces (Andueza et al., 2014). However, there has been limited study on using NIRS for online real-time determination of progesterone concentration in bovine milk during milking.

For this reason, the objective of this study was to develop an online real-time NIR spectroscopic sensing system for bovine milk progesterone determination during milking.

6.1 Materials and methods

6.1.1 NIR spectroscopic sensing system

An empirical online NIR spectroscopic sensing system was designed for analyzing milk progesterone concentration of each cow during milking. The system consisted of an NIR spectrum sensor, NIR spectrometer, milk flow meter, milk sampler and a laptop computer. The system was fixed between a teatcup cluster and a milk bucket of the milking system. Non-

homogenized milk from the teatcup cluster flowed continuously across a bypass into the milk chamber of the NIR spectrum sensor. Excess raw milk flowed past the milk flow meter and was then released through a line tube into the milk bucket. The NIR spectrum sensor consisted of three Halogen lamps namely; halogen lamps A, B and C. The optical axes of halogen lamps A and B and the optical fiber were set at the same level, but the optical axis for halogen lamp C was set at 5 mm higher than the optical fiber. The volume of milk sample in the milk chamber was approximately 30 mL. The spectrum sensor acquired absorbance spectra through the milk. Spectra were obtained in the wavelength range of 700 nm to 1050 nm at 1-nm intervals every 20 s during milking. The milk flow rate was simultaneously recorded.

6.1.2 Cow and milk samples

In this study, milk samples were obtained from four Holstein cows at the Experimental Farm of Field Science of Northern Biosphere, Hokkaido University, Japan. The cows (cow number 1221, 1239, 1250 and 1263) were used in the experiment during different lactation periods (Table 2). Cow number 1263 was treated by prostaglandin to promote estrus artificially. Measurements were performed in two consecutive milkings, milking in the evening and milking the following morning from July to August 2016. This experiment was carried out for 28 days because it covers the beginning of one period of estrus (period of sexual receptivity and fertility) to the beginning of the next period of estrus, which is known as 21 days of estrus cycle. The ovulation date was determined using rectal palpation method by a veterinarian. A pipeline milking system was used for milking the cows. Two cows were milked at the same milking time and each cow was examined for about 23 milking times. Milk spectra data were recorded and then milk samples were collected from the milk sampler every 20 s during milking. The milk samples obtained were then stored in a freezer at a temperature of -80°C and were later thawed and used for progesterone chemical determination. The experiment was conducted to cover variation in milk spectra caused by cow individuality, calving times and lactation stage.

Table 6.1 Information on cows used in the experiment

Cow number	Date of birth	Date of latest calving	Calving times	Ovulation date during experiment
1221	Oct. 22, 2006	Mar. 31, 2016	6	Jul. 24, 2016 and Aug. 15, 2016
1239	Nov. 25, 2008	Nov. 22, 2015	5	Aug. 12-13, 2016
1250	Jul. 05, 2012	May 15, 2016	2	Aug. 4, 2016
1263	Mar. 02, 2014	May 08, 2016	1	Aug. 1, 2016

6.1.3 Hormone assay for reference (chemical) analyses

Progesterone concentration in bovine milk was evaluated using competitive double-antibody enzyme immunoassays (EIA) according to the method described by Yanagawa et al.

(2015) modified to bovine milk. The primary antisera used assay were rabbit anti-progesterone-3-CMO-BSA serum (KZ-HS-P13, Cosmo Bio, Tokyo, Japan). Goat anti-rabbit IgG antiserum (111-005-003, Jackson Immuno Research, PA, USA) was used as the secondary antiserum. All samples were assayed in duplicates. The intra- and inter-assay coefficients of variation were 2.2 and 3.3%, respectively. The concentration of progesterone under 0.1 ng/mL was regarded as 0.1 ng/mL in this assay. The results obtained were used as reference (chemical) data for chemometric analyses.

