Use of electrical bioimpedance analysis to evaluate the quality of bovine raw milk

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Abstract

The objective of the present study was to use electrical bioimpedance analysis (BIA) as an alternative method of evaluating the quality of bovine raw milk. Fourteen lactating purebred Dutch cows were used. For the analyses of milk quality and electrical bioimpedance, 140 ml milk per mammary quarter and a homogeneous sample of each animal were collected, totalling 70 samples. The criteria used to evaluate milk quality consisted of somatic cell count (CCS), total bacterial count (CBT), fat, protein, lactose, total solids (ST) and non-greasy solids (SNG) as required by IN No 62/2011. A monofrequential 50 kHz and 800 µA alternating current generator was used for electrical BIAs. The temperature of the raw milk was 5 °C for all samples. Primary resistance and reactance readings were obtained. From these values, the values of impedance, conductance and phase angle were obtained. Correlation analyses were performed to determine the variables used by the regression analyses. The regression analysis was performed using raw milk components as dependent variables and BIA measurements as independent variables. Lactose, ST and SNG were the components of milk that correlated best and presented moderate prediction equations in relation to BIA variables. The prediction equations using resistance showed R^2 of 0.42 and 0.45 for ST and SNG, respectively. In the prediction equation for ST and SNG, the conductance resulted in R^2 of 0.45, and 0.45, respectively. Regarding impedance, the R^2 of 0.42 and 0.45 were obtained for the predicted equations ST and SNG, respectively. Thus, it can be concluded that BIA has the potential to estimate the composition and quality of bovine raw milk.

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Introduction

Milk production is one of the most important chains in the Brazilian agroindustrial complex. The agribusiness of milk and its derivatives plays an important role in the supply of food, job and income generation for the population (Embrapa, 2012).

To ensure the quality of the raw material and dairy products that arrive on the shelves, and to help producers to meet the standards demanded by the international market, the analyses that are required by Regulatory Instruction 62 of 2011 (IN 62/2011) are of extreme importance to be able to standardize a profile of the milk produced in the country.

With the objective of searching for new technologies and methodologies that can contribute to and assure the identification of milk quality, the use of BIA is a possibility for analysing the electrical properties of biological objects (Eickemberg *et al.*, 2011). It can contribute to promoting an advance in the immediate diagnosis of the quality of the bovine raw milk and to producing high-quality milk that can compete in the international market.

Electrical bioimpedance is a non-invasive easy-to-perform fast method to evaluate milk quality. Besides, it is portable and inexpensive compared with other traditional methods (Berg *et al.*, 1996). BIA is based on the principle that tissues can be considered a circuit, which is formed by elements of resistance and capacitance, in which they offer different oppositions to the passage of alternating electric current, called impedance (Z) (Baumgartner *et al.*, 1988, Piccoli *et al.*, 2002). Impedance is determined by the primary resistance (Rs) and capacitive reactance (Xc) measurements that are obtained through the bioimpedometer.

The resistance vector measures the direct opposition of the electric current flow when it passes through the intra and extracellular means (Eickemberg *et al.*, 2011). Muscle tissues and proteins are considered electrical conductors and can measure conductance (C). That is, they have low electrical resistance (Avril *et al.*, 2013). On the other hand, Mabrook & Petty (2003) demonstrated that milk lactose has little effect on electrical conductivity (EC), whereas the presence of fat results in a decrease in milk conductance. Because lactose and fat present a lower amount of liquids and electrolytes, this causes a decrease in conductance and, as a result, a high resistance value.

Reactance is the opposition to the electric flow, which is caused by capacitance. It is believed that cell membranes function as electrical capacitors because there is a fat layer between two protein layers with intense biological activity and a hydrophilic property (Cintra *et al.*, 2010) where energy is stored in the form of electrons for a short period. This cell membrane structure causes a delay in the electric current, which can be measured by the BIA equipment as Xc, and can be used as an indicator of the amount of lean mass in humans (Scheunemann *et al.*, 2008).

