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Comparative studies on the souring process of milk by means of enzymatic and electrochemical sensors

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1. Introduction

In recent years, the demand for quick and specific analytical tools for food analysis has increased and is still expanding. Both the industry and government health agencies require a variety of different analytical methods in the quality assurance of food materials [1]. Analysis is needed for monitoring nutritional parameters, food additives, food contaminants, microbial counts, shelf life assessment and other olfactory characteristics like smell and odour [2]. Enzymatic and electrochemical sensors are of great importance in food analytics [3]. Especially in dairy farming they are widely used for the quality control of products as well as for monitoring and optimising of production processes, whereby as most interesting parameters the pH value, the chloride and lactate concentrations and the conductivity are determined [4, 5, 6, a]. Furthermore, electrochemical measuring procedures can be helpful for the health care of animals. Thus, e.g. pathogens in the cow's udder can be detected in a simple procedure by conductivity measurement [7]. The aim of the paper presented here is to give a short survey on possibilities of application of biosensors and electrochemical sensors and measuring methods in dairy farming.

2. Experimental

As sample materials served fresh, untreated cow's milk immediately after getting as well as pasteurised milk and buttermilk. During the investigations the test material was permanently stirred and held on constant temperature (20 °C).

The pH value, oxygen, chloride and carbon dioxide concentrations were measured by means of miniaturized electrochemical sensors. All these sensors were developed and manufactured at the Kurt-Schwabe-Institute (KSI). The lactate concentration was measured by means of an amperometric thick-film biosensor. This sensor was also manufactured at the KSI. Its enzyme immobilized membrane was prepared by using a coating method, which had been developed at the Forschungszentrum Sensorik e.V. Greifswald [8].

For the operation of the sensors and the analysis of their signals the pCO_2, pH und pCl sensors were combined with the microprocessor pMX 3000/ION meter (WTW). The miniaturized oxygen sensor and the lactate sensor require special electronic measuring units, which had been developed at the Kurt-Schwabe-Institute. In order to avoid interferences between the different sensors, which are inserted simultaneously into the same measuring medium, the electrical circuits for operation and signal conditioning of the amperometric sensors (lactate, oxygen) had been insulated from each other with special care.

For measuring the conductivity and the impedance commercially available measuring instruments were used. The conductivity was determined by the microprocessor conductivity meter LF 3000 with the measuring cell LTA 1 (WTW). Electrochemical impedance measurements were performed with the measuring system IM6d of the Zahner-elektrik GmbH.

In Tab. 1 the miniaturized sensors, which had been used in the investigations of the souring process of milk, are presented and characterized.

Table 1 Technical parameters of the sensors, which had been used in the experiments to the souring process of milk.

Measuring parameter	**Sensor type**	**Measuring range**	**Accuracy**	**Service life** months	**Response time** τ_{90}
pH	potentiometric	1 ... 11	ΔpH < 0.05	12	7 s
pO_2	amperometric	0.1 mg/l ... sat.	1 %	12	30 s (25°C)
pCl	potentiometric	1 ... 35000 mg/l	1.75 mg/l $\pm$ 2 %	12	20 s
pCO_2	potentiometric	0.2 ... 2000 mg/l	$\pm$ 1 mg/l $\pm$ 4 %	> 6	4 min (25 °C)
Lactate	amperometric enzyme	0 mM ... 5 mM	$\pm$ 2 %	3 (storage) 0.5 (stability)	90 s

Fig. 1 shows as an example the general construction and the dimensions of the potentiometric carbon dioxide sensor. The CO_2 permeable polymer membrane at the tip of the sensor separates the electrochemical sensor system from the substances to be measured. CO_2 permeates through this membrane into the sensor electrolyte and causes there a defined, reproducible pH shift, which is measured by means of a pH glass electrode. The output voltage signal of the CO_2 sensor is proportional to the logarithm of CO_2 concentration. It amounts to approx. 55 mV/dec. at room temperature. Provided that the sensor had been carefully calibrated its measuring accuracy is better than ±1 mg/l ±4 %; the long-term drift of the sensor signal within the measuring periods of few days is <1 mg/l without intermediate calibration.