6.1.4   Chemometric analyses

Chemometric analyses were carried out to develop calibration models for progesterone concentration of each cow and to validate the precision and accuracy of the models. Spectra data analysis software (The Unscrambler ver. 10.3, Camo AS, Trondheim, Norway) was used for the analyses. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. One data set was obtained for each cow from the experiment conducted using four cows. Full cross validation method was used to validate the calibration models. The data set for each cow was used to develop calibration models and the same data set was used for validation of the calibration models. The best model was obtained when we used the original milk spectra data. Thus, pretreatment techniques such as multiplicative scatter correction, 2nd derivative and smoothing were not used.

6.1.5   Progesterone concentration at one Milking time

Bucket milk progesterone concentration at one milking time was calculated by taking NIR-predicted progesterone value and milk flow rate obtained every 20 s and then compared it with the reference chemical values of bulk milk progesterone per each milking time.

6.2 Results and discussion

Near-Infrared spectra

An example of original NIR spectra of raw milk at one milking time is shown in Figure 4. The NIR spectra showed two bands peaks at around 740 nm and 840 nm indicating the overtone absorptions by C-H strings and C-C strings that are related to the distinctive absorption bands of milk constituents such as fat, protein, lactose and progesterone. There was a strong absorption peak of O-H functional group in water such that band around 960 nm were prominent spectra.
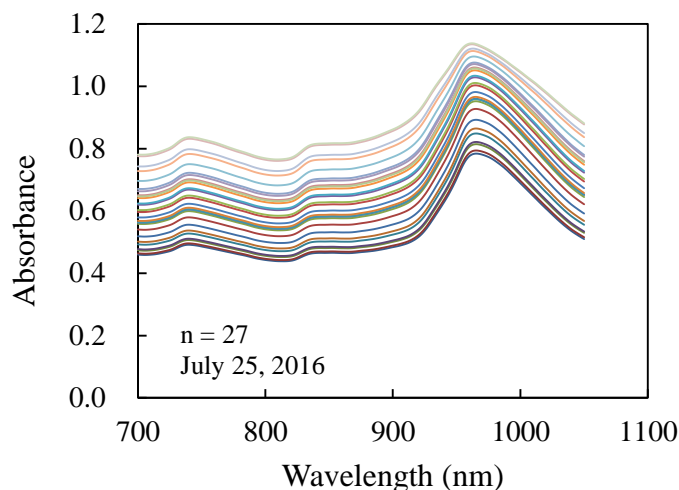
Figure 6.1 Original spectra of non-homogenized milk from cow number 1221 during milking
on July 25, 2016

6.2.1 Validation of measurement accuracy of progesterone concentration in real-time

The validation statistics of the NIR sensing system for the determination of progesterone concentration at every 20 s during milking for each of four cows are shown in Table 6.2.

Progesterone is a steroid hormone that is produced in bovine milk. It plays vital role in cow's reproductive cycle such that it prepares the uterus for pregnancy and so progesterone has been globally accepted as the ideal cow reproductive status indicator. The coefficient of determination (r^2), standard error of prediction (SEP) and bias of the validation set for progesterone for cow number 1221, 1239, 1250 and 1263 were 0.73, 1.12 ng/mL and 0.05 ng/mL; 0.62, 1.78 ng/mL and 0.02 ng/mL; 0.64, 1.64 ng/mL and -0.04 ng/mL; and 0.60, 3.05 ng/mL and -0.02 ng/mL respectively. The r^2 values obtained for each cow showed a good fit with the points lying close to the straight line and the SEP values were considered to be sufficiently low. These validation statistics indicated that this calibration models were much more accurate compared to that reported by Garnsworthy and Mann (2001). These results obtained indicate that there were sufficient levels of precision and accuracy for predicting the progesterone concentration of each cow. Thus, these values obtained by NIR sensing system for progesterone concentration indicate the ability of the calibration models to predict cow ovulation (Table 6.2).

Validation of measurement accuracy of progesterone Concentration at One Milking Time

The validation statistics of the NIR sensing system for determination of progesterone concentration at one milking time of each cow are summarized in Table 6.3. The r^2, SEP and bias of the validation set for progesterone for cow number 1221, 1239, 1250 and 1263 were 0.70, 1.35 ng/mL and -0.30 ng/mL; 0.69, 1.85 ng/mL and -0.13 ng/mL; 0.58, 1.94 ng/mL and -0.39

ng/mL and 0.70, 2.76 ng/mL and -0.38 ng/mL respectively. This validation statistics showed that these calibration models were sufficiently accurate for predicting milk progesterone concentration at one milking time.