For more than a decade, bioimpedance has been investigated in order to explore the electrical properties of animal tissues. Several methods that are based on the concepts of bioimpedance are widely used in fields of knowledge such as electrochemistry, biology, medicine, evaluation of the bacteriological quality of raw milk, adulterations and evaluation of the bovine, ovine, and buffalo carcasses.

However, few studies have been conducted with the intention of exploring the electric bioimpedance technique in bovine milk. Therefore, this experiment was developed with the objective of using bioimpedance analysis to evaluate the quality of bovine raw milk.

Material and Methods

The milk samples were collected in a single day in April 2018 at the Dairy Cattle Laboratory of the Department of Animal Science of the Federal University of Santa Maria (UFSM), Santa Maria, RS. The data were obtained from 14 purebred Black and White Dutch cows, which had milk samples collected in the morning milking between 8h00 and 9hoo by mechanized milking, using four sets of individual collection cups.

During the collection of the samples, the first milking jets were rejected. Soon afterwards, milk samples were collected to perform the California mastitis test (CMT). Following this test, udder hygiene was performed.

The procedure of milk collection was conducted in this sequence: pre-disinfection of the teats with iodine solution; drying of the teats with a paper towel; and manual collection of 140 mL of milk from each udder. A sample of each teat and a homogenous sample of each animal were obtained to carry out the bioimpedance tests. The milk was packed in a clean dry plastic container. From the total volume of milk, 40 ml were collected for analysis of the bacterial count. These were inserted in a sterile container with an azidiol tablet for preservation. Five samples were collected from each animal, totalling 70 samples.

After collection, the samples were conditioned in a thermal box and taken to the laboratory for analyses by electrical bioimpedance. In the first analysis, the milk was at a temperature of 28 °C. After the first measurement, the samples were kept in the refrigerator until they reached a temperature of 5 °C. At that temperature, new bioimpedance measurements were performed.

The BIA measurement was performed with a single-frequency device (Model RJL Quantum II bioelectrical body composition analyser) alternating current generator of 50 kHz (high frequency) and 800 µA (low amplitude). This device has four electrode terminals (four-pole technique), which are fixed by electrical cables that are coded by colour. The black electrodes are configured as inductors and the red ones as current detectors.

For the bioimpedance analysis, a system was developed in which raw milk samples were inserted into a plastic container measuring 13 x 7 cm. The electrodes were connected to Singer® needles, measuring 6 cm. To guarantee standardization of the distances between the electrodes, these needles were inserted in a piece of polystyrene measuring 9 x 5 cm. The 1.5 cm contact with the milk was maintained for the transmission of the current without interrupting the signal. The inducing electrodes were inserted into the polystyrene piece a distance of 2 cm from the wall of the container material. The distance between the inductive and receptor electrodes was also 2 cm, and the distance between the inductive electrodes was 6 cm (Figure 1).
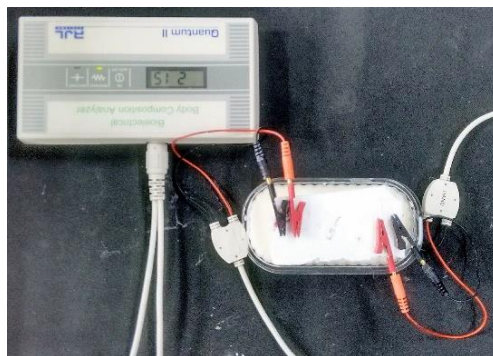
Figure 1: Representative methodology of electrical bioimpedance measurements in bovine raw milk
Source: author's personal archive

At the end of the BIA measurements, the milk from each sample was inserted in a bottle containing the preservative Bronopol. All the samples were stored in thermal boxes containing recyclable ice and sent to the Dairy Herd Analysis Service (SARLE) of the University of Passo Fundo (UPF). The analyses of fat, protein, lactose, total solids (ST), and non-greasy solids (SNG) were performed through the infrared method. Somatic cell counts (CCS) and plaque counts (CBT) were performed using the flow cytometry method.