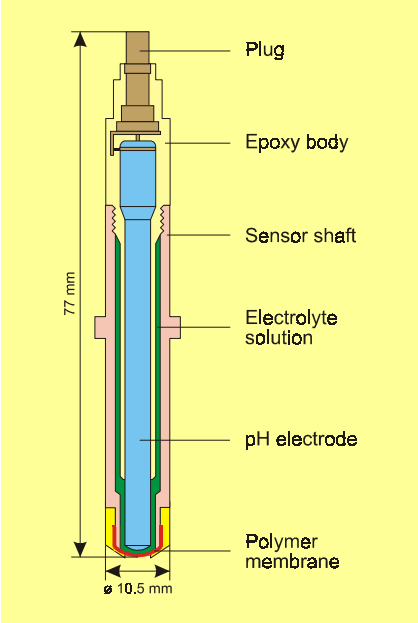
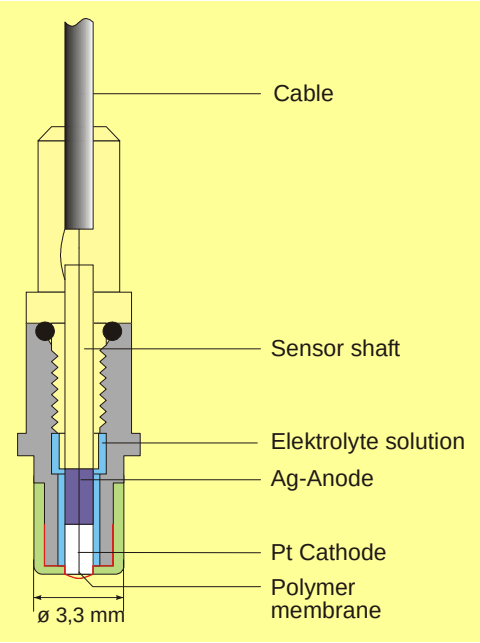


Fig. 1 Construction and dimensions of the electrochemical carbon dioxide sensor

The pH sensor is designed as a two-part measuring chain, consisting of a miniaturized pH-glass electrode and a miniaturized Ag/AgCl reference electrode. The practical measuring properties of this miniaturized pH electrode corresponds to those of conventional electrodes. Its sensitivity is about 57 mV/pH at 25 °C. For the potentiometric pH and chloride sensors one common Ag/AgCl reference electrode is used. The chloride-selective electrode has a moulding of precipitated silver chloride of 3 mm diameter as sensitive membrane. The moulding is coated with heat-hardening, electrolytically impermeable epoxy resin.

For the present application a special oxygen sensor having particularly small limiting diffusion current must be used in order to minimize oxygen consumption from the medium by cathodic reduction of oxygen. The miniaturized oxygen sensor, which is shown in Fig. 2, is based on an amperometric measuring principle.



It consists of a Pt cathode as working electrode and an Ag/AgCl counter-electrode, which simultaneously acts as reference electrode. The electrode system is separated from the measuring medium by a 20 µm polypropylene membrane. The oxygen consumption from the measuring medium by cathodic oxygen reduction was reduced to the sufficient low value 5,25 pg O_2/min. and the sensor signal is not influenced by moving the medium. These properties are achieved by using a Pt micro-cathode (diameter 30 µm). The sensitivity is about 100 pA/(mg/l O_2) at 25 °C.

Fig. 2 Scheme of the miniaturized electrochemical oxygen sensor

In Fig. 3. the structure and dimensions of the reusable amperometric thick-film biosensor are shown schematically. This sensor works according to the method of H_2O_2 oxidation at the polarisation voltage of +700 mV versus an internal reference electrode (Ag/AgCl on chip). The enzyme lactate oxidase was deposited upon the sensor structure [9]. A covalent bond is formed between the proteins (BSA and LOD) with the bifunctional reagent glutaraldehyde as cross-linker. The sensor was covered by a second membrane which is permeable to lactate. As material for this membrane a copolymer of methacrylate esters was used. The membranes have been applicated on the sensor by means of the Dispenser 1500 XL (GLT Gesellschaft für Löttechnik mbH, Pforzheim).

Regarding a later production, the thick film technique is a very economical manufacturing procedure for biosensors, since numbers of items of the sensors between 10,000 and 1 million per year are to be expected [10, 11]. Another important criterion is the sensor price in comparison to the lifetime of the sensor. Since the lifetime of enzyme layers is generally not very high, biosensors on thick-film basis with relatively small lifetime are an economical alternative to more expensive sensors with long lifetime [12].

Before starting the measurements the lactate sensor had been calibrated in Imidazol buffer (pH = 7) with defined lactate concentrations 0.5 and 5 mmol/l L(+)lactic acid (Fluka) at the measuring temperature in milk ϑ = 20 °C.

