Possibility of using near infrared spectroscopy for prediction of bacterial contamination in raw milk

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ABSTRACT: The possibility of using near infrared spectroscopy (NIRS) to determine the amount of bacterial contamination in raw milk was investigated. The transmission mode of NIR measurements in the short wavelength region from 700 nm to 1100 nm were done using two types of test tubes (external diameter: 16 mm and 20 mm) as sample cells. To improve efficiency of bacterial determination, samples were also modified using dilution and filtration techniques for reducing colloidal particles presented in raw milk. The spectra of raw milk in the bigger test tube (20 mm) could provide better calibration results compared with that in a smaller test tube (16 mm). The attempts in reducing colloidal particles by either dilution or filtration could not success in approving the system precision. The calibration results from spectra of those treated samples showed poorer results than the results of raw milk. It is suggested that, nondestructively, spectra of raw milk samples carried in 20(2)-mm test tubes could provide high efficiency of the NIR-log Total Bacterial Count determination.

Keywords: NIR, raw milk, bacteria, milk quality, safety

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Introduction

Milk is an important part of many people's diet for a long time. As its high nutritive value, it is a favorable growth medium for numerous microorganisms, primarily concerns bacteria, and then susceptible to be contaminated. Evaluation of bacterial amount contaminated in raw milk is one of the important procedures required by the World Health Organization. Presently, the culture method requires 48 hours that is time-consuming. For a decade, near infrared spectroscopy (NIRS) has been widely used in quality evaluation of feed and food products, because of its advantages as a rapidity, nondestructive measurement as well as a great potential for on-line analysis (Williams and Norris, 2001). In this study, we aimed to find the possibility of using NIRS for the bacteria measurement in order to replace the conventional analysis. Due to the low concentration of bacteria and high scattering condition of raw milk, proper pathlength and sample modification techniques were examined.

Materials and Methods

Samples: Raw milk samples collected from dairy cows were used. The milk samples were divided into the test tubes and the samples were separated and incubated at 32°C for 0, 3, 4.5, 6, 9 and 12 hours in a shaking water bath to obtain various levels of bacterial amount. For NIR measurement, raw milk was diluted 10 times with the sterilized 0.85% saline solution and then filtered bacteria and big particles with the sterilized syringe filters (cellulose acetate membrane; pore size = 0.45 µm). Filters filtered were dried in oven vacuum at 45°C for 3 hours and kept in a desiccator.

Sample preparation: This work consists of 3 sample series which sample conditions were gradually modified to be suitable for NIR analysis. During the NIR measurement, raw milk samples, the 1st series, were kept in small test tubes [16(Ø) x 105(h)-mm] and big test tubes [20(Ø) x 125(h)-mm], hereafter called RM+S and RM+B, respectively. The 2nd set, to reduce scattering materials, ten-fold diluted milk solution kept in big test tubes, filter filtered before (WF) and after drying (DF) were used for the measurement. The 3rd set, to increase pathlength, raw milk, diluted milk solution and filtered solution kept in big test tubes; hereafter to be called RM+B, DM+B and FM+B, respectively were measured. Prior the measurement, sample temperature was controlled by dipping the samples into a water bath at 25°C for exactly 20 minutes except the dried filters kept in desiccator were moved to storage in the instrument laboratory (25°C ambient) for 1 hour. Statistical characteristics of calibration and validation sets are shown in Table 1.

Spectral acquisition: NIRS6500 with a fibre optic “Transmittance Probe” (FOSS NIRSystem, Silversprings, USA) was used to measure NIR spectra in the short wavelength from 700-1100 nm. Two sizes of Pyrex-glass test tube as described in sample preparation used as sample cells were measured with a suitable size of aluminium block test tube holder. A 3.0-mm thick ceramic plate was measured as a reference for solution samples but filtered solution samples. Filter samples were placed in sample chamber for the measurement and a 1.0-mm was measured as a reference.
Unscrambler® program (CAMO, Oslo, Norway) was used to derive calibration equations.

**Results and Discussion**

**Effect of pathlength:** Second derivative spectra of raw milk kept in the small tubes (RM+S) and the big tubes (RM+B) are shown in Figure 1. Baseline shift in original spectra due to the variations in light scattering levels of milk could be significant reduced by second derivative pretreatment. However, the water peak at 966 nm of the RM+B samples still had higher intensity compared with the RM+S as the number of water molecules on the light path of the RM+B
was higher. PLS calibration results for log Total Bacterial Count (TBC) are shown in Table 2. Satisfied results could be obtained only with the RM+B samples \([R = 0.93, SEP = 0.71 \log (\text{CFU/ml})]\), while only poor calibration \([R = 0.62, SEP = 1.05 \log (\text{CFU/ml})]\) could be obtained for the RM+S. The failure of RM+S was believed to be caused by the insufficient amount of optical information presented in the light path. Scatter plots between actual and predicted log TBC values of the RM+B validation set is shown in Figure 2. Regression coefficient plot of this calibration was quite complex, indicating that there were many factors, such as lactic acid, lactose and fat, involved in the model (Figure 3).

**Figure 1** Second derivative \([d^2 \log(1/R)]\) spectra of raw milk samples kept in the small tubes (RM+S) and the big tubes (RM+B).

**Figure 2** Scatter plots for validation sets of actual TBC vs. NIR-predicted TBC developed from the RM+M spectra.
Effect of scattering materials: The NIR spectra of RM+S were studied as a preliminary condition for bacterial determination but could not provide good results. It might be due to the severe scattering effect from a lot of fat globules (Chen et al., 2002). To reduce the effect of scattering materials, RM+B were modified to DM+B, WF and DF but they were not suitable for the NIR measurement. None of the methods could provide superior results over that of the raw milk (RM+B). Water seems to play a vital role in this determination as strong coefficient could be noticed at 766 nm and the relationship between spectra of filter samples and bacterial amount could hardly be harvested (R = 0.30 for WF and R = 0.34 for DF). The 980-nm peak of coefficient plots might be related with lactose corresponding to the peaks observed by Tsenkova et al. (1999) at 734, 750, 786, 812, 908, 974, 982 and 1064 nm (Figure 3). The band assignments of other strong peaks were difficult as the actual concentration of bacteria in milk was very small.

Conclusions

By the NIR measuring system developed, it became possible to predict the amount of bacteria contaminated in raw milk. Therefore, there was possibility to monitor quality and safety of raw milk in dairy farms. Nondestructively, spectra of raw milk samples carried in big test tubes could provide high efficiency of the NIR-log Total Bacterial Count determination.

References