The primary values of BIA were obtained in values of resistance (Rs) and reactance (Xc) through the bioimpedanciometer. From these measurements it was possible to calculate the conductance (C), which is the inverse of the resistance (1/Rs) and the impedance (Z), calculated as the sum of the resistance and reactance squares (Z = Rs2 + Xc2) (Lukaski *et al.*, 1985). The phase angle was obtained through the arc-tangent relationship of Xc and Rs, where the result obtained, expressed in radians, was multiplied by 180 °/π = 57,296 to convert to degrees (Lukaski, 2013). Thus, the AF formula is expressed as AF = [tan-1 (Xc/Rs) x 180 °/π] (Baumgartner *et al.*, 1988).

The milk variables and BIA measurements were tabulated in an Excel spreadsheet and analysed for correlation observation at the (P <0.05) probability level. In addition, regression studies with significant correlations were performed using the PROC REG procedure through the statistical software SAS (version 9.4, 2016) (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Table 1 shows the average, minimum, maximum standard deviation and coefficient of variation (CV) of bioimpedance measurements in bovine raw milk at a temperature of 5 °C and 28 °C.

Table 1 Bioimpedance evaluations of bovine raw milk samples at 5 ° C and 28 ° C of the mammary quarters

BIA components (5 °C)	Average	Minimum	Maximum	Standard deviation	CV (%)
Resistance, Ohms	271,54	174	384	47	17,30
Reactance, Ohms	-0,6	-3	0	0,69	115
Impedance, Ohms	271	174	384	47,55	17,34
Conductance, Ohms	0,003	0,002	0,005	0,0006	20
Phase angle°	-0,11	-0,4	0	0,12	109
BIA components (28 °C)	Average	Minimum	Maximum	Standard deviation	CV (%)
Resistance, Ohms	149	101	215	28	18,79
Reactance, Ohms	0	0	0	0	0
Impedance, Ohms	147	0	215	33	22,44
Conductance, Ohms	0,006	0,004	0,009	0,001	16,66
Phase angle	0	0	0	0	0

[1]CV: coefficient of variation

The largest variations (CV 115–109%) were found for reactance (5 °C) and phase angle (5 °C), respectively. For the values of conductance (28 °C), resistance (5 °C) and impedance (5 °C), the CV

variations were lower (16.66–17.30–17.34%, respectively). The coefficient of variation values for resistance (28 °C), conductance (5 °C) and impedance (28°C) were moderate (18.79%, 20% and 22.44%, respectively).

The electrical impedance results for resistance and reactance of the cooled (5 °C) raw milk were higher (271 and 0.6) than the results for the raw milk at 28 °C (149 and 0) (Table 1). These differences were expected because conductance (1/resistance) decreases proportionally with the reduction of temperature (Berg *et al.*, 1997).

Similar results were found by Mabrook et al. (2003), in which the electrical conductance of milk samples, measured at 100 kHz, showed a linear increase with rising temperature. Similarly, Nielen (1992) reported that there is a positive linear relationship between the increase in temperature of milk samples and the increase in EC (0.113 mS/ºC) when evaluating milk temperature between 15 ºC and 40 ºC.

The bioimpedance technique, when used to evaluate the quality of the body composition of cattle, swine, sheep, buffaloes and rabbits, showed that the characteristics of the carcass, morphology, electrode positioning, and homogeneity of the parameters directly influenced the measurements (Altmann *et al.*, 2004; Avril *et al.*, 2013; Schäff *et al.*, 2017).

According to Bertemes-Filho *et al.* (2010), an impedance value of 89 ohms was found when analysing bioelectrical impedance for bovine milk. In the same study, in which distilled water or hydrogen peroxide were added to detect possible adulterations, impedance values were found of 114 and 82 ohms, respectively.

The decrease in the impedance of the milk with hydrogen peroxide can be because the dissolution of the hydrogen peroxide in the milk increases the concentration of free ions in the sample. However, in the samples to which distilled water was added, there was an increase of Z. This is because there is a decrease in the concentration of ions in the sample, because the ions are removed when distilling the water. Consequently, the conductivity of the solution decreases and the impedance increases.

