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Infrared or ultrasonic milk analysis can affect its results?

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ABSTRACT: *Constant monitoring of milk quality by the processing industries demands the use of methodologies that add agility and low-cost analysis, such as the use of electronic equipment based on infrared or ultrasonic spectroscopy. In the present study, quality of raw milk received by dairy farms from western region of Santa Catarina state, Brazil, was evaluated throughout two methodologies of analysis of chemical composition and compared, being that both equipment used standard procedures for calibration, considering as reference data from the classic analytical methods recommended in Brazilian legislation. Milk samples from 45 producers were analyzed for SCC, TBC and physicochemical analysis, performed at dairy's laboratory and at the official laboratory. Infrared and ultrasound methodologies for chemical composition of fat, protein and lactose were compared and correlated. The comparison of the methods showed that there was no significant difference for the mean values of fat (P=0.06); however, they presented significant difference for protein (P=0.001), higher for IR (3.23 vs. 3.33), and lactose (P<0.0001), higher for US (4.47 vs. 4.83). Correlation was positive and significant for fat (r=0.73, P<0.0001), protein (r=0.47, P=0.001) and lactose (r=0.51, P=0.0003). Considering the minimal composition required, TBC and SCC levels, 95.5%, 84.1% and 64.4% of the milk producers met the current Brazilian legislation, respectively. It was concluded that the methodologies have affected results, which may have been influenced by the correction factor used for equipment calibration, specifically for analyzed samples during the study period.*
Key words: *composition, fast methods, fat, milk quality, protein.*

A análise infravermelha ou ultrassônica do leite pode afetar seus resultados?

RESUMO: *O monitoramento constante da qualidade do leite por parte das indústrias beneficiadoras demanda o uso de metodologias que agregam agilidade e baixo custo por análise, como a utilização de equipamentos eletrônicos que podem ter como princípio a espectroscopia por infravermelho ou por ultrassom. No presente estudo, avaliou-se a qualidade do leite recebido por um laticínio da região extremo oeste catarinense e comparou-se duas metodologias de análise de composição química desta matéria prima, sendo que ambos os equipamentos utilizaram procedimentos padrões para calibração, considerando como referência os dados oriundos dos métodos analíticos clássicos preconizados na legislação Brasileira. Foram comparadas amostras de leite de 45 produtores para análise físico-química, de CCS e CBT, realizadas no laboratório do laticínio e em laboratório oficial. As metodologias por infravermelho (IR) e ultrassom (US) foram comparadas e correlacionadas para composição química de gordura, proteína e lactose. Os resultados demonstraram que não houve diferença significativa para as médias de gordura (P=0,06), entretanto, apresentaram diferença para proteína (P=0,001), maior para IR (3,23 vs. 3,33), e lactose (P<0,0001), maior para US (4,47 vs. 4,83). A correlação foi positiva e significativa para gordura (r=0,73, P<0,0001), proteína (r=0,47, P=0,001) e lactose (r=0,51, P=0,0003). Os resultados das análises de qualidade, considerando composição, CBT e CCS, atenderam a legislação vigente em 95,5%, 84,1% e 64,4% dos produtores, respectivamente. Concluiu-se que as metodologias afetaram os resultados, e estes podem ter sido influenciados pelo fator de correção, utilizado para calibração, sendo específicos para as amostras analisadas durante o período do estudo.*
Palavras-chave: *composição do leite, gordura, métodos rápidos, proteína, qualidade de leite.*

INTRODUCTION

Use of electronic methodologies in milk quality monitoring has gained space in the dairy chain because it provides quick and efficient responses on milk composition (HOLROYD, 2013) either for the dairy producer (TSENKOVA et al., 2000), or for the industry, in the receiving platform, in order to reach the best destination of the stocked milk and also the adequate use of parameters for quality payment

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(PONSANO et al., 2007).Besides that, they are non-destructive methods that allow a large amount of analysis in a short period of time (BUCKIN et al., 2003, TSENKOVA et al., 2000). Rapid milk analysis equipment can be based on the methods of infrared and ultrasonic spectroscopy.

Ultrasonic spectroscopy has as its principle the application of high frequency sound waves that print intermolecular forces to the materials of the system. Compression and decompression oscillating in the ultrassonic wave cause oscillations in the molecular arrangement of the sample, which responds with attraction or repulsion of intermolecular forces (BUCKIN et al., 2003); therefore, analyzed by transmission or reflection of the signals generated (BHARDWAJ, 2002). On the other hand, the infrared spectroscopy is based on the ability of the specific chemical groups of some milk components to absorb infrared radiation at different wavelengths, such as fat, protein and lactose (BIGGS et al., 1987). Infrared spectroscopy is widely used to determine milk composition (HOLROYD, 2013), and is the standard method adopted by the Brazilian laboratories government to analyzed milk quality, denominated "Rede Brasileira de Laboratórios de Controle de Qualidade de Leite" (RBQL), as well as the method being adopted for use in the identification of adulterated milks (POONIA et al., 2017).