The precision and accuracy of progesterone concentration at every 20 s during milking and at one milking time were almost the same. In other words, we were able to determine progesterone concentration at each milking time with almost the same accuracy as the predicted progesterone concentration in real-time milking. Therefore, by taking records of this predicted progesterone value at every milking time and monitoring the continuous changes in progesterone concentration, it is possible to predict each cow ovulation status and diagnose the early pregnancy of each cow.

Table 6.2 Validation statistics of near-infrared sensing system for the determination of progesterone concentration at every 20 s during milking

Cow number	n	Range (ng/mL)	r^2	SEP (ng/mL)	Bias (ng/mL)	Regression line
1221	194	0.10 - 9.07	0.73	1.12	0.05	y = 0.99 x - 0.03
1239	172	0.10 - 13.42	0.62	1.78	0.02	y = 0.97 x + 0.08
1250	212	0.10 - 11.33	0.64	1.64	-0.04	y = 0.99 x + 0.07
1263	178	0.10 - 17.74	0.60	3.05	-0.02	y = 0.96 x + 0.21

n: number of validation samples. r^2: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).

Table 6.3 Validation statistics of near-infrared sensing system for the determination of progesterone concentration at one milking time

Cow number	n	Range (ng/mL)	r^2	SEP (ng/mL)	Bias (ng/mL)	Regression line
1221	22	0.10 - 8.67	0.70	1.35	-0.30	y = 1.01 x + 0.28
1239	22	0.10 - 12.00	0.69	1.85	-0.13	y = 1.14 x - 0.38
1250	18	0.10 - 10.56	0.58	1.94	-0.39	y = 1.09 x + 0.13
1263	23	0.10 - 16.28	0.70	2.76	-0.38	y = 1.09x - 0.07

n: number of validation samples. r^2: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).

Comparison of chemical and NIR-predicted progesterone concentration trend

The calculated results by the calibration models having negative NIR-predicted values were changed to zero since progesterone values are always positive values. Figures 6.1 and 6.2 show chemical and NIR-predicted milk progesterone concentration trend for cow 1221. Figures 6.3 and 6.4 show the same for cow 1239.

The changes of (trend in) the chemical and NIR-predicted progesterone were almost the same for each cow. There were similar chemical and NIR-predicted progesterone concentration reductions on the day of ovulation (Jul. 24 and Aug. 15, 2016; Aug. 12-13, 2016) for cow number 1221 and 1239 respectively. The similarity of chemical and NIR-predicted progesterone concentration trend was good for each cow. The trend monitoring of progesterone concentration of each cow was much better than that previously reported by Garnsworthy and Mann (2001). These results suggested that NIR spectroscopic sensing system developed in this study could be used for online real-time monitoring of milk progesterone concentration of each cow during milking.

