Disease diagnosed and described by near infrared spectroscopy

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Introduction

A mammary gland is made up of tissue, which has the capability of producing a large volume of secretion, i.e. milk, under normal and healthy conditions. When bacteria enter the gland and establish an infection or inflammation it initiates an udder disease called mastitis.[1] It is accompanied by an influx of white cells from the blood stream into the milk, altered secretory function and changes in the volume and composition of secretion. Cell counts in milk are closely associated with inflammation and udder health. Somatic cell count (SCC) has been accepted as the international standard measurement of milk quality in dairy and for mastitis diagnosis.[2] In previous studies near infrared (NIR) spectroscopy has been successfully applied for non-invasive mastitis diagnosis performed by qualitative raw milk spectral analysis[3,4] when an expert's diagnosis has been used as a reference.

The purpose of this investigation was to evaluate the accuracy of mastitis diagnosis based only on near infrared spectra of milk when somatic cell count (SCC) has been used as a standard reference method. Two-dimensional correlation spectroscopy (2-DCOS) was applied for further understanding of the disease on a molecular level.

Materials and methods

Samples

A total of 189 composite milk samples from seven Holstein cows were analysed. The samples were collected for 28 days, consecutively, beginning on 7th day after calving. All cows were fed the same rations, twice daily and always had access to drinking water. The average BW of the cows was 552 kg.

Each milk sample was divided into two subsamples. One was subjected to spectral analysis and the other was analysed for SCC by using the fluoro-opto-electronic method using a Fossomatic 400 (Foss-Electric A/C, Hillerød, Denmark). Somatic cell count standards were used to calibrate the Foss instrument throughout the study. The repeatability coefficients of variation of this method are 4 to 5% for the region between 400,000 and 500,000 cells mL^{-1}and 5 to 10% for the region between 100,000 and 200,000 cells mL^{-1} and over 500,000 cells mL^{-1} (IDF Standard 148A, 1995).[5] Log_{10}SCC was calculated to normalise the SCC distribution. Samples were also analysed for fat, total protein and lactose content[1] by Milko Scan (Foss-Electric A/S, Hillerød, Denmark). Three of the examined cows were healthy, with SCC lower than 137,000 cells mL^{-1}. One cow was mastitic during the entire experimental period. Her measured SCC varied from 204,000 to 11,876,000 cells mL^{-1}. Three cows had mastitic and healthy periods (SCC was between 80,000 and 4,737,000 cells mL^{-1}).

NIR spectra

Near infrared transflectance (T) spectra were obtained using an the InfraAlyzer 500 spectrophotometer, (Bran+Luebbe, Nordestedt, Germany), in terms of optical density log (1/*T*) in a

Near Infrared Spectroscopy: Proceedings of the 10th International Conference

wavelength range from 1100 to 2500 nm. A flow cell with pathlength of 0.2 mm, connected with automated liquid sampling system and taking alternatively milk samples and cleaning solution, was used. Before the spectral analysis, each sample was warmed up to 40°C in a water bath with a temperature control of ± 0.1°C. During the analysis, the same temperature was controlled through the use of an integrated water-jacketed holder of the flow cell connected to the water bath.

NIR analysis

A commercial software program (Pirouette Version 2.6, Infometrics, Inc., Woodinville, WA, USA) was used to process spectral data.

To develop a regression equation for logSCC, spectra were randomly divided into a set of 128 calibration samples and a set of 68 validation samples. Both data sets covered similar ranges of each investigated parameter.

Methods used for preliminary examination of the data included smoothing the spectral data, multiplicative scatter correction, standard normal variance correction, baseline correction and first or second derivative transformation of log (1/*T*) data. The smoothing and derivative transformations were based on the Savitzki–Golay second-order polynomial filter.[6]

Calibration for SCC was performed by partial least square (PLS) regression using the calibration set of samples. Calibration and validation statistics for each regression model included standard error of calibration, coefficient of multiple correlation, standard error of prediction, correlation coefficient between measured and NIR-predicted values. Statistical parameters were used to evaluate the accuracy of NIR for SCC determination.