With the challenges currently found in the milk chain, these and other analyzes are essential for determining the quality and safety of the end products. In addition, they should be able to identify fraud or milk quality problems. The objective of this study was to evaluate the quality of the milk received by a dairy, compare and correlate two methodologies for analyzing the chemical composition of this raw material. Secondly, verify if the milk samples collected complies the actual legislation of Brazil.

MATERIALS AND METHODS

Samples were collected between September 11 to 14, 2016, from five municipalities of the extreme west of Santa Catarina state, Brazil, as a probabilistic sampling carried out in 45 dairy producers, which delivery milk to the same dairy company. Samples were collected by trained dairy company staff, directly from the milk cooling tank of each dairy producer, where the following aliquots were collected: a sample with 300 mL of milk for the composition analyzes in the dairy company laboratory; a sample with 40 mL of milk in a sterile vial containing Bronopol (2-bromo-2-nitropropane-1,3-diol concentration between 0.017% to 0.023% m/v, and, natamycin between 0.00060% to 0.00093% m/v) for analysis of somatic cell count (SCC), fat, protein and lactose; and a sample with 40 mL in a sterile vial containing Azidiol (chloramphenicol concentration between 0.00045% to 0.00051% m/v, and, sodium azide between 0.011% to 0.012% m/v)for analysis of total bacterial count (TBC).Both of 40 mL vials were sent for analysis at the Dairy Herd Analysis Service (SARLE), University of Passo Fundo, to a laboratory accredited by the Ministry of Agriculture, Livestock and Supply, integrant of the Brazilian Network of Laboratories for Quality Control of Milk (RBQL). The vials containing the conservants (Azidiol and Bronopol)were homogenized by inversion until complete dissolution. All samples were remained under refrigeration (1-10 °C) until analysis.

Additionally, in the samples sent to the SARLE, analyzes of composition (fat, protein, lactose and total dry matter) were carried out using the mid-infrared radiation method (IR) (INTERNATIONAL..., 2013) and somatic cell count by flow cytometry (CF) (INTERNATIONAL..., 2006), by means of the electronic equipment *Bentley Combi System 2300®*, consisting of *Bentley 2000* (BENTLEY..., 1998) and *SomaCount 300* (BENTLEY..., 1997), respectively. The analysis of TBC was performed by the same laboratory, using the technique of flow cytometry (CF) (INTERNATIONAL..., 2004), by means of the electronic equipment *IBC BactoCount 150* (BENTLEY..., 2007).

In parallel, the replicate of each sample was used for determination of fat, protein, lactose and density by the ultrasound method (US) using the portable ultrasonic milk analyzer (*Ekomilk Total®)* at dairy company laboratory. In addition to these, titratable acidity tests (BRASIL, 2006) and pH (with bench potentiometer, *AKSO®*) were performed.

The instruments calibration and operation for raw milk analisys were performed according to ABNT NBR 17025 (2005) standards and BENTLEY (1997, 1998) manufacturer's instructions. The official laboratory calibrates its equipment twice a month, while the company laboratory calibrates its equipment weekly. Both laboratories calibrate the equipment more often when necessary. The lab has a testing routine for sample quantity obtained from the supplier, comparing to the data bank values, to determine the calibration coefficient according to the Ministry of Agriculture, Normative Instruction No. 68 (Brazil, 2006).

Data on milk quality were presented in a descriptive way. To perform the mean tests, Student's

T or WillCockson tests were used, according to the normality and homoscedasticity of the variances analyzed by the Shapiro-Wilk and Bartlett tests, respectively. When possible, data transformations were performed using quadratic or logarithmic transformations. Pearson correlation was performed between the fat, protein and lactose variables, considering the infrared and ultrasound methods of analysis.

