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Identification of Mastitis Pathogens in Rabbit Milk by Near Infrared Spectroscopy and SIMCA Classification Method

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Abstract. The purpose of this study was to investigate the potential of near infrared spectroscopy combined with multivariate analysis for determination of bacterial species, caused mastitis infection, based on NIR spectra of milk. Experimental mastitis was induced in 12 milking rabbits. The rabbits were infected with Staphylococcus aureus and E. coli bacteria, isolated from mastitis cows by injection of 0.5 ml of bacterial suspension with different concentration into the base of the teats. After the inoculation of bacterial strains into the teats, the infected rabbit milk was collected at different time intervals – 24, 48, 72, 96, 120 and 144 hours. Spectra of diluted milk were collected using a USB4000 visible-near-infrared spectrometer (OceanOptics, USA) over the wavelength range 450–1100 nm using transmission through a 10 mm quartz cuvette. The instrument was first set up with healthy milk as a reference. Spectra of 37 milk samples from rabbits, contaminated with Escherichia coli, and 28 milk samples from rabbits, contaminated with Staphylococcus aureus, were used in the investigation. Soft Independent Modeling of Class Analogy (SIMCA) was implemented to create models for discrimination of milk according to bacterial infection. SIMCA models correctly classified from 81.08 to 100% of milk samples from rabbits, infected with Escherichia coli, and from 89.28 to 100% of samples from rabbits, infected with Staphylococcus aureus, depending on used spectral region and spectral data transformation. Models, based on spectral region from 456 to 960 nm allowed 100% correct identification all samples. The information of SIMCA models was used for investigation of spectral information, related to presence and action of Escherichia coli and Staphylococcus aureus bacteria in milk. The most important spectral region for detection of Escherichia coli infection was found to be 720–750 nm, and for Staphylococcus aureus infection – from 920 to 960 nm, respectively. The results demonstrated that near infrared spectroscopy in combination with multivariate chemometrics technique offers an alternative approach to traditional methods with large potentials for a rapid and reliable identification in microbiology and biodiagnostics.

Keywords: near-infrared spectroscopy, milk, bacteria


Introduction

Food quality and safety, as well as bacteria identification, have become very important issues. The quality of processed milk and milk products are influenced by the quality of the raw milk and animal health. Mastitis (intramammary infection) is a major problem for the global dairy industry and causes substantial economic losses from decreasing milk production, considerable compositional alteration in milk, reducing milk quality, medical treatment and labour costs, and increasing risk of early culling of the cows (Harmon, 1994). Current methods used for diagnosis, as bacteriology examination, somatic cell count determination etc. are destructive, expensive, time and labor consuming. Fast and non-destructive methods for monitoring and control are highly desirable in the dairy industry.

Near-infrared spectroscopy (NIRS) has proved to be an efficient and advanced tool for analysis of different agricultural products and food processed food. (Williams and Norris, 2001; Woodcock et al., 2008). The utility of this technique arises mainly from its ability to perform fast, accurate, nondestructive and simultaneous measurements of chemical components in complex sample matrices. Additionally, it can provide information about structural and physical properties of biological materials.

NIR spectroscopy studies of milk are connected with analysis of milk constituents (fat, protein, lactose) for the purpose of obtaining information of milk composition, and analysis of attributed such as somatic cell count which may be used for disease detection, mainly mastitis (Tsenkova et al., 1998; Tsenkova et al., 2001; Pravdova et al., 2001; Kawamura et al., 2007). Somatic cell count is a recognized indicator of mastitis, but not showed specific mastitis-causing bacterial pathogens. Saranwong and Kawano (2008) reported fairly precise level of determination of amount of aerobic bacteria in terms of TBC by NIR analysis of raw milk. Tsenkova et al. (2006) reported first results for NIR spectroscopy identification of Staphylococcus aureus, Coagulase negative staphylococcus and Streptococcus bacteria in quarter cow milk in small-scale experiment.

The aim of this study was to investigate the potential of near infrared spectroscopy in short-wave near-infrared region combined with multivariate analysis for determination of bacterial species, caused mastitis infection, based on NIR spectra of milk.

Material and methods

Experimental mastitis was induced in 12 milking rabbits. The rabbits were infected with Staphylococcus aureus and E.coli bacteria, isolated from mastitis cows by injection of 0.5 ml of bacterial suspension with different concentration (10^7, 10^8, 10^9 and 10^10 cfu/ml) in each pair of teats. After the inoculation of bacterial strains into the teats, the infected rabbit milk was collected at

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* e-mail: atanassova@uni-sz.bg
different time intervals – 24, 48, 72, 96, 120 and 144 hours. Spectra of diluted milk were collected using a USB4000 visible near-infrared spectrometer (OceanOptics, USA) over the wavelength range 450-1100nm using transmission through 10mm cuvette. The instrument was first set up with healthy milk as reference. Spectra of 37 milk samples from rabbits, contaminated with Escherichia coli, and 28 milk samples from rabbits, contaminated with Staphylococcus aureus, were used in the investigation. Milk samples were grouped into two classes as follow: milk from rabbits infected with Staphylococcus Aureus (class Staphylococcus), milk from rabbits infected with Escherichia coli, (class E.coli).

