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Evaluation of a novel chemical sensor system to detect clinical mastitis in bovine milk

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Abstract

Automatic detection of clinical mastitis is an essential part of high performance and robotic milking. Currently available technology (conductivity monitoring) is unable to achieve acceptable specificity or sensitivity of detection of clinical mastitis or other clinical diseases. Arrays of sensors with high cross-sensitivity have been successfully applied for recognition and quantitative analysis of other multicomponent liquids. An experiment was conducted to determine whether a multisensor system ("electronic tongue") based on an array of chemical sensors and suitable data processing could be used to discriminate between milk secretions from infected and healthy glands. Measurements were made with a multisensor system of milk samples from two different farms in two experiments. A total of 67 samples of milk from both mastitic and healthy glands were in two sets. It was demonstrated that the multisensor system could distinguish between control and clinically mastitic milk samples ($p = 0.05$). The sensitivity and specificity of the sensor system (93 and 96% correspondingly) showed an improvement over conductivity (56 and 82% correspondingly). The multisensor system offers a novel method of improving mastitis detection.

Keywords: Mastitis; Milk; Electronic tongue; Conductivity; Potentiometric chemical sensors

1. Introduction

Bovine mastitis is the inflammation of the bovine mammary gland caused by pathogen infection. The bovine udder consists of four mammary glands, or quarters; each quarter may contract mastitis independently of the others. This disease is one of the largest production concerns in the dairy industry worldwide. The cost of mastitis to the dairy industry is associated with lost yield, discarded milk, cost of veterinarian treatment, herdsman's time, extended calving intervals and extra services per conception, and culling (Miller et al., 1993). Mastitic milk contains both pathogens and bacterial toxins and its consumption may directly or indirectly increase the risk of ingestion and transmission of foodborne pathogens and ingestion of potentially harmful toxins (Oliver et al., 2003). Thus, the detection of clinical infectious disease is specified by hygiene regulations in most developed countries as a legal requirement for milk for human consumption. In robotic milking systems, mastitis detection is currently done by a combination of human inspection of animals, by electrodes in the milking system to detect changes in the conductivity of milk (electrical conductivity), and by analysis of data in herd management software to detect changes in milk yield and other factors (Hogeveen and Meijering, 2000). However, there is no evidence to suggest that such systems that attempt to dispense with all human inspection will detect all infectious diseases that are implied by the EU directives 92/46 (Anon, 1992) particularly mastitis, while there is evidence to suggest that it is not possible to detect all types and degrees of mastitis by changes in conductivity even in combination with other data (De Mol et al., 1997). De Mol and Ouweltjes (2000) reported results of single and combined measures of 29,033 milkings to detect clinical mastitis and concluded that early warning is not reliable with sensors and software currently on the market.

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Enzymes and ions are released as a result of the animal's immune response against infection and changes in cellular membrane chemistry. For example, it was suggested to use *N*-acetyl β-D-glucosaminidase (NAGase) (Kitchen, 1981; Urech et al., 1999; Mottram, 1997; Mottram et al., 2000), serum amyloid A and haptoglobin and other acute phase proteine (Eckersall et al., 2001) for mastitis detection.

Another consequence of clinical mastitis is change of concentrations of sodium, chloride and potassium in milk (Kitchen, 1981; Bramley, 1992). The changing ion concentrations are largely due to different inflammatory responses of the mammary gland. Changes of ion concentrations induce changes in electric conductivity of milk, which has been used as an indicator of mastitis for almost 60 years (Davis et al., 1943). Electrical conductivity is an inexpensive technique and can be easily implemented on-line. Conductivity is the most widespread method of automated detection of mastitis, e.g. in milking robots. However, number of drawbacks of conductivity measurements was reported, for example failure to detect mastitis caused by *Streptococcus uberis* (Hillerton and Walton, 1991; Milner et al., 1996). There are also a high number of false positives when healthy milk is incorrectly classified as mastitic.