RESULTS AND DISCUSSION

Considering the milk physicochemical composition, the comparison between ultrasonic (US) and infrared (IR) analysis did not show significant difference for milk fat (P>0.05). However, there was a significant difference for protein and lactose (Table 1). The results showed positive and significant correlations for fat (r=0.73, P<0.0001), protein (r=0.47, P=0.001) and lactose (r= 0.51, P=0.0003).In the US method, the parameters used in the analysis of the variation of the components are the attenuation and the velocity of the emitted sound (DUKHIN et al., 2003). The concentration of fat is directly related to attenuation property, which is determined by the dispersion of ultrasonic waves in non-homogeneous samples, such as emulsions and suspensions (BUCKIN et al., 2003). Besides the lipid concentration, the degree of homogenization also influences the attenuation of the ultrasound, due to thermal losses resulting from the transfer of heat from the aqueous medium to the fat globules and contrariwise (MILES et al., 1990). On the other hand, by the IR method the amount of radiation absorbed in the transmittance at specific wavelengths is recorded in the mid-infrared region (INTERNATIONAL..., 2013), in which the carbonyl groups (C=O) of the triglyceride ester bonds absorb radiation at the wavelength of 5.723 μm and the carbon-hydrogen groups (C-H) of 3.48 μm (BARBANO & CLARK, 1989; LYNCH et al. 2006). Despite the possible influence of the homogenization of the sample on the results, there was no significant difference (P>0.05) between the methods for the means of fat (P=0.06), indicating absence of the influence of this factor in the present research. The results of the comparison of the evaluated methods for fat disagree with the results obtained by PINTO et al. (2008), who found a significant difference in the comparison of the same methods (P=0.0005). On the other hand, its correlation was strong and positive (r=0.98) for this component, which agrees with the similar positive and significant correlation obtained in this study (r=0.73, P<0.0001).Similarly, in another study by MELO et al. (2018), using buffalo milk, it was possible to identify that there was also a significant difference in the comparison between the infrared and ultrasound in fat analysis, however, the correlation was also high and positive (r=0.84108, P<0.0001), which determines that ultrasound spectroscopy, can also be used in the evaluation of this component.

For protein, in the ultrasound spectroscopy the concentration of colloids is determined from the evaluation of the mechanical disturbances caused by the sound waves when passing through the sample (MILES et al., 1990; BUCKIN et al., 2003; PONSANO et al., 2007). Somehow, it can be affirmed that the ultrasound is scattered and absorbed while propagates through the heterogeneous system (DUKHIN et al., 2003), since milk is characterized as an emulsion of fat globules and a suspension of casein micelles in an aqueous phase, along with lactose, serum proteins and solubilized minerals (GONZÁLEZ, 2001). In the IR method, the amide groups (CONH) of the peptide bonds of the proteins absorb radiation at the wavelength at 6.465 μm (BARBANO & CLARK, 1989; LYNCH et al., 2006). The mean protein levels differed (P=0.001) between the two methods, but the correlation was positive and significant (r=0.47, P=0.001. As in fat, the mean protein content was higher in IR method compared to the US method. According to DÜRR et al. (2001), in the determination of protein by the IR method, the lactic acid present in the sample is interpreted as protein, which may cause an overestimation of this total component, and therefore explain the significant difference found in the present research.

As well as the protein, the lactose is the dispersed phase in aqueous medium, being evaluated in the same way in the US method. But by the IR method, the hydroxyl groups (OH) of lactose absorb radiation at wavelengths of 9.610 μm (BARBANO & CLARK, 1989; LYNCH et al., 2006). There was also a significant difference (P<0.0001) between the methodologies evaluated for this component, being the parameter that had the largest difference between the means and the only one that there was a decrease in the percentage in the IR in relation to the US. In fact, lactose is the component that is most altered with storage time due to its depletion in lactic acid, because of the action of microorganisms (DÜRR et al., 2001). However, in this study the correlation between the methods was moderate (r=0.51, P=0.0003).

According to MARTINS et al. (2009), the effect of the Bronopol can be influenced by the level of contamination of the sample, because according to its study, the conserve has as much bacteriostatic

Table 1 - Comparison of fat, protein and lactose content of raw bovine milk, evaluated by infrared *versus* ultrasound method (n = 45).

Parameter	Mid-infrared[1]	Ultrasonography[1]	*P*
Fat, %[2]	4.11 ± 0.36	4.03 ± 0.41	0.06
Protein, %[2]	3.33 ± 0.21	3.23 ± 0.18	0.001
Lactose, %[3]	4.47 ± 0.11	4.83 ± 0.27	<0.0001

[1]Mean followed by standard deviation; [2]T Test; [3]WillCockson Test.

effect in samples of milk with TBC below 10^5 UFC/mL and bactericide in samples with TBC higher than 10^6 UFC/mL. Physiologically, the concentration of lactose is constant because it acts in the control of water inflow, due to its role in the maintenance of osmotic balance between milk and mammary gland (GONZÁLEZ, 2001), due to this fact, a low variation around the mean of the analyzes already corresponds to the statistical difference.

It is important to note that in ultrasonic spectroscopy it is possible to verify that the measurement of the transmitted energy is not affected by the multiple scattering, which occurs in infrared spectroscopy, for example. The forward ultrasonic propagation pulse precedes multiple scattering, however, it only decays (attenuation) due to single scattering, completely eliminating the multiple scattering problem, being one of the major advantages of ultrasound based techniques in relation to light, such as the infrared (DUKHIN et al., 2003).