The results in this study are important because for the first time, an online real-time NIR spectroscopic sensing system could predict progesterone concentration (cow ovulation) of each cow during milking. Thus, by installing the NIR spectroscopic sensing system developed in this study into a milking robot, it could monitor milk progesterone concentration every day.
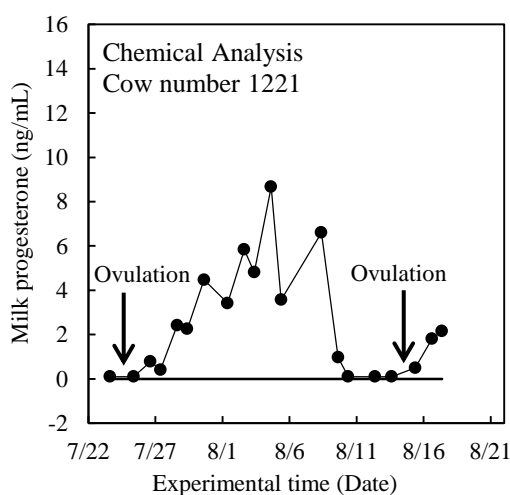
Figure 6.2 Milk progesterone trend for cow number 1221 by chemical analysis
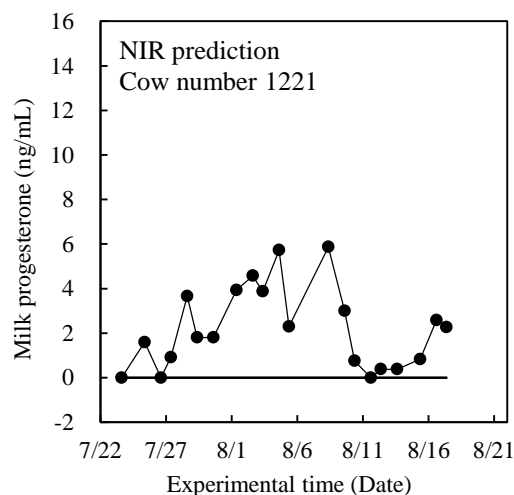
Figure 6.3 Milk progesterone trend for cow number 1221 by NIR sensing system
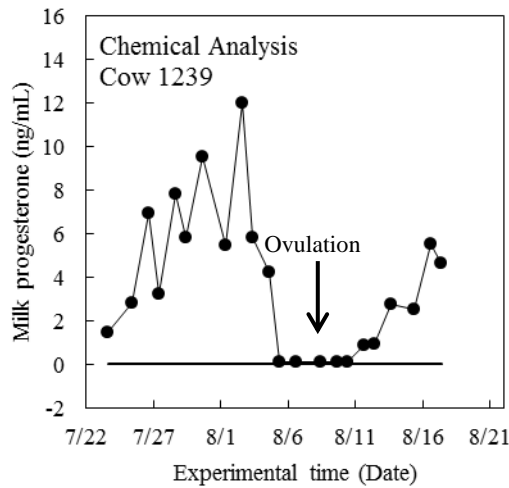
Figure 6.4 Milk progesterone trend for cow number 1239 by chemical analysis
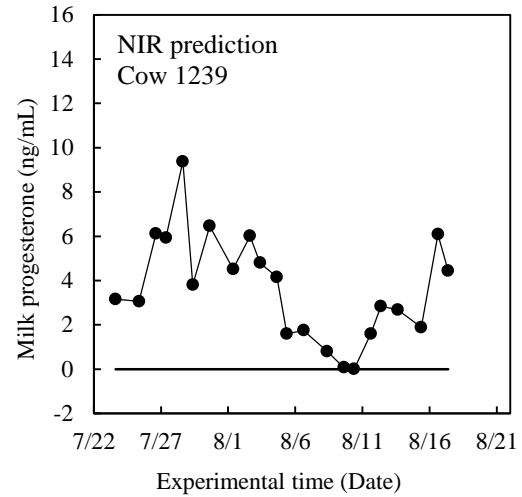
Figure 6.5 Milk progesterone trend for cow number 1239 by NIR sensing system

6.3 Conclusion

This study suggested that the NIRS sensing system we have developed has the potentials to predict milk progesterone concentration of each cow during milking. The results obtained in this study could provide screening capability for progesterone determination in bovine milk. Thus, putting the importance of both study areas into consideration, our results show the possibility for future study on the combination of milk progesterone and NIRS methodology.

7. **Chapter VII: Measurement Accuracy of Near-Infrared Spectroscopic Sensing System in a Milking Robot**

Summary

In this study, I investigated the precision and accuracy of NIRS sensing system installed into an automatic milking system for the prediction of milk quality indicators in cow milk during milking. The objective of this study was to investigate one milking time accuracy of the three major milk constituents (fat, protein, and lactose), SNF, moisture content, MUN and SCC of twenty-six Holstein cows using reference and NIR-predicted data of one milking time.

Introduction

Dairy farming involves a lot of work such as feeding, milking, livestock management, feed crop production and manure treatment. As usual, extensive dairy farmers manage their livestock in groups which is a system known as herd management (Svennersten-Sjaunja et al., 1997). However, a system known as individual cow management is essential for monitoring milk composition quality of each cow which is important for animal breeding, effective cow usage and feed management. Thus, this is the reason for the recent need for a technique that will enable dairy farmers to determine milk quality of individual cows during milking.