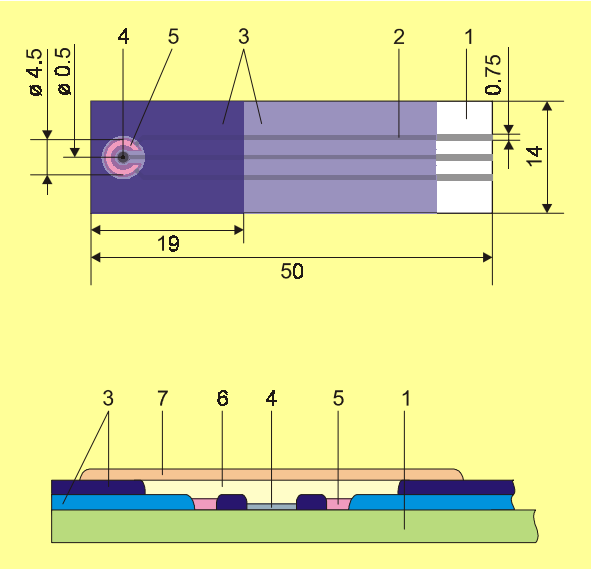


Fig. 3 Thick-film lactate sensor
above: Electrode configuration and dimensions
below: Layer structure (schematic drawing, not to scale)

1 Al_2O_3 ceramic substrate, 0.6 mm thick
2 Ag contacts
3 Glass paste – insulating layer
4 Pt electrode (anode)
5 Ag/AgCl electrode
6 Lactate oxidase, enzyme immobilized
7 Diffusion membrane

Measurements in milk require special experimental care. It is to be made certain that all parameters are measured at the same time under uniform conditions and that the test results are not impaired or falsified by deposits on the active surface of the sensors.

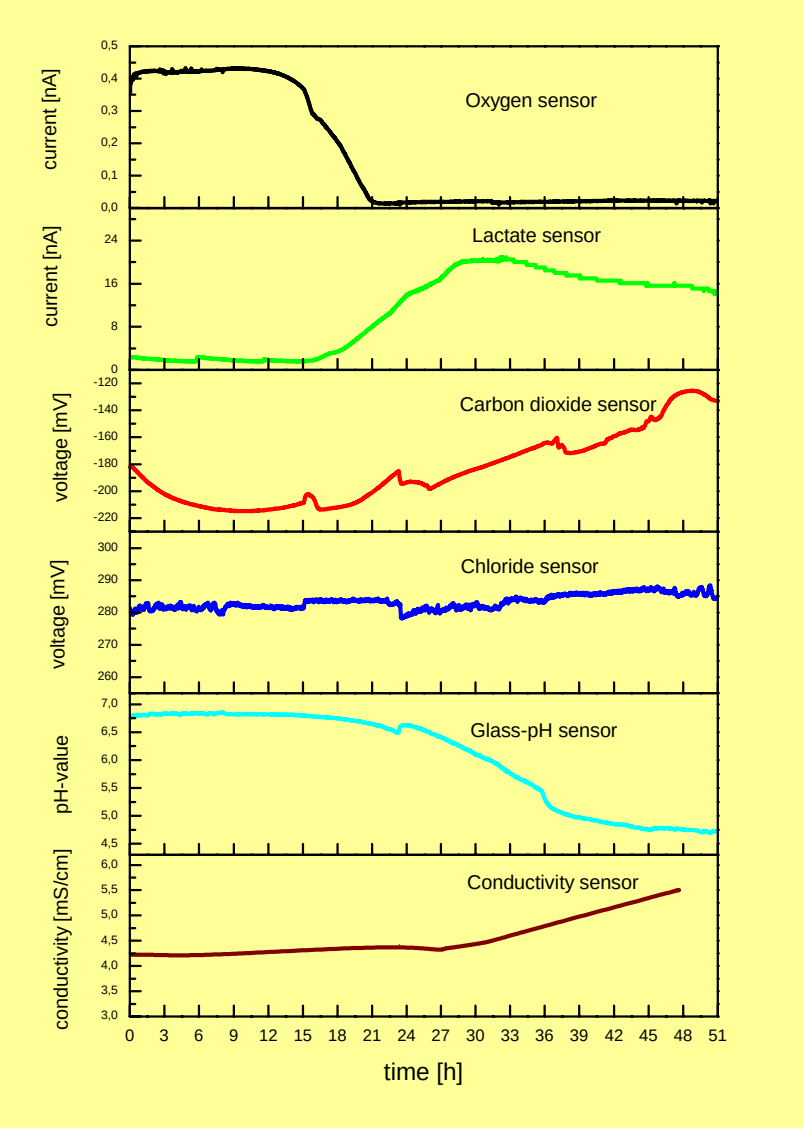
3. Results

In the following table the temporal changes of the various measured parameters within the test period of 48 hours are summarized. The initial values had been determined two hours after getting the milk.