An analytical technique that may be used for mastitis detection is a multisensor systems (electronic tongue). Multisensor systems for liquid analysis have been intensively developing during last decade. Electronic tongues based on various types of sensors were described in literature (Arrieta et al., 2004; Legin et al., 2003; Winquist et al., 2003). In particular, successful applications of such system based on potentiometric chemical sensors to quantitative analysis as well as recognition and classification of multicomponent media were reported. In particular, the electronic tongue was used for the discrimination and classification of various foodstuffs (Legin et al., 2003), human urine (Di Natale et al., 2000), growth media fermented using different microorganisms (Soderstrom et al., 2005) as well as for follow-up of biotechnological processes and quantitative analysis of broths (Legin et al., 2004). Therefore, we suppose that the use of a multisensor system based on potentiometric sensors sensitive to inorganic and organic ions may be an appropriate method for mastitis detection.

The objective of this study was evaluation of the electronic tongue multisensor system based on potentiometric chemical sensors as a method for mastitis detection that is for discrimination between milk samples from cows with clinical mastitis and from healthy ones.

2. Materials and methods

2.1. Samples

Two sets of milk samples were obtained. In both cases, milk samples were taken on the day of diagnosis from cows with moderate clinical mastitis, identified by the presence of clots in the milk along with observable inflammation in the infected quarter, such as heat, pain, redness or swelling.

The first set of milk samples was taken at the experimental farm of the Veterinary School of the University of Glasgow. Samples from nine cows with mastitis were taken. From four cows milk was sampled from mastitic quarter and the opposite healthy quarter. From further four cows with mastitis milk was taken from only mastitic quarter and one sample was taken from a healthy cow. A total of 14 samples including 9 mastitic and 5 controls were collected. Mastitis in these cows was caused by a variety of pathogens including *S. uberis*, *Escherichia coli* and *Arcanobacter pyogenes* being identified by routine bacteriological investigation.

A second larger set of milk samples was taken at another farm. Overall 54 milk samples from 17 cows were taken during the period of 3 weeks. Each time two samples were taken from each cow: from mastitic quarter and opposite healthy quarter. Thus, a total of 27 paired samples were collected. All milk samples were frozen and kept in the refrigerator before measurements.

2.2. Measurements

All milk samples from both sample sets were measured using an electronic tongue multisensor system.

Electronic tongue used in this experiment consisted of 15 potentiometric chemical sensors. An array comprised sensors with plasticised PVC membranes with cross-sensitivity to inorganic and organic cations and anions, chalcogenide glass sensors, chloride-, potassium- and sodium-selective electrodes, and glass pH electrode. Details of the compositions of the sensor membranes and sensor preparation methodology were reported earlier in (Legin et al., 1999).

Potentiometric measurements were carried out using custom-made multichannel voltmeter with high input impedance connected to the PC. Potential values of each sensor of the array were measured versus conventional Ag/AgCl reference electrode. Schematic of the experimental set-up used in this experiment is shown on Fig. 1.

Before measurements milk was defrosted and 10-fold diluted with the aim to decrease contamination of sensor membranes by lipids and proteins and to adjust sample volume to the necessary for the analysis. Measurement time was 3 min. Three replicated measurements were run on each sample. Between measurements sensors were washed with distilled water until stable potential readings were reached.

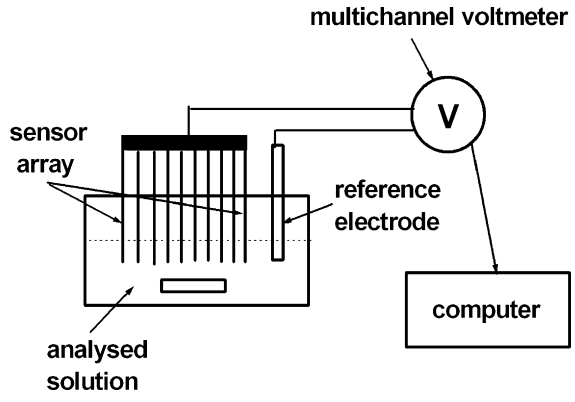

Fig. 1. A schematic diagram of the sensor array operating in static mode.