The non-destructive, rapid, easy to use, time saving and pre-treatment free nature of near-infrared spectroscopy (NIRS) makes it an effective tool for analyzing milk quality during milking process. NIRS has been used to obtain qualitative and quantitative information of food and agricultural commodity such as rice (Kawamura et al., 2003a; Natsuga and Kawamura, 2006), wheat (Natsuga et al., 2001), and fruits and vegetables (Lakshmi et al., 2017). NIRS has been practically used in automatic rice-quality assessment in Japan (Kawamura et al., 2002; Kawamura et al., 2003a). NIRS has also been use for milk quality determination (Sato et al., 1987; Tsenkova et al., 2001; Kawamura et al., 2003b; Tsenkova et al., 2006; Kawamura et al., 2007; Kawasaki et al., 2008; Tsenkova et al., 2009; Iweka et al., 2016). However, the application of NIRS for online real-time monitoring of milk quality of individual cow has not been achieved.

In this study, an experimental online near-infrared (NIR) spectroscopic sensing system was designed for milk quality determination. Iweka et al., (2016) reported that the NIR spectroscopic sensing system can be used for real-time determination of milk quality during milking with sufficient precision and accuracy. As a result of our findings, the NIR spectroscopic sensing system was installed in an automatic milking system.

Also, dairy farmers want to know the average milk quality of one milking but as usual, raw milk from one cow is mixed with milk from other cows after milking. In other words, cow raw milk contains information that could be used to monitor the physiological and nutritional

status of each cow but cow raw milk after milking is immediately cooled by a bulk cooler. The bulk cooler contains and mixes raw milk of other cows and for this reason, milk quality determination of each milk quality indicator produced by each cow is possible only during milking. Usually, milk whey fluctuates during milking and so to obtain milk of one milking time, the milk quality and milk yield must be measured at regular intervals during milking and calculated by taking the average of bulk milk per milking time.

7.1 Objectives

Thus, this study was centered on

1. Examining the accuracy of the NIR spectroscopic sensing system for milk quality determination in an automatic milking system.

2. Investigating the measurement accuracy of one milking time using near-infrared spectroscopic sensing system installed into an automatic milking system for milk quality determination during milking.

7.2 Materials and methods

7.2.1 Near-Infrared spectroscopic sensing system

An experimental online near-infrared (NIR) spectroscopic sensing system was designed for analyzing milk quality of each cow during milking. The system consisted of an NIR instrument (NIR spectrum sensor and NIR spectrometer), milk flow meter, milk sampler and a laptop computer (Figure. 7.1). The system was installed in a milking robot system (GEA Farm Technologies, Westfaliasurge, Germany). Non-homogenized milk from the milking robot flowed continuously across a bypass into the milk chamber of the NIR spectrum sensor. Excess raw milk flowed past the milk flow meter and was then released through a line tube into the bucket. The volume of a milk sample in the chamber was about 30 mL. The optical axes of halogen lamps A and B and the optical fiber were set at the same level, but the optical axis for halogen lamp C was set at 5 mm higher the optical fiber. The NIR instrument acquired absorbance spectra through the milk. Spectra were obtained in the wavelength range from 700 nm to 1050 nm at 1 nm intervals every 20 s during milking. The milk flow rate was simultaneously recorded in the laptop computer.
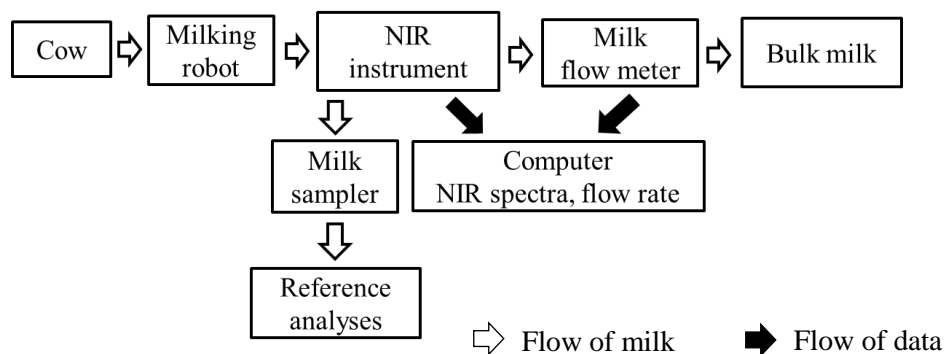
Figure 7.1 Flow chart of an on-line near-infrared spectroscopic sensing system for determining milk quality in an automatic milking system