Table 2 Results of measurements in fresh, untreated cow's milk

Time of the measurement	ϑ °C	pH	$[Cl^-]$ mg/l	$[O_2]$ mg/l	$[CO_2]$ mg/l	[lactate] mmol/l	σ mS/cm	$\lvert Z \rvert$ Ω
Initial value	20	6.8	1600	4.0	30	0.1	4.2	220
After 48 h	20	4.8	1600	0.2	277	> 3	5.5	180

As can be seen from Fig. 4 the souring process of the untreated raw milk begins gradually after approximately 15 hours. This is indicated especially by significant changes of the parameters pH value, oxygen, carbon dioxide and lactate concentration as well as conductivity, while, as expected, the chloride concentration remains nearly unchanged in the investigated milk. The very low initial lactate concentration proves the excellent milk quality of the supplier.



The biochemical reactions taking place in the milk during the souring process result in a considerable decrease of the oxygen concentration. The measuring signal of the oxygen sensor decreases already after 15 hours noticeably. At the same time the lactate concentration, as is to be seen in Fig. 4, rises after 18 hours continuously. However, the strongly dropping oxygen content in the medium is a limiting factor for the long-term applicability of the lactate sensor.

Fig. 4 Results of measurement of the souring process of raw milk

For long-term measurements an electron mediator should be immobilized on the surface of the electrode system in order to attain that the enzyme reaction becomes independent from the oxygen content.

After 18 hours changes of the pH value and of the CO_2 concentration in the milk sample are registered, too. The pH value decreases with increasing milk souring as expected, while the CO_2 concentration rises.

The electrochemical sensors and biosensors described here permit direct tests and control in foodstuff without addition of reagents. Furthermore, as is evident from the service lifetimes of the sensors which are specified in table 1, they can be applied to continuous measurements and process control.

From Fig. 5 it becomes clear that also online impedance measurements permit conclusions on the condition of the milk. The process of the milk souring reflects itself in the changes of the impedance spectra, which had been measured at different times. The measured conductivity correlates with the electrolyte resistance in the impedance spectrum.

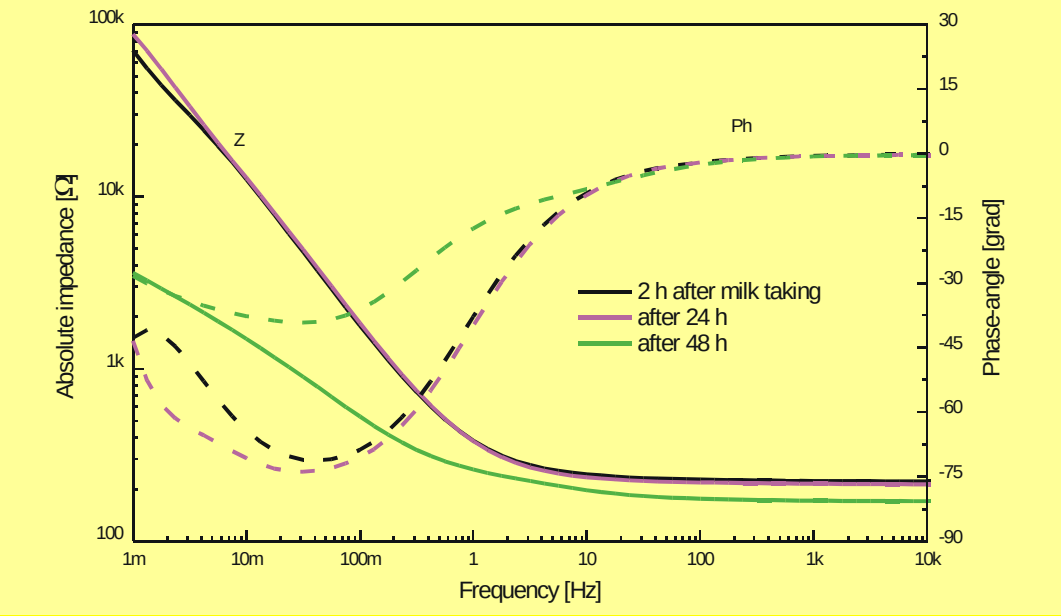


Fig. 5 Bode plots of measurements in milk during the souring process

4. Conclusions

By simultaneous application of electrochemical sensors for different measuring quantities and biosensors it was proven that the state of milk can be characterized by various parameters. Therefore, the combination of different sensors in a multi parameter probe results in an improvement of the validity and reliability of the measuring values. This method is applicable for quick tests as well as for continuous *in situ* measurements.

The enzymatic lactate sensor, which had been prepared in thick film technique, was used successfully in food analytics. In this application this technique seems to be advantageous concerning price and user friendliness in comparison with conventional sensor manufacturing techniques. Especially multi-parameter probes can be prepared easily by combining various coating materials [13].

Acknowledgements

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