Measurements using a conductivity probe were carried out in the milk samples from the second set. Only 40 milk samples out of 54 were available for the conductivity measurements due to the restricted sample volume. Measurements were made in raw milk without any sample preparation. Three replicated measurements were made on each sample.

2.3. Data processing

Three data sets were considered: conductivity data, responses of the three electrodes selective to chloride, potassium and sodium ions, and electronic tongue data, which included responses of 15 sensors. It is important to note that three aforementioned ion-selective electrodes were a part of the sensors array and thus, electronic tongue data included measurements with these three electrodes as well.

Data processing was aimed at recognition of mastitic and healthy milk samples. For this purpose, classification models were made and then class membership prediction was performed. Cross-validation was employed for the validation of the classification models in all cases. Reported results were obtained for the cross-validation data sets.

Soft Independent Modelling of Class Analogy method (SIMCA) based on Principal Component Analysis was applied to processing of the data from the sensor array. Commercial software Unscrambler (CAMO ASA, Trondheim, Norway) was used for SIMCA calculations. A classification model for the conductivity measurements was made using Linear Discriminant Analysis (LDA). Classification model using three sensors selective to chloride, sodium and potassium was also made using LDA. Detailed description of data processing techniques can be found elsewhere (Esbensen, 2001).

3. Results and discussion

Initially, the capability of the electronic tongue to distinguish between mastitic and healthy milk was tested on the small number of milk samples that is the first sample set. The measurements with the electronic tongue in the first set of milk samples were processed using PCA. Score plot of the first and second Principal Components is shown in Fig. 2. All mastitic samples can be easily distinguished from healthy ones. Two replicated measurements in each sample are plotted. Reproducibility of the sensor response was good enough to ensure separation of the samples on the Principal Component score plot.

Furthermore, classification of mastitic and healthy milk was done using SIMCA. In SIMCA, classification models are calculated separately for each class using PCA and the number of Principal Components necessary for adequate class description is determined. Afterwards unknown or validation samples are projected on each classification model and a conclusion about new sample class membership is made. Two criteria are used for the prediction of the class membership in SIMCA: sample to model distance and Leverage. Sample to model distance is a Euclidean distance between the centre of the model describing class and unknown sample. Leverage describes how "similar" is an unknown sample to samples used for calculation of classification model. Values of these two parameters for unknown samples should be lower than certain limit values if the sample belongs to the class. If they are higher than limit values the sample does not belong to this class. Limit values of sample to model distance and Leverage are defined using Fisher distribution for $\alpha = 0.95$. It was observed that samples of healthy milk formed a tighter cluster on the PCA score plot compared to mastitic milk samples (Fig. 2). Thus, only one class of healthy milk was modelled. Results of class membership prediction for the samples from the first set are shown in Fig. 3. All milk samples from this set were classified correctly.

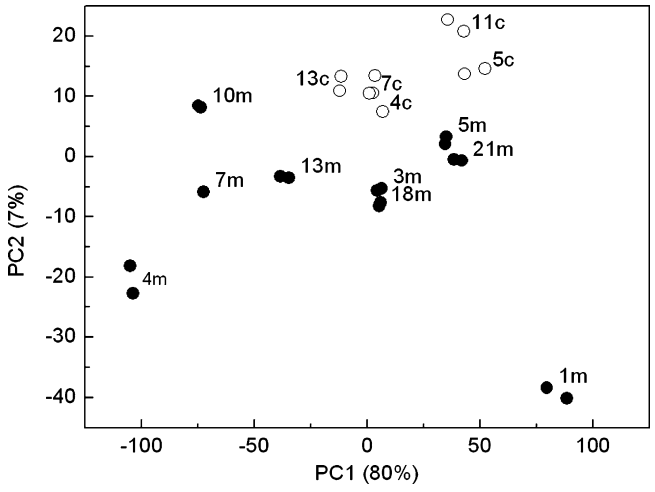

Fig. 2. PCA score plot of the first set of mastitic (●) and healthy (◯) milk samples.