Classification of milk samples in class "healthy" or class "mastitic" was performed using soft independent modeling of class analogy (SIMCA) and various spectral data pretreatments. Two levels of SCC—200,000 cells mL^{-1} and 300,000 cells mL^{-1}, respectively, were used and compared as thresholds to discriminate between healthy and mastitic cows.

Two-dimensional correlation (2-DCOS) analysis of NIR milk spectra was done to assess the changes in milk composition, which occur simultaneously with the variation of SCC.

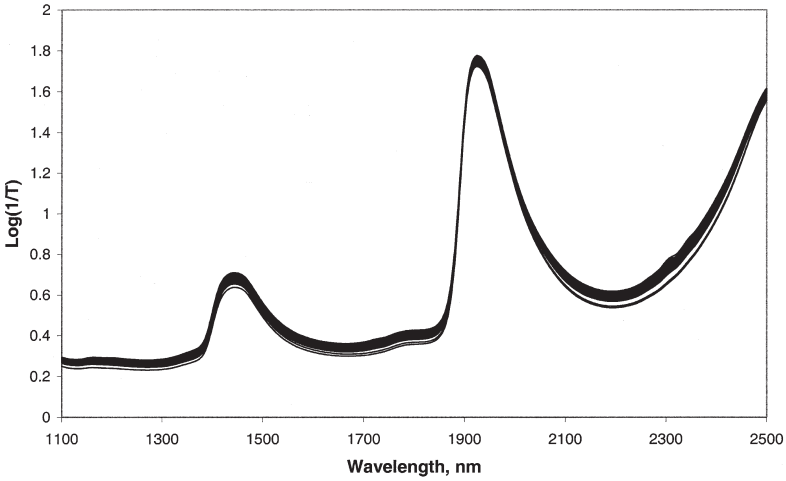

Figure 1. NIR milk spectra, 1 mm cell.

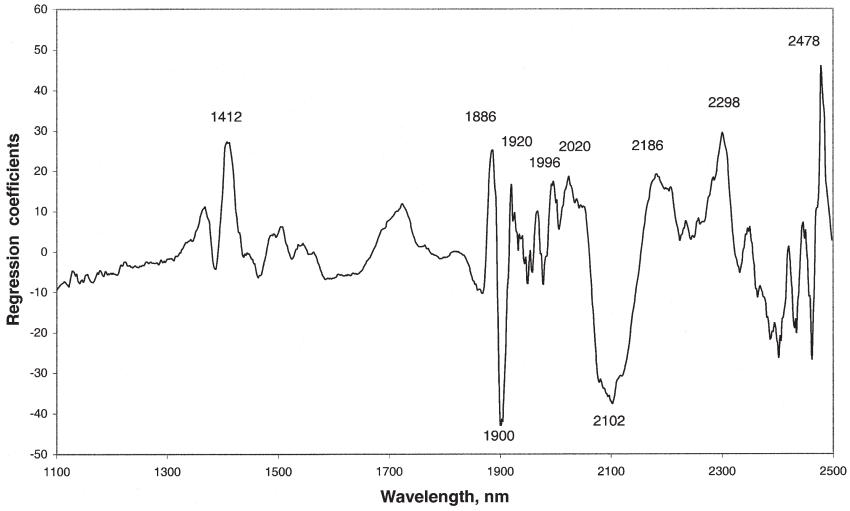

Figure 2. Regression vector for determination of logSCC based on milk spectra.

Results and discussion

A PLS model for prediction of logSCC, based on NIR milk spectra (Figure 1), was developed. The best accuracy of determination for the 1100–2500 nm range was found using smoothed absorbance data and 10 PLS factors. The standard error of prediction for independent validation set of samples was 0.382, correlation coefficient—0.854 and the variation coefficient—7.63%. It has been found that SCC determination by NIR milk spectra was indirect and based on the related changes in milk composition. From the spectral changes and the regression vector (Figure 2), we learned that when mastitis occurred, the most significant factors that simultaneously influenced milk spectra were alteration of milk proteins seen around 2100 nm and changes in ionic concentration of milk that appeared around 1412 nm and 1900 nm. This result was consistent with the milk compositional changes observed in mastitic milk[7] and the results we obtained further when 2-D correlation spectroscopy was applied (Figure 3).