7.2.2   Cows and milk samples

Twenty six Holstein cows belonging to a dairy farm at Tochigi Prefecture, Japan were used for this study. These cows were at their different lactation stages. The experiment was conducted throughout the whole day for two consecutive days, that is; on the 22nd and 23rd of February 2018. Milking was automatically started as soon as a cow walked into the milking robot. Milk samples were collected from the milking sampler every 20 s during milking.

7.2.3   Reference analyses

Three major milk constituents, SNF, moisture content and SCC of non-homogenized milk were measured as milk quality items in this study. The milk constituents, SNF and moisture content were determined using a MilkoScan instrument (Foss Electric, Hillerod, Denmark) and SCC was determined using Fossomatic instrument (Foss Electric, Hillerod, Denmark). The total number of samples used for reference analyses were 377 for each milk quality indicators.

Chemometric analyses

Chemometric analyses were carried out to develop calibration models for each milk quality indicator and to validate the precision and accuracy of the models. Spectra data analyses software (The Unscrambler ver. 10.3, Camo AS Trondheim, Norway) was used for the analyses. The total reference samples were used to develop calibration models. The total reference samples were randomly divided into two sample data sets such that two-third of the total sample sets were used as calibration set and the remaining (one-third) sample set as validation set. The statistical method of partial least squares (PLS) was used to develop calibration models from the absorbance spectra and reference data. The best model was obtained when we used the original spectra data thus, pretreatment techniques such as multiplicative scatter correction, 2nd derivative and smoothing was not used.

Milk quality determination at one milking time

Bucket milk at one milking time was calculated by taking NIR-predicted progesterone value and milk flow rate obtained every 20 s and then compared it with the reference values of bulk milk per each milking time.

7.3 Results and discussion

7.3.1 Precision and accuracy of calibration models in real-time

The validation statistics of the NIR spectroscopic sensing system for determination of milk quality for real-time milking and one milking time are shown in Tables 7.1 and 7.2 respectively. Figure 7.2 and 7.3 shows the correlations between reference and NIR-predicted values of SCC for real-time milking and one milking time respectively.

High precision and accuracy were indicated by the high coefficient of determination values, low standard error of prediction values and the negligible bias values (almost zero) for predicting milk constituents, SNF and moisture content. The performance of calibration models for milk fat and moisture content were excellent. The reason for the extremely high accuracy was due to the fact that NIR spectra had much information on the carbon-hydrogen strings of triacylglycerol and water molecules respectively. These results indicated that the three major milk constituents, SNF and moisture content which are the main factors of determining milk quality of individual cow can be monitored in real-time using the NIR sensing system developed in this study.

SCC is a recognized standard for mastitis diagnosis and it is a very important indicator for health and milk quality. Milk SCC can show the level of cow infection and it consequence on the mammary gland of dairy cows which is related to mastitis (Satu, 2003). Milk produced from the udder of a healthy cow contains less than 100,000 somatic cells per mL (i.e., 4logSCC/mL) while cows with subclinical mastitis produce milk containing more than 200,000 somatic cell per mL (i.e., 5.3logSCC/mL) (Satu, 2003).

The precision and accuracy for predicting SCC was sufficiently high. This means that the calibration models developed for SCC could be used for the diagnosis of subclinical mastitis, as SCC is a world standard indicator for cow disease. Thus, we can monitor individual cow physiological condition in real-time by using the NIR sensing system installed into an automatic milking system in our study.