With the aim to confirm obtained results the same measurements were repeated on the much larger set of samples. PCA score plot of 54 milk samples of the second set is shown in Fig. 4. In this case, separation of mastitic milk samples from healthy ones is less clear than in the previous experiment. This might be expected with bigger sample set since milk composition during mastitis may change depending on infection

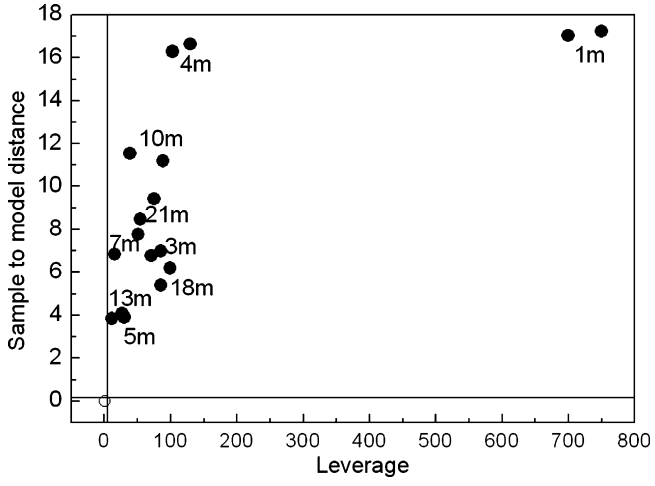

Fig. 3. Results of class membership prediction for the first set of milk samples. Classification was done using SIMCA method. Sample to model distance and Leverage are two parameters defining class boundaries for the class of healthy milk. Samples of mastitic milk (●) and healthy milk (◯).

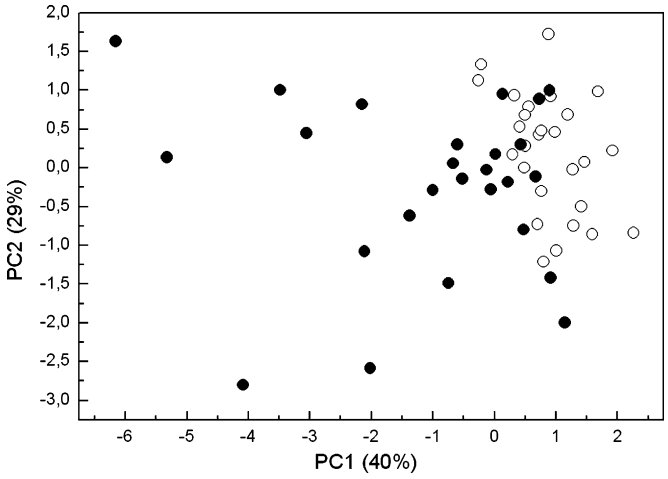

Fig. 4. PCA score plot of the second set of mastitic (●) and healthy (◯) milk samples.

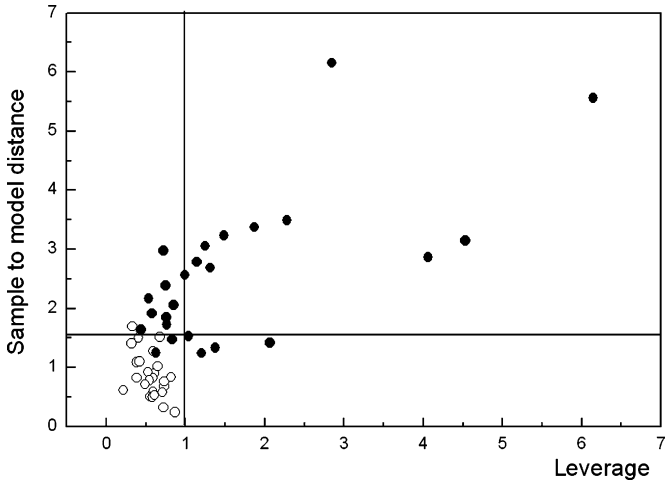

Fig. 5. Results of class membership prediction for the second set of milk samples. Classification was done using SIMCA method. Sample to model distance and Leverage are two parameters defining class boundaries for the class of healthy milk. Samples of mastitic milk (●) and healthy milk (◯).

type. The natural differences between cows may also to some extent mask changes related to mastitis.

