Christine Bürger, Dresden, and Thomas Nacke, Heilbad Heiligenstadt

Cell Count Determination in Crude Milk

The somatic cell content in crude milk is an important quality criterion. It allows conclusions about animal- and udder health to be drawn, influences the utilizability of crude milk and thus determines the efficiency of milk production. For this reason, a technique from biotechnology for the on-line determination of the cell count in a nutrient solution has been examined with regard to its applicability in milk production.

Dr.-Ing. Christine Bürger is a scientist working at the Chair of Agricultural Machinery of the Technical University of Dresden, Bergstr. 120, D-01069 Dresden (director: Prof. Dr.-Ing. habil. Gerd Bernhardt); e-mail: *buerger@landmaschinen.tu-dresden.de*
Dipl.-Ing. Thomas Nacke is a scientist working at the Institute of Bioprocess- and Analytical Measuring Technology, Rosenhof, D-37308 Heilbad Heiligenstadt (director: Prof. Dr. Dieter Beckmann); e-mail: *thomas.nacke@ba-heiligenstadt.de*.
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Keywords

Somatic cell count, milk constituents, electrical impedance spectroscopy

Literature

Literature references can be called up on the internet under LT 05313: http://www.landwirtschafts-verlag.com/landtech/local/literatur.htm.

In conventional milking parlours, the Milk Hygiene Decree requires that a visual examination of the milk condition in each udder quarter be carried out before milking. In automatic milking parlours, where visual pre-milking examination is no longer possible, the on-line measurement of electric conductivity is used for the examination of the milk condition in each udder quarter. This technique is also employed for the examination of the whole milking in conventional milking parlours. This measuring method does not enable alterations in the crude milk as the result of udder diseases to be clearly detected.

For the examinaton of the pre-milking in conventional milking parlours, practicable solutions in real time are not yet available. An on-line measuring technology suitable for practice is required which allows beginning mastitis resulting in an increase in the somatic cell count ($\geq 300 \cdot 10^3$ cells/ml) to be detected.

Initial Situation

In biotechnology, the growth of cells (biomass) during fermentation or in suspensions is sucessfully measured using the methods of electrical impedance spectroscopy (determination of alternating current resistance) [1, 2, 3, 4, 5, 8]. At cell concentrations above 100,000 cells/ml, the dependence between measurable frequency-dependent electrical properties, such as impedance, capacity, conductance, phase shift, and cell count in the frequency range between 10 kHz and 10 MHz is represented well. From the viewpoint of measuring technology, the growth of cell mass as well as the cell count/volume unit (e.g. the growth of yeast cells in beer production) are determined well in real time.

Comprehensive studies on the electrical properties of cells and the frequency ranges where meaningful measurement results can be obtained are available.

These electrically measurable properties are based on the great resistance of the cell membrane. Every living cell is surrounded by a cell membrane, which separates the electrolyte in the interior of the cell from the electrolyte outside the cell. If alternating tension is applied to the trial suspension using electrodes, the cells behave like little capacitors, and a capacity depending on the cell count is measured.

Today, the measurement of cell concentration in suspensions is already possible at cell counts of 10,000 per ml. This was the starting point for the concept of using this method to measure cells in crude milk and to find mathematical/physical connections. For tests, an existing measuring place for cell suspensions was used. Capacity C, phase shift ϕ, conductance G, effective resistance R (real part of impedance Z), and reactive

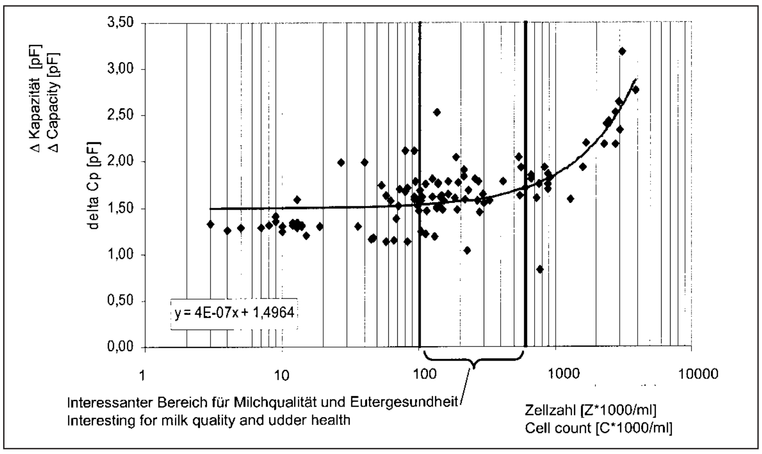

Fig. 1: Change of capacity as a function of the somatic cell count (ΔC: 400 kHz - 10 MHz)