7.3.2 Precision and accuracy of calibration models at one milking time

One milking time precision and accuracy for predicting each milk quality indicator was sufficiently high (Table 2). The different between one milking time accuracy and real-time

milking accuracy was not significant except for fat and moisture content. Nevertheless, comparing between the real-time milking results and the one milking time results, we were able to predict milk quality at one milking time with almost the same accuracy as predicted value of milk obtained every 20 seconds. This means, by recording this predicted value every milking and monitoring the continuous change of each milk quality indicator, it becomes possible to use it for predicting cow physiological condition such as the diagnosis of mastitis and nutritional condition of cow.

Dairy precision farming

The installation of NIR spectroscopic sensing system developed in our study into an automatic milking system would facilitate the monitoring of milk constituents and diagnosis of mastitis of individual cows in real-time during milking. The NIR sensing system could provide dairy farmers and veterinarians useful information on milk quality and physiological status of each cow and thus, give them assessment control for improving dairy farm management. The application of this NIR sensing system could take dairy farm management to the next level of dairy precision farming on the basis of individual cow information.

7.4 Conclusions

The NIR spectroscopic sensing system developed in this study can be used for online real-time monitoring of fat, protein, lactose and SCC during milking by an automatic milking system with sufficient precision and accuracy. By application, the NIR sensing system would enable dairy farmers to be able to produce high-quality milk and dairy precision farming will be actualized.

Recommendations

Further studies is recommended in order to improve the calibration models of each milk quality indicator which will consequently increase the precision and accuracy of the calibration models, thus bringing smart farming to realization in the nearest future.

Table 7.1 Validation statistics of near-infrared sensing system for the determination of milk quality at every 20 s during milking

Milk quality items	n1	n2	Range	r^2	SEP	Bias	RPD	Regression line
Fat (%)	252	125	0.98-8.54	0.97	0.24	-0.01	6.27	y = 1.00 x + 0.01
Protein (%)	252	125	2.76-4.46	0.75	0.24	0.00	2.01	y = 1.02 x - 0.06
Lactose (%)	252	125	3.99-4.97	0.57	0.15	0.00	1.53	y = 1.06 x - 0.27
SNF (%)	252	125	8.15-10.09	0.74	0.24	0.00	1.96	y = 1.00 x + 0.03
Moisture (%)	252	125	82.38-90.40	0.96	0.33	0.01	5.19	y = 0.99 x + 0.44
SCC (log SCC/mL)	252	125	3.70-6.47	0.64	0.45	0.01	1.66	y = 1.00 x - 0.01

n: number of validation samples. r^2: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).

Table 7.2 Validation statistics of near-infrared sensing system for the determination of milk quality at one milking time

Milk quality items	n	Range	r^2	SEP	Bias	RPD	Regression line
Fat (%)	25	1.96-5.79	0.76	0.56	-0.03	2.01	y = 0.81 x + 0.81
Protein (%)	25	2.89-4.33	0.66	0.27	-0.12	1.42	y = 0.98 x + 0.20
Lactose (%)	25	4.14-4.85	0.56	0.15	0.02	0.79	y = 1.37x - 1.71
SNF (%)	25	8.29-9.94	0.70	0.25	-0.06	1.56	y = 0.97 x + 0.32
Moisture (%)	25	84.81-89.46	0.82	0.61	0.09	2.29	y = 0.90 x + 8.43
SCC (log SCC/mL)	25	4.00-6.47	0.68	0.41	-0.10	1.28	y = 1.13 x - 0.51

n: number of validation samples. r^2: coefficient of determination. SEP: standard error of prediction. Regression line: Regression line from predicted value (x) to reference value (y).
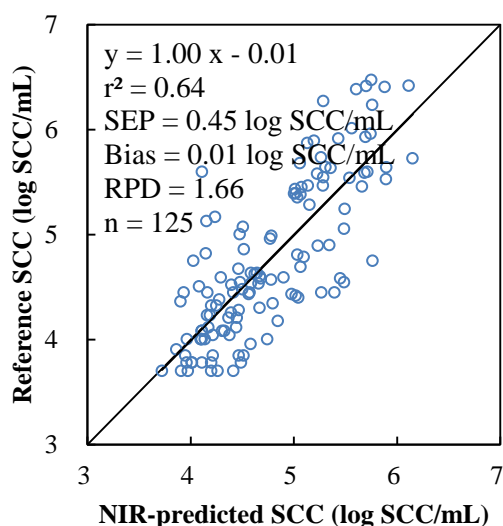