Different thresholds for SCC were set up and SIMCA classification performed on milk spectra was used to find out the most appropriate one to be used in further mastitis diagnosis based on NIR spectra of milk. Two levels of SCC—200,000 cells mL^{-1} and 300,000 cells mL^{-1}, respectively—were set up and compared as thresholds to discriminate between healthy and mastitic cows. The best detection accuracy was found with 200,000 cells mL^{-1} as the threshold for mastitis and smoothed absorbance data:—98% of the milk samples in the calibration set and 87% of the samples in the independent test set were correctly classified (Table 1). When the spectral information was studied it was found that the successful mastitis diagnosis was based on reviling the spectral changes related to the corresponding changes in milk composition.

Two-dimensional correlation analysis of NIR milk spectra was done to assess the changes in milk composition, which occur when somatic cell count (SCC) levels increase with mastitis. The synchronous correlation map revealed that when SCC increases, protein levels increase, while water and lactose levels decrease. Results from the analysis of the asynchronous plot (Figure 3) indicated that changes in water and fat absorption occur before the changes with other milk components. In addition, the same technique was used to assess the changes in milk spectra during a period of time when SCC

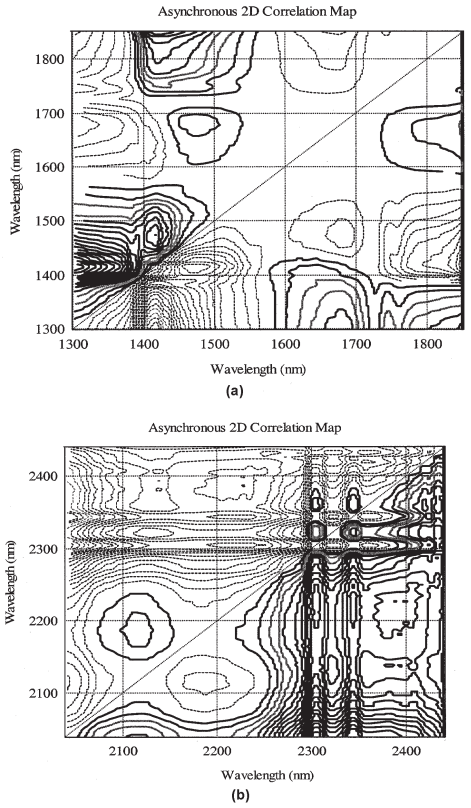

Figure 3. (a) 1300–1900 nm 2-D synchronous and (b) 2000–2450 nm asynchronous correlation map constructed from milk spectra.

levels did not have substantial variations. Results indicated that milk components were in equilibrium and no appreciable change in a given component was observed with respect to another. This was found in both healthy and mastitic animals. However, milk components were found to vary with SCC content regardless of its range. This important finding demonstrates that 2-D correlation analysis may be used to track even subtle changes in milk composition of individual cows, as well as to explain these changes on a molecular level.

Conclusion

NIR spectra of milk subjected to different ways of data mining can provide a fast and accurate alter-

Table 1. SIMCA classification results based on milk near-infrared spectra and two different somatic cell count levels used as thresholds for mastitis diagnosis.

		Calibration set			Validation set		
Threshold	PC factors	Correct classification		Incorrect classification	Correct classification		Incorrect Classification
SCC (cells mL^{-1})	Cal set/val set	*n*	%	kind	*n*	%	Kind
200,000 cells mL^{-1}	11/11	124	98	2 false positive	55	87	4 false negative 2 false positive 2 non classified
300,000 cells mL^{-1}	11/11	117	93	7 false negative 2 false positive	49	78	8 false negative 4 false positive 2 non classified

native to current methods for mastitis diagnosis and a new insight into understanding the disease at a molecular level.

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