According to Santos (2005), the EC of the milk of healthy cows varies from 4 to 5 (mS/cm). Norberg (2005) found that in subclinical cases of mastitis, EC presented 5.75 mS/cm and in clinical cases 6.73 mS/cm. In the present study, the results of C are expressed in ohms and not in siemens (mS/cm). Even though these units are reciprocal, the values are below those presented in the literature.

Variations in the phase angle indicate changes in body composition, cell membrane function and health status. Lower phase angle values represent low reactance and high strength, and may be associated with cell death or some change in the membrane selective permeability. On the other hand, higher values of FA represent high reactance and low resistance, which can be associated with the greater quantity of intact cellular membranes (Gupta *et al.*, 2004).

The correlations between the BIA measurements and the components of the raw milk composition are presented in Table 2. When the results were analysed in terms of temperature, the best responses were found at 5 °C.

Table 2 Pearson coefficient of correlation between qualitative components of the milk at 5 °C and measurements of bioimpedance

	Rs, Ohms	Xc, Ohms	Z, Ohms	C, Ohms	AF,°
CCS x (1000 Cél/ml)	-0,26*	0,2^{ns}	-0,26*	0,27*	0,21^{ns}
CBT x (1000 UFC/ml)	-0,28*	0,22*	-0,28*	0,29*	0,23*
Fat (g/100 g)	0,07^{ns}	-0,11^{ns}	0,07^{ns}	-0,08^{ns}	-0,08^{ns}
Protein (g/100 g)	0,24*	-0,22^{ns}	0,24^{ns}	-0,23*	-0,22^{ns}
Lactose (g/100 g)	0,46**	-0,26*	0,46**	-0,49**	-0,28*
ST (g/100 g)	0,45**	-0,34**	0,45**	-0,48**	-0,34*
SNG (g/100 g)	0,53**	-0,37**	0,53**	-0,56**	-0,38**

AF: phase angle; C: conductance; CBT: total bacterial count; CCS: somatic cell count; Rs: resistance; SNG: non-greasy solids; ST: total solids; Xc: reactance; Z: impedance
^{ns} not significant; *P <0,05; ** P <0,01

The CCS variable of milk composition had a positive correlation of 0.27 with conductance (P >0.05) and negative correlation -0.26, -0.26 for Rs and Z (*P* >0.05), respectively. On the other hand, the AF and Xc presented no correlation with the CCS.

The correlation between CCS and C was expected. By raising the level of CCS in the milk, damage is caused in the epithelial tissue, which results in the destruction of the active system of ion transport and, as a consequence, a decrease of the solid components of the milk and the permeability of the tissues, leading to the reabsorption of lactose and infiltration of blood serum. Thus, milk saline conductance increased, lowering the concentration of lactose and K and increasing Na + and CL- ions (Nielen *et al.*, 1992), which pass into the alveolar lumen of the mammary gland, interfering with the stability of the milk.

The increase in the concentration of minerals should not be considered favourable to the quality of the milk, since more noble mineral elements, such as Ca and K, are replaced with other minerals, such as Na + and Cl-, which have less nutritional importance (Neville *et al.*, 1983; Vargas *et al.*, 2014).

Santos *et al.* (2015) emphasized that EC can be used to evaluate mastitis, provided fat is taken into account. When Nascimento *et al.* (2010) conducted the study on the use of EC measures for the detection of the addition of water, sodium chloride and caustic soda in milk, it was proved that EC measures could be used for fraud verification in milk, and for the early diagnosis of mastitis.

CBT was positively correlated (0.22 and 0.29) (P <0.05) with Xc and C and negatively (-0.28, -0.28, -0.23) with the measurements of Rs, Z, and AF, respectively. Piton *et al.* (1998) used impedance variations to evaluate changes in milk composition during microbial growth, and concluded that Z and C can be used for the detection of bacteria in milk. Sokolinska *et al.* (2017) used impedance in the production of kefir to estimate and control the quality of the fermentation and the metabolism of the activity of the bacterial microflora, since, for its production, it is necessary to add bacteria for the lactic fermentation.

