NON-DESTRUCTIVE METHODS OF MILK QUALITY ASSESSMENT

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Abstract
Monitoring the quality of raw cow milk is essential to maintain food safety and human health. To guarantee the quality and safety of milk, easy-to-use non-destructive analysis methods are available. Non-destructive methods have made progress, being useful for obtaining quantitative and qualitative data, destroying the sample, and offering several advantages, such as high sensitivity, adequate response time, and minimal sample preparation. Conventional methods are laborious, destructive, toxic, and time-consuming, require fully equipped laboratories and skilled personnel, extensive sample preparation, and are not suitable for real-time continuous monitoring of milk quality. The rapid development of non-destructive methods for the evaluation of milk quality consists of applications based on imaging, near-infrared spectroscopy, conductivity, biosensors, and ultrasound. This paper presents different non-destructive methods applied to determine the physicochemical parameters of raw milk (fats, proteins, lactose, casein, dry matter, acidity, density), the number of somatic cells, and the microbiological load.

Key words: raw milk, non-destructive methods, physico-chemical parameters.

INTRODUCTION

The quality of raw milk has always been the subject of research at the national and international levels, through different analysis techniques, having a direct influence on health and food safety (Oarga A.C.D, 2010).

The determinations made in Romania regarding the physico-chemical quality of raw milk are based on globally standardized analysis methods. At the national level, the physico-chemical quality parameters of raw milk are regulated by STAS 2418:2008-Full raw milk. Potentially non-destructive methods, such as imaging analysis, NIR spectroscopy, NMRI, ultrasound, X-ray imaging, and biosensors may supplement or replace many of the traditional time-consuming destructive methods (Narsaiah K., *et al*, *2012*). Over the past 30 years, Near Infrared Spectroscopy (NIR) has proven to be one of the most influential and advanced tools for continuous monitoring and quality control of dairy products. There is a growing demand from consumers in the food industry sector to have fast-measuring devices that allow the characterization of raw materials or food. Conventional chemical methods cannot determine the parameters of dairy products in a short time (Růžičková J. *et al., 2006*; Bondoc I., Șindilar E.V., 2002). The identification of microorganisms in the dairy industry relies mainly on conventional standard methods such as plate count and culturing, microscopy, flow cytometry, or on advanced immunological techniques (e.g. biochemical kits, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) (Mandal *et al, 2011*; *Law et al, 2015*; Zhao *et al, 2014*). Commonly used as the gold standard, conventional plate count and culture procedures are very sensitive (10-100 CFU/ml), affordable, and capable of providing qualitative and quantitative data on the quantity and kind of microorganisms. However, they are labor-intensive, time-consuming (the time needed for pathogen identification often takes more than a week) (Zhao *et al, 2014*), and unsuitable for real-time, on-site applications. Since they can reduce the amount of time needed for detection following enrichment to as little as 1-3 hours, immunological techniques using robotic and automated ELISAs are frequently used. However, the findings can only be available in 1-2 days due to sampling pre-processing and enrichment stages. PCR-based methods enable the identification of bacteria through their genetic makeup and do not necessitate a step involving bacterial culture (Law JW-F. *et al, 2015*; Mortari A., Lorenzelli L., 2014). Although PCRs take between 30 and 90 minutes to complete, the entire process including sample

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preparation, DNA extraction, and amplicon analysis—can take from 6 to 8 up to 48 hours.

The inability of PCR-based approaches to differentiate between living and dead cells is a significant flaw that could result in overestimation and false-positive results. This paper presents different non-destructive methods applied to determine the physicochemical parameters of raw milk, the number of somatic cells and the microbiological load.

MATERIAL AND METHOD

Materials: milk, combiscope kit (decon, triton, coloration solution), Neogen kit, Clear Milk test plates.

Methods

The evaluation of the density of cow's milk was carried out with the help of the analytical balance to which a specific determination kit was attached.

Preparation of the sample to be analyzed: Carefully pour the milk into a 250 ml Berzelius glass, held in an inclined position, to avoid the formation of foam or air bubbles. After balancing, insert the Berzelius glass with milk inside the balance. The kit for determining the density is easily inserted, and then the obtained value is displayed on the screen of the analytical balance *(Figure 2).*

Evaluation of protein content by the Dumas Method - The Dumas method consists in burning the sample of known mass in a high-temperature range of 800-900 °C in the presence of oxygen, nitrogen, and helium. The gases are then passed through special columns that absorb carbon dioxide and water.

Preparation of the sample to be analyzed: Weigh the sample on the analytical balance. For the analysis of liquid products, an absorbent/hygroscopic substance is used, in which a maximum of 100 mg of milk is added with a pipette. Weighed samples are folded in tin foil and added to a numbered rotor (weigh 3-5 samples on average). After every 4 minutes, the result of each sample expressed in total nitrogen content is obtained, and by multiplying it with the correction factor of cow's milk 6.38, the protein content is obtained (*Figure 1*).