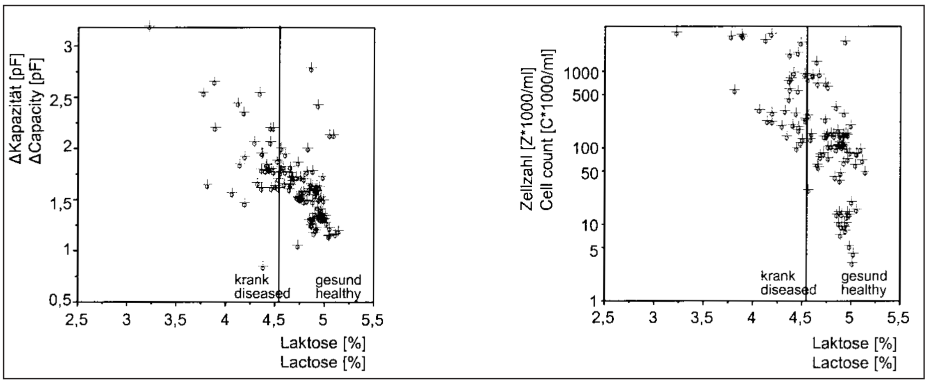

Fig. 2: Change of capacity and somatic cell count as a function of lactose content

impedance X (imaginary part of impedance Z) were measured in a frequency range from 10 kHz to 10 MHz in crude milk with different cell concentrations. For evaluation and representation, the difference of capacities at 400 kHz and 10 MHz is formed and shown as a function of the cell count (*Fig. 1*).

In the range up to 500,000 cells, which is interesting for milk quality, there is no satisfactory correlation between the measurement value ΔC and the cell count. Above 600,000 cells/ml, a connection becomes apparent which increases with growing cell counts. Later, this correlation was always found at high cell counts.

Experimental Studies

Systematic studies of cell count determination in crude milk pursued two goals:

- Optimization of measuring technology and measuring ranges
- Determination of the influence of milk constituents

Measuring Technology and Measuring Range

Thanks to the permanent improvement of this measuring principle (worldwide) in biotechnology and medical technology, the theoretical fundamentals are very highly developed, and measurement-technological solutions for various areas of application are available [3]. For crude milk as a multidisperse biological system, the task is the development of fundamentals and the processing of available results. Studies in the following areas are necessary:

- Hard- and software components of measuring instruments
- Electrodes - number, form, material, and arrangement in the measuring chamber
- Measuring chamber - geometry, volume, material
- Measuring temperature and frequency range

The alteration of one factor requires comprehensive studies in order to prove its influence on the measurement result. The measurement values of capacity are in the order of 10-12 F. Every change which contributes to the improvement of measuring accuracy is important.

Results

In different media, impedance and relative permittivity sink with increasing temperature at frequencies of more than 50 kHz. In the frequency range above 2 MHz, impedance in a KCl solution and crude milk grows again. Here, more systematic studies are necessary.

At a constant cell count, capacity difference ΔC increases with growing measuring volume. If the cell count is increased while the measuring volume is constant, the measurement value ΔC grows as well.

In studies on the influence of temperature and the sample volume, opposing behaviour in different frequency ranges was observed in some cases. Here, comprehensive studies on the functional connections are required.

Milk Constituents

The different concentrations of the main constituents in the crude milk (fat, protein, lactose) from the individual cows are reflected in the entire measured frequency range of impedance and the measurement values which were calculated from it. The measured capacity- and resistance values are influenced by the milk constituents and lead to different values at identical cell counts. Milk poses the following problems:

- The concentration of the constituents and the somatic cells changes during milking.
- The concentration of the constituents and the somatic cells changes from one milking time to the next.
- Milk samples age (acidification, alteration of the protein fractions).
- Milk samples are individual for each animal.

Systematic studies on sampling and the influence of milk constituents are necessary.

Results

Impedance-spectroscopic studies on the influence of protein (casein) and fat (a mixture of butter having a fat content of 83% and 0.08% lecithin as an emulsifier) both solved in a buffer solution did not lead to any result. One reason may be that fractions were used (protein) and that constituents were altered (fat).

The fat globules in crude milk exert a great influence on dielectric properties, in particular conductivity. Even though they cannot be polarized like the cells, they impede the migration of free charge carriers within an electric field due to an increase in viscosity.

In the case of mastitis, the lactose content of the crude milk sinks (while the cell count increases). 4.55% is considered the limit [6]. In lactose, the frequency-dependent electric properties change with growing lactose content: impedance Z increases, whereas conductance G and capacity diminish (*Fig. 2*).

This result has already been able to be discerned in the tests. In reference [7], a correlation of conductance and lactose concentration of 0.966 at a determinateness of 92.2% was reached after the measuring equipment had been optimized (*Fig. 3*).

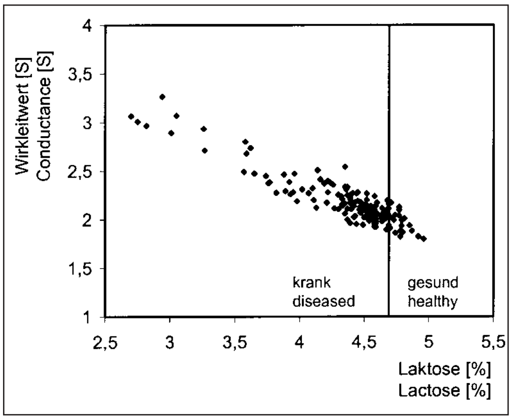

Fig. 3: Dependence of conductance from lactose content [7]

The evaluations of the behaviour of impedance and the phase angle allow the individual cows to be discerned over the course of the curve in the low frequency range.

Capacity has a wide scattering range; the course of the curves for individual cows overlap. Currently, it cannot be used for evaluations.