The percentage of milk fat did not correlate with BIA variables (P >0.05). According to Mucchetti (1993), total milk fat takes the form of large globules covered with a thin non-conductive membrane, which prevents the mobility of ions (Nielen, 1992; Mabrook & Petty 2003).

The result presented in this research can be explained because the milk samples had low fat percentage, and there were few resistive obstacles to impede the passage of ions. Santos *et al.* (2015), when analysing electrical conductivity and CCS by simple linear regression, did not find any difference between the coefficients, but when considering the multivariate regression, the fat item presented a positive association (R2 = 0.32).

According to Veiga & Bertemes-Filho (2012), when analysing the bioelectrical impedance of bovine milk fat, found impedance values for whole milk of 219.56 ohms, semi-skimmed at 203.57 ohms and skimmed at 170.08 ohms. The same authors also reported that by decreasing the milk fat content, the conductivity increased. The percentage of proteins was not correlated (P >0.05) with reactance, impedance and phase angle. If the variables resistance and conductivity are considered, there was a positive correlation of 0.24 for both (P >0.05). In the study by Juozaitienė *et al.* (2015), when correlating electrical conductivity and protein, a strong but negative correlation between the variables was found (r = -0.77) (P <0.001).

Although casein has low conductance in relation to milk salts, it may still have an influence on conductivity. This occurs because milk-insoluble salts, especially calcium phosphate, are associated with casein micelles in the colloidal phase. In certain conditions, these salts can pass into the soluble phase, becoming free and increasing the conductivity of the milk (Mabrook & Petty 2003).

The lactose presented a positive correlation (P >0.05) with the resistance (r = 0.46) (P <0.01) of the variables and a negative correlation with reactance (r = -0.26) and with the phase angle (r = -0.28) (P >0.05). The measurements of impedance and conductance were not correlated with lactose. Mabrook & Petty (2003) reported that there is little contribution of lactose to the values of milk conductance.

Total solids presented a positive correlation (r = 0.45) (P <0.01) with Rs and Z and negative correlation (r = -0.34, -0.48 (P <0.01), -0.34 (P <0.05) with Xc, C, and AF, respectively. The content of SNG showed negative correlations (r = -0.37, -0.56, -0.38 (P <0.01), with Xc, C and AF, respectively, and a positive correlation (r = 0,53; P <0,01) with those found for Rs and Z. These results are explained by the direct relation of impedance to - resistance and reactance (opposition to the current flow caused by the capacitance produced by the cell membrane) and the inverse relationship of the conductance with the resistance.

The high values of CCS increase the concentration of minerals Na and Cl, which therefore may influence the other variables of the BIA and the milk (Veiga *et al.*, 2012). According to Lima *et al.* (2006), in acute clinical or subclinical mastitis, lactose tends to decrease owing to the lower synthesis of milk caused by the destruction of secretory tissue. Thus, there is an increase in the permeability of the membrane and the use of lactose by intramammary pathogens.

According to Noro *et al.* (2006), when there is an increase in CCS levels, the levels of fat increase as well. This may be because the possible reduction in milk production is more pronounced than the reduction in fat synthesis. However, these results were not confirmed in the present study.

According to Lima *et al.* (2006), the total protein in milk with high CCS remains unchanged or undergoes minor changes. However, modifications of the protein content take place when serious clinical

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signs of mastitis appear, with a decrease in the fraction of casein through proteolysis and alteration of the permeability of the blood capillaries that allow the influx of serum proteins (albumin and immunoglobulins) into the mammary gland (Pereira *et al.*, 1999, Machado *et al.*, 2000).

The content SNG decreases with the elevation of CCS, since it may be associated with the behaviour of lactose. However, the ST content increases with high levels of CCS, which is related to the result that was found for milk fat (Vargas *et al.*, 2014).

According to some researchers, when milk has high CCS values, CBT has a tendency to increase or decrease, since there is no correlation between these variables. This reinforces the different behaviour, considering the microorganisms that cause the inflammation of the mammary gland (Harmon, 1994; Vargas *et al.*, 2014).