Figure 1 Rotor of the Automatic Analyzer – Dumatherm

The evaluation of the physico-chemical parameters and the number of somatic cells - may be done with the Combiscope type analyzer consisting of Lacto-Scope FTIR and Soma Scope - MK2. The Combiscop is a fast and complex analyzer whose principle of analysis is based on infrared measurement. It is composed of two parts: LactoScope FTIR used for measuring the physicochemical parameters of milk and SomaScope used for counting somatic cells in raw milk.

Preparation of the sample to be analyzed: - the samples are inserted into Falcon-type tubes with a capacity of 50 ml. On the label, data regarding the registration number of the animal from which the respective sample was collected and the date of collection are mentioned. Subsequently, the samples are heated beforehand at 37 ^{0}C in a water bath, then placed on a special rack. The rake with samples moves on the band of the device and the syringe draws a quantity of 1 mL of milk from each Falcon tube.

The sample is analyzed for 1 minute, then the results are displayed on the computer software.

LactoScope FTIR - uses special kits both for determining the physico-chemical composition of milk (basic compounds: protein, fat, dry matter, lactose, casein), but also for other additional parameters (urea, free fatty acids, density). ***Somascope*** - Specific kits are used to determine the total number of somatic cells/mL of milk.

Evaluation of the total number of germs (TVC) with *Soleris*® *System (Neogen)* - this technique

consists of determining pH, metabolic activity during growth of the microorganism, and other biochemical reactions within 24 hours. One mL of the milk sample is inoculated into the Soleris vial containing a selective growth medium and a pH indicator (NB-100, Total Viable Count Medium-TVC), followed by incubation for 18–24 hours at 35°C. The system consists of a single incubator with 32 independently monitored and temperature-controllable locations, with a range between 15°C and 60°C.

During testing, in less than 24 hours, the Soleris software reported positive test findings (Crivei I.C*. et al, 2022)*. The absence of detection within 24 hours was considered a negative result. In the case of positive samples, the growth curves are examined, and the visual validation of medium color change is also performed. Also, randomly, for some of the positive results, standard methods were used for confirmation.

Evaluation of the microbiological load - can be evaluated with Clear Milk test plates made of - Three-section Petri dish with chromogenic agars, a tube for milk collection inoculation wand for transferring milk to the Petri dish, napkin for disinfecting the teat before sampling. The milk sample is taken in a sterile test tube; the milk is applied with the inoculation wand to the surface of all three agars of the Petri dish; The plate is incubated for 22-26 hours at 37.5°C and the species of the pathogen are assessed according to the atlas https://www.clearmilk.cz/en

RESULTS AND DISCUSSIONS

The analyzed milk was collected weekly from the collection tank of the farm S.C.D.C.B. Dancu, Iași, and the processing of the analyzed matrix was carried out periodically. The physico-chemical parameters and the microbiological load (density, acidity, fat, protein, lactose, dry matter, NCS, NTG) were evaluated, applying different non-destructive methods. The evaluation of the density of cow's milk was carried out with the help of the analytical balance to which a specific determination kit was attached *(Figure 2)*, the results obtained falling within the range of 1.028 - 1.031 g/cm^3, with a general average of 1.030±0.001 and a coefficient of variation of 0.11 %. The obtained results were also verified by the classic method (with thermolactodensimeter), the results being similar. The density of milk is also given by the automatic analyzer - CombiScope 600/300 FTIR System.

Figure 2 Specific kit for measuring milk density

Determination of protein content by the Dumas method

The first method developed for determining the nitrogen content of milk, including food, was developed in 1826 by Jean-Baptiste Dumas. The instrument must be carefully standardized using a compound of known nitrogen content (eg EDTA, Glycin used to calibrate the thermal conductivity detector). The Dumas method is easy to use and fully automatic *(Figure 3).* It was developed as a much faster method than the Kjeldahl method, and the determination of total nitrogen content can be determined in a few

minutes, compared to 6 hours or even more in the case of the Kjeldahl method. It also does not use toxic substances or catalysts. As with the Kjeldahl method, it does not provide a measurement of protein, as it records non-protein nitrogen, and different correction factors are required depending on the type of matrix analyzed, as they have different amino acid sequences.

Figure 3 Illustration of the Dumas Analyzer

The evaluation of the physico-chemical parameters and the number of somatic cells - can be done with the Combiscop Analyzer consisting of Lacto-Scope FTIR and Soma Scope - MK2 *(Figure 4).*

Establishing the health status of the udder, as an indicator of the quality of the raw milk, was achieved by determining the number of somatic cells and the physical-chemical parameters. Following the analysis carried out in the hot and cold seasons, on the samples collected from the tank, the results were the following:

Dry matter content and fat content varied accordingly. Thus, in milk samples with an average fat of over 4.0%, the dry matter content exceeded 12.5% on average, and in the warm season, when the percentage of milk does not exceed 3.8%, the dry matter content was on average 12.05 %.