A commercial program Pirouette Version 2.02 (Infometrics, Inc., Woodinville, WA, USA) was used for qualitative analysis, i.e. classification of samples. Soft Independent Modeling of Class Analogy (SIMCA) was implemented to create models of the respective classes based on milk spectra. SIMCA develops models for each class based on factor analysis, i.e. principal components (PC) that describe the variations of the spectral data. In this work, models to describe bacterial species were developed for various wavelength ranges and using raw, first-derivative, and second-derivative spectra. The derivative transformations were based on the Savitzky-Golay second-order polynomial filter (Savitzky Golay, 1964).

Results and discussion

SIMCA models for discrimination of milk from infected with Staphylococcus Aureus or Escherichia coli rabbits were developed for different spectral regions and spectral data transformations. Results of SIMCA models for spectral region from 456 to 960nm, based on smoothed raw data, first or second derivative spectra transformation, were presented in Table 1. Correct classification of all samples was obtained with models, based on first and second derivative data transformation. Models, based on smoothed raw data classified correct all samples from class E.coli and 92.86% of samples from class Staphylococcus. The interclass distance represents the distance between the classes in SIMCA models.

Table 1. Results of SIMCA classification of tested milk samples, spectral region 456-960nm.

<table>
<thead>
<tr>
<th>Spectral data transformation</th>
<th>Principal components in SIMCA models</th>
<th>Interclass distance</th>
<th>Classification class E.coli (n=37) % correct classification</th>
<th>Classification class Staphylococcus (n=28) % correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth</td>
<td>10,10</td>
<td>2.73</td>
<td>100%</td>
<td>92.86% 2 – determined as class E.coli</td>
</tr>
<tr>
<td>First derivative</td>
<td>10,10</td>
<td>2.64</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Second derivative</td>
<td>10,10</td>
<td>2.91</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2. Results of SIMCA classification of tested milk samples, spectral region 540-805 and 920-960nm.

<table>
<thead>
<tr>
<th>Spectral data transformation</th>
<th>Principal components in SIMCA models</th>
<th>Interclass distance</th>
<th>Classification class E.coli (n=37) % correct classification</th>
<th>Classification class Staphylococcus (n=28) % correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth</td>
<td>10,10</td>
<td>3.22</td>
<td>100%</td>
<td>92.86% 2 – determined as class E.coli</td>
</tr>
<tr>
<td>First derivative</td>
<td>10,10</td>
<td>4.70</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Second derivative</td>
<td>10,9</td>
<td>5.43</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3. Results of SIMCA classification of tested milk samples, spectral region 720-750nm.

<table>
<thead>
<tr>
<th>Spectral data transformation</th>
<th>Principal components in SIMCA models</th>
<th>Interclass distance</th>
<th>Classification class E.coli (n=37) % correct classification</th>
<th>Classification class Staphylococcus (n=28) % correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth</td>
<td>10,10</td>
<td>15,06</td>
<td>100%</td>
<td>92.86%, 2 – determined as class E.coli</td>
</tr>
<tr>
<td>First derivative</td>
<td>10,10</td>
<td>9.45</td>
<td>100%</td>
<td>96.43%, 1- determined as class E.coli</td>
</tr>
<tr>
<td>Second derivative</td>
<td>10,9</td>
<td>13.25</td>
<td>100%</td>
<td>89.28%, 3 – determined as class E.coli</td>
</tr>
</tbody>
</table>

Greater the distance between two classes the greater is the difference in composition of samples belonging to those clusters. As a rule of thumb, a distance of over 3 indicates that the samples are well separated. Parameter “Interclass distance” from SIMCA procedure between class Staphylococcus and class E.coli was from 2.64 and 2.91, which showed good separated classes. Graphical presentation of obtained result was presented in Figure 1.

The information of SIMCA models was used for investigation of spectral information, related to presence and action of bacteria in milk. The most important spectral information for discrimination between classes were found in the range 544-546nm, 721-728nm, 740-743nm, 920-922nm, and 953-958nm. SIMCA models for discrimination of milk were developed using some of above important spectral regions. Results of SIMCA models for spectral region from 540 to 805 nm combined with region from 920 to 960nm; spectral region from 720 to 750nm; and from 920 to 960nm, were presented in table 2-4, respectively.