In Table 3, the prediction equations of the raw milk components are presented from the bioimpedance measurements. The variables lactose and ST explained R2 0.31% and 0.42% (P <0.05), respectively, and the SNG accounted 45% (P <0.01) of variation from the resistance variable. The raw milk variables were estimated by increasing linear equation.

Table 3 Predictor equations of bovine raw milk components from bioimpedance measurements

Equações	R^2	CV (%)	P>F
Resistance (Rs)			
Lactose (g/100 g), Y = 2,91 + 0,005. Rs	0,31	7,59	0,0119
Total solids (g/100 g), Y = 6,82 + 0,01. Rs	0,42	5,34	0,0381
SNG (g/100 g), y = 6,16 + 0,009. Rs	0,45	4,65	0,0081
Reactance (Xc)			
Total Solids (g/100 g), Y = 9,48 - 0,65. Xc	0,32	5,79	0,0344
SNG (g/100 g), y = 8,38 - 0,45. Xc	0,25	5,45	0,0666
Conductance (C)			
Total solids (g/100 g), Y = 13,02 - 824,30. C	0,45	7,63	0,0082
SNG (g/100 g), y = 11,12 - 549,82. C	0,45	4,63	0,0078
Impedance (Z)			
Lactose (g/100 g), y = 2,91 + 0,005. Z	0,31	7,59	0,0381
Total solids (g/100 g), y = 6,82 + 0,01. Z	0,42	5,34	0,0119
SNG (g/100 g), Y = 6,16 + 0,009. Z	0,45	4,65	0,0081
Phase angle (AF)			
Total solids (g/100 g), Y = 9,46 - 3,83. AF	0,32	5,76	0,0325
SNG (g/100 g), Y = 8,35 - 2,74. AF	0,27	5,38	0,0559

CBT: total bacterial count; CCS: somatic cell count; SNG: non-greasy solids; R^2: coefficient of determination; CV: coefficient of variation
*P <0,05; ** P <0,05.

The equation of the ST estimated from the measurement of reactance was explained with R2 of 32% (P <0.05), presenting an increasing linear effect. Although CBT, CCS, lactose and SNG showed correlation with Xc, this variable does not have enough interaction to explain the variation of milk components.

Items ST and SNG were estimated by linear decreasing equations with variable C. This result demonstrates that by decreasing the levels of ST and SNG, conductance increases. Regarding lactose components, ST and SNG were estimated by the equation with R2 of 0.31, 0.42 (P <0.05), and 0.45 (P <0.01), respectively, resulting in a linear one. In the study by Santos *et al.* (2015), when the multivariate regression analysis was calculated between the CCS, EC, and SNG items, no significant equation was found. It is observed that in the prediction equations of the raw milk components from the phase angle measurement, 32% (P <0.01) of the total milk solid total variation was explained, causing a linear decreasing effect.

The measurements obtained from the BIA as conductance, resistance and impedance are commonly used to verify the quality and composition of the milk, mainly CCS and CBT (Mucchetti *et al.*, 1993;

Bertemes-Filho *et al.*, 2010; Veiga *et al.*, 2012;). However, the reactance and the phase angle are variables that are considered unprecedented in the literature for evaluation of milk composition and quality. These variables are commonly used to measure body composition and indicator of survival prognoses in some clinical situations in human beings (Berbigier *et al.*, 2013; Ferreira *et al.*, 2015). However, these measurements had moderate correlations with the components of raw milk.

In this sense, more studies are needed on the use of new technologies to estimate milk composition or quality.

Therefore, more research should be developed to increase the accuracy of this technique and make it able to estimate the composition of raw bovine milk.

Conclusion

BIA is a potential method to estimate the composition and quality of raw bovine milk. However, more research is necessary to increase technical precision.

Authors' Contributions

LLS designed, collected and drafted manuscript; JV, SN and TJT analysed the data, drafted and revised the manuscript; LR, LC, ABM and SSR critically evaluated the manuscript.

Conflict of Interest Declaration

Conflict of Interest Declaration The author declares that she has no conflict of interest.

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