The amount of fat (total lipids) was in the range of 3.5% - 4.25%, with an average of 3.80%. In the cold season, the values were between 3.6% - 4.25%, with an average of 3.9%, a fact due to the fodder ration administered, and in the warm season, the values were between 3.5% - 3.8%, with an average of 3.7%.

The protein content of the analyzed milk samples fell within the range of 3.15% - 3.4%, with an average of 3.368±0.057%. The determination of the lactose content was carried out at Combiscope and the average values vary depending on the fat and protein content, the variation range being between 3.36% - 5.21%, with an average of 4.717±0.112%

The casein content varied according to the protein values, falling between 2.75% - 3.1%, with an average of 2.8±0.12%. Establishing the state of health of the udder, as an indicator of the quality of milk - raw material, was achieved by determining the number of somatic cells, using the FTIR CombiScope 600/300 System.

Thus, the determinations made indicated that the milk - raw material, from the experimental biobase of SCDCB Dancu, falls within the norm recommended by the EU regarding the quality of fresh milk (maximum 400 000 cells/mL), having values between 249 000 – 361200 cells/mL, with an average value of 305 100 cells/mL milk – raw material.

Figure 4 Automated Analyzer – CombiScope 600/300 FTIR System (LactoScope FTIR 600/300 and SomaScope LFC 600/300)

Evaluation of the total number of germs (TVC) with *Soleris® System (Neogen*)

Determination of the quality of raw milk in terms of its degree of sanitation was carried out by determining the total number of germs (TVC), using the rapid microbial detection system Soleris® System (Neogen) *(Figure 5; 6).* The determination of the quality of the milk in terms of the degree of sanitation was achieved by determining the total number of germs. The maximum duration of sample analysis can be up to 12 hours, depending on the microbial load of the raw milk samples.

Establishing the total number of germs by applying this technique consists in determining the pH, the metabolic activity during the growth of the microbial load, and other biochemical reactions, within 24 hours. Following the testing of milk samples from the experimental biobased of

S.C.D.C.B. Dancu found that the milk corresponds from a hygienic point of view, falling within the norm recommended by the EU (TVC < 100.000 CFU/mL), with values between 29.000 – 62.000 CFU/mL, with an average value of 45.500 CFU/mL milk - raw material. Thus, compared to the serial dilution method (classical method), this rapid technique offers the advantages of reduced analysis time and costs.

Figure 5 Graphical representation of the detection curve of the total number of germs generated in real time

Figure 6 System for determining the total number of germs (TVC) – SOLERIS

Evaluation of the microbiological load – The Clear Milk test plates were used for the rapid detection of pathogens in milk. The presence of more than one colony of major pathogens such as *Streptococcus agalactia, and Staphylococcus aureus* indicates a positive result. The presence of more than five colonies of other pathogens such as *Streptococcus uberis*, and *Streptococcus dysgalactia* is likewise, an indicator of positive results.

Figure 7 shows a plate inoculated with milk collected from a cow with clinical mammity in which a high number of somatic cells (6 502 000 cells/mL) and the presence of Streptococcus dysgalactia pathogens were identified.

This type of on-farm culturing systems enables veterinarians to establish a proper treatment approach in order to ensure a the recovery of the mammary gland and prevent antibiotic resistance.

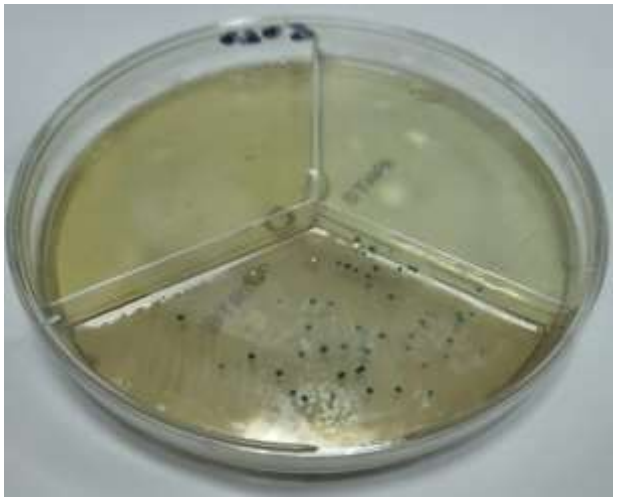

Figure 7 Milk sample from cow with mastitis cultured on Clear milk test plate – Streptococcus dysgalactia colonies visible

CONCLUSIONS

NIR spectral techniques can provide results with a high degree of accuracy in a short time, offering advantages in terms of working time, price, and human resources.

The development and use of non-destructive or portable instruments follow the idea of green analytical chemistry which involves a drastic reduction in costs, analytical steps, sample pre-treatment, energy, and reagents, and for this reason, the use of these devices should be promoted to help cattle farmers to monitor milk quality and be able to have low costs in this regard.

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