When spectral data from 805 to 920nm were excluded from
data set, the accuracy of SIMCA models remained the same as for models for whole spectral region from 456 to 960nm (Table 2). At the same time the parameter “interclass distance” for SIMCA models was bigger – from 3.22 to 5.43. Obtained high values of parameter “interclass distance” showed well separated classes. These results confirming assumption that spectral information, connected with infection and presence of bacteria in milk, were not distributed uniformly in the visible and near-infrared spectra, and exclusion of irrelevant information improved discrimination between classes. Results of SIMCA models, obtained using very short spectral region –30 or 40nm, supported these findings.

Using a short spectral region from 720 to 750nm in SIMCA models allowed correct classification of all samples from class E.coli and from 89.28% to 96.43% of samples from class Staphylococcus (Table 3). Opposite results were found for spectral region from 920 to 960nm. SIMCA models, based on spectral information in that region, correct classified all samples from class Staphylococcus and from 81.08 to 86.65% of samples from class E.coli (Table 4). Parameter “interclass distance” from SIMCA procedures was bigger than 4.4 for both spectral regions. Therefore milk spectra in spectral region from 720 to 750nm contained information, important for detection of E.coli bacteria infection, while the spectral region from 920 to 960nm is significant for detection of Staphylococcus aureus bacteria infection.

Differences in important spectral regions for detection of

<table>
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<th>Principal components in SIMCA models</th>
<th>Interclass distance</th>
<th>Classification class E.coli (n=37) % correct classification</th>
<th>Classification class Staphylococcus (n=28) % correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth</td>
<td>10,10</td>
<td>4,41</td>
<td>81.08%, 6-determined as class staphylococcus, 1- nonclassified</td>
<td>100%</td>
</tr>
<tr>
<td>First derivative</td>
<td>10,10</td>
<td>4,46</td>
<td>83.78%, 6- determined as class staphylococcus</td>
<td>100%</td>
</tr>
<tr>
<td>Second derivative</td>
<td>10,10</td>
<td>6,75</td>
<td>86.65%, 5- determined as class staphylococcus</td>
<td>100%</td>
</tr>
</tbody>
</table>
infection caused from two investigated bacteria could be connected with differences in bacteria metabolism, produced toxins and shape of bacterial cells. For example S. aureus bacteria are spherical and create agglomerations; therefore significant scatter effect is expected in the spectra. E. coli is rod-shaped bacterium in contrast to S.aureus. E.coli is an intra-cellular toxin producer (toxins are released into the environment only after cell destruction); while S. aureus is an extra-cellular toxin producer (toxins are released into the environment as they are produced). E.coli fermented lactose. S. aureus bacteria produced both lipases and proteases. Most strains of S.aureus digest casein, breaking it down to peptides and amino acids.

Importance of spectral regions 720 - 750nm and 920 to 960nm for detection of bacteria in different products were reported by several authors. Saranwong and Kawano (2008) pointed significance of milk absorption in the region 912-1000 nm for determination of total bacteria count in milk. Similarly, Al-Qadiri et al. (2007) reported apparent spectral variations at 744 and 960 nm in spectra of milk samples, stored at 37°C for 30 h due to the high degree of spoilage, and assigned spectral variations at those wavelengths to absorption of water OH groups. Suthiluk et al. (2008) investigated possibility of NIRS for measurement of bacterial contamination in shredded cabbage. The biggest coefficients in the regression vector for determination of bacteria amount were found at 924 and 952nm.

The absorption at 740 and 960nm might be assigned with vibration of O-H group of water; and at 747 and 930nm with absorption of C-H group (Workman and Weyer, 2008). Sasic and Ozaki (2001) assigned absorption at 960nm in milk with second overtone of O-H stretching of water or water interacting with protein. Wu et al. (2008) proposed wavelength assignment of milk power spectra in the 800-1050nm region. Absorption at 920, 945 and 958 were connected with lactose, and at 949nm with protein in milk.

Observed spectral regions important in creation of SIMCA models showed that differentiation between milk, infected with Staphylococcus aureus or E.coli bacteria was based mainly on alternation of a milk chemical composition. The main observed changes could be connected with alteration of ions and lactose concentration and influence of these changes on water bonds, as well as changes in protein fractions of the milk from bacteria.

Conclusion

Near infrared spectroscopy in combination with multivariate chemometrics technique - soft independent modelling of class analogy (SIMCA) offers an alternative approach to traditional methods with large potentials for a rapid and reliable identification in microbiology and biodiagnostics. It was possible to establish SIMCA models for identification of raw milk samples, infected with S.aureus or E.coli/bacteria. The obtained results indicated that NIRS could be used for development of a new method for detection of bacteria, casing mastitis in cows.

Acknowledgements

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References


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