The chemical composition analyzes performed in the study (Table 2) showed that the results met the minimum parameters required by current legislation (BRASIL, 2011) in 95.5% (43) of the producers. In TBC, it was observed that the median was below the mean, which justifies the high standard deviation. However, the results were satisfactory, since 84.1% (37) of the samples were within the parameters required by the legislation (<300000 UFC/mL) and only 15.9% (7) of the samples were above the permitted level (>300000 UFC/mL) (BRASIL, 2016).

According to TRONCO (2010), for a normal udder state, the ideal is less than 250000 SC/mL. Only 15.6% of the samples could be considered inside the

Table 2 - Physical-chemical and microbiological quality of milk, according to analyzes performed in an official laboratory of the Brazilian Network of Laboratories for Quality Control of Milk (RBQL, by the mid-infrared radiation method) and in a laboratory of a commercial dairy (by ultrasound method).

Parameter	Mean ± SD	Max.	Min.	Median	*n*
----------Lab of RBQL (Mid-infrared method)----------					
Fat (% w/w)	4.11 ± 0.36	4.89	3.56	4.03	45
Protein (% w/w)	3.33 ± 0.21	3.79	2.86	3.31	45
Lactose (%)	4.47 ± 0.10	4.64	4.03	4.48	45
Total dry matter (%)	12.87 ± 0.56	14	11.53	12.82	45
SCC (mi SC/mL)[1]	480 ± 271	1579	86	434	45
TBC (mi CFU/mL)[1]	197 ± 519	2447	3	24.5	44
----------Dairylab (Ultrassoundmethod)----------					
Fat (%)	4.03 ± 0.41	4.87	3.3	4	45
Protein (%)	3.23 ± 0.18	3.41	2.21	3.27	45
Lactose (%)	4.83 ± 0.27	5.09	3.29	4.87	45
Dornic (°D)	15.7 ± 0.8	18	13	16	45
pH	6.85 ± 0.05	6.92	6.72	6.85	45
Density (g/mL)	1.031 ± 0.0008	1.0319	1.0274	1.031	44
Cryoscopy (°H)	-0.540 ± 0.006	-0.527	-0.559	-0.539	45

[1]Flow cytometry method.

ideal state standards for the mammary gland. When the individual values are compared with current legislation, 64.4% (29) samples were inside the allowable parameters (<500000 SC/mL) (BRASIL, 2016), 26.7% (12) between 500000 and 750000 SC/mL, and only 8.9% (4) were above 750000 SC/mL. According to VOGES et al. (2015), there is a positive relationship between TBC and SCC, which determines that the same factors that affect the microbiological quality of milk affect the health of the mammary gland, justifying that properties with hygiene problems are very likely to have problems also with subclinical mastitis. It can be confirmed by the CHRAMOSTOVÁ et al (2016) study, that found a positive and significant correlation ($P<0.05$) between the total mesophilic bacteria count and somatic cell count (SCC) ($r=0.3126$).

In this sense, of the 7 producers with high TBC, all of them also presented SCC above adequate levels for a healthy mammary gland, which according to TRONCO (2010), 250000 SC/mL. In addition to the sanitary parameters involved in high SCC, it also influences the accuracy of determination by infrared spectroscopy, where studies by TSENKOVA et al. (2001) concluded that the use of high SCC milk samples in a dataset for calibration or prediction strongly influenced the accuracy of fat, protein and lactose determination, increasing the standard error of prediction and significantly reducing the correlation coefficient. Therefore, it is necessary to use milk with low SCC for calibration of equipment based in IR method, situation which suggests research on the influence of SCC on the analysis also by US.

MELO et al., (2018) indicate that the differences between results of the determination of protein and lactose of buffalo milk for the infrared method and ultrasonic analyser is possibly due to calibration, use of preservatives and time between the collection and samples analysis. In this way the observed difference between the results obtained by the infrared ultrasonic methodology can be related to the calibration correction factor, used for each method, considering each analytical procedure, as well as, the samples used to compose this factor, which are not necessarily from the same group

CONCLUSION

The comparison of the ultrasonic and infrared methodologies in the milk analysis showed differences in the results, and the positive correlation between the methodologies is regular. Considering that two out of three evaluated parameters differed (protein and lactose).

The ultrasonic and infrared methodologies comparison in the milk analysis showed differences in the results, and the positive correlation between the methodologies is regular. Considering that two out of three evaluated parameters differed (protein and lactose), it is suggested that the processing industries use only one of the methodologies for decision-making process. Yet more research about the influence of composition, time of the year and frequency of collection for analysis on both methods, is required.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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