Figure 7.2 Correlation between reference SCC and NIR-predicted SCC for one milking time
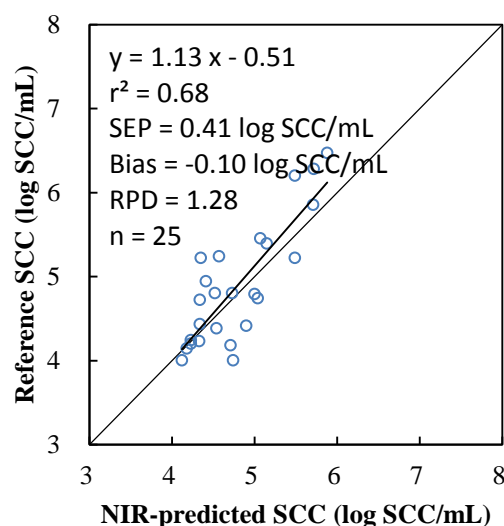
Figure 7.2 Correlation between reference SCC and NIR-predicted SCC for real-time milking

8.   Chapter VIII: General Conclusions and Recommendations

In this study, online real-time NIR spectroscopic sensing system was developed for milk quality determination during milking. This study has provided useful information for predicting milk quality indicators such as fat, protein, lactose, SNF, moisture content, MUN and SCC of each cow during milking.

The investigation on the factors affecting the performance of calibration models provided useful information for designing more efficient technologies for online real-time milking using NIR spectroscopic sensing system. The information could contribute to the development of transfer of technology approach to reduce individual cow health problems such as mastitis through early detection of this disease. This information can help to provide preventive measures for improving cows` hygiene and milking routine. Globally, this would aid improving the quality and quantity of milk production where milk and milk products are important source of food and income.

The potentials of the NIR spectroscopic sensing system for the determination of progesterone concentration were examined. Based on the results obtained, NIR technology developed on this study could be used to monitor each cow ovulation status during milking.

The installation of NIR spectroscopic sensing system developed in our study into an automatic milking system would facilitate the monitoring of milk constituents and diagnosis of mastitis of individual cows in real-time during milking.

The main empirical findings, how these empirical findings respond to the research goal and the conclusions have been summarized within the respective sections in Chapter IV, V, VI and VII.

Conclusively, the information obtained in this study could be useful for designing NIR spectroscopic sensing system required in the milk industry, such as obtaining a non-destructive, time saving, quick analysis and reliability of determinations of the quality of non-homogenized milk during milking. It could also be useful for improving the performance of the calibration models developed for each milk constituents, thus helping to increase the efficiency of the NIR spectroscopic sensing system for online real-time milk quality determination during milking. The NIR spectroscopic sensing system developed in this study could be used for monitoring milk constituents such as fat, protein and lactose, MUN and SCC during milking with sufficient precision and accuracy. It could also be used to detect mastitis disease in cows and for assessing the physiological condition of each cow in real-time during milking. This system can give livestock farmers' feedback control for upgrading dairy farm management such as overcoming

the difficulty in individual cow management and consequently enable them to produce high-quality milk. Thus, the system will enable realization of precision dairy farming.

Outstanding headway has been made in the milk industry since studies were first made into using NIR spectroscopy for milk quality determination, but there is still a need for further studies in order to achieve a practical application method to determine the milk quality of each cow during milking.

Recommendations

Therefore, it is recommended that further studies should be carried out on other factors affecting the accuracy of calibration models in order to cover the data set variations caused by these factors. These factors include cow individuality, milking season, calving times, lactation stage, and feeding stage as this would help to improve the performance of calibration models of each milk quality indicator. The kind of information obtained from these further studies would help in improving the precision and accuracy of the calibration models developed which will consequently help in the designing of NIR spectroscopic sensing system for milk quality evaluation during milking and precision farming will be realized.

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DEVELOPMENT OF NEAR-INFRARED SPECTROSCOPIC SENSING SYSTEM

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(搾乳時乳質のオンラインリアルタイム評価のための

近赤外分光センシングシステムの開発)

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