Classification model for healthy milk samples was made as before using SIMCA. The results of classification of all milk samples are shown in Fig. 5. With the exception of the three samples—one healthy and two mastitic, all other milk samples were correctly classified.

Subsequently, the performance of the electronic tongue system was compared with conductivity measurements and three ion-selective electrodes. Measurement of conductivity is currently the most widely used method for on-line mastitis detection and, thus, was considered as a standard against which to compare new technology. It was reported in the literature that observed conductivity changes in mastitic milk are caused by the change of ion concentrations, mostly the concentrations of chloride, sodium and potassium. Thus, it was supposed that three sensors selective to sodium, potassium and chloride might be used for this purpose. Since these three sensors were included in the electronic tongue sensors array, their responses were simply extracted into the separate data set and used for the modelling. Capability to discriminate mastitic and healthy milk samples was compared for the following three types of data: conductivity measurements, reponses of the three ion-selective electrodes and electronic tongue.

Classification models for conductivity and three ion-selective sensors were made using LDA. The results of classification using all three data sets, i.e. conductivity probe, three discrete ion-selective sensors and electronic tongue are shown in Table 1. It was demonstrated that on the given set of samples the sensor array ensured the best performance in the discrimination of healthy and mastitic milk samples. In milking systems, absolute measurements of electrical conductivity are not used because of large variability of conductivity between cows, for the same cow during lactation periods, during milking, etc. Instead differential conductivity measurements together with previous conductivity data for the same cow are used for classification of mastitic and healthy milk (Fernando et al., 1982; Nielen et al., 1995). Differential measurements, comparison with historical data and the use of fuzzy set logic as described by De Mol and Ouweltjes (2000), should also improve performance of the electronic tongue system.

It can be proposed that with a suitable sampling system an array of chemical sensors can be used to detect clinical and possibly sub-clinical mastitis in a milking system. The system could take a sample from the foremilk of a cow, dilute it and pass it to a sensor chamber. An output from the sensors could

Table 1
Summary of classification of healthy and mastitic milk using conductivity measurements, measurements with three discrete ion-selective electrodes and the electronic tongue

	Conductivity (LDA, 40 samples)	Cl, Na and K ion-selective electrodes (LDA, 54 samples)	Electronic tongue—15 sensors (SIMCA, 54 samples)
Healthy as mastitic	4	5	1
Mastitic as healthy	8	8	2
Sensitivity[a] (%)	56	70	93
Specificity[b] (%)	82	81	96
Correct classification[c] (%)	70	76	95

[a] Sensitivity = True Positives/(True Positives + False Negatives).
[b] Specificity = True Negative/(True Negatives + False Positives).
[c] Correct classification—percentage of cases assigned to correct classes.

be measured and the milk sample classified. An automatically controlled system could be attached to a milk meter and form a key component in monitoring the health of cows in milking systems. The details of such a system need further research before a practical system could be installed on a milking system.

The specification of an automatic mastitis detection system will be greatly improved if the sources of variability of chemical composition of milk (diet, stage of lactation, breed of cow, type, stage of infection, etc.) were determined and compared with response of multisensor system for the same samples. The effect of sub-clinical mastitis on the milk composition in the days and hours before clinical signs appear needs further study before on-line measurement can be routinely used to determine the need for treatment.

4. Conclusions

A multisensor array was used to discriminate between milk from healthy and infected glands in two separate experiments. An array of fifteen chemical sensors (the electronic tongue) was the best detection method (sensitivity 93%, specificity 96%) above an array of three ion-selective electrodes (sensitivity 70%, specificity 81%) and conductivity (sensitivity 56%, specificity 82%). The system needs to be tested on fresh milk samples to determine its potential as on-line measurement system.

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