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Research Article

Innovative method for early detection of bovine mastitis through dielectric spectral analysis

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Abstract

The present work is aimed to study the effect of somatic cell counts (SCC) on the dielectric properties *viz.* dielectric constant (ε′) and dielectric loss factor (ε″) that could be correlated with the detection of mastitis in dairy cattle. A total of 100 samples were analyzed and found to have a mean SCC (10^3) ±SD of 106±97, 384±264 and 957±858 for healthy, subclinical and clinical mastitis samples collected from Hardhenu cows. The values of ε′ and ε″ of raw milk were measured over the frequency range from 4 to 2954 MHz at the ambient temperature (25°C) using a vector network analyzer and an open-ended coaxial-line probe. The results showed that the ε′ increased up to the frequency range of 4 to 804 MHz, beyond which the values decreased with increasing frequency in milk samples of healthy and mastitis animals. However, ε″ decreased linearly with increasing frequency range of 4 to 2954 MHz. The value of ε′ was significantly lower ($p<0.05$) as compared to subclinical and clinical mastitis milk samples. However, ε″ increased significantly ($p<0.05$) with increased SCC in milk samples. The present work concludes that the ε′ and ε″ could be a promising technique for quick identification of the presence of mastitis in the dairy cattle and provide information for developing the mastitis detector that being suitable for routine laboratory analysis or real-time quality monitoring based on dielectric properties of raw milk.

Keywords: California mastitis test, Dielectric constant, Dielectric loss factor, Mastitis, Somatic cell count

Highlights

- Milk samples had a mean somatic cell count of 106±97 and 384±264 for healthy and subclinical cases. Dielectric constant was significantly lower when compared to subclinical and clinical mastitis milk
- Dielectric loss factor increased significantly with increased somatic cell count in milk
- Dielectric constant (ε′) and dielectric loss factor (ε″) could be a promising technique for quick identification of mastitis in dairy cattle

INTRODUCTION

Mastitis affects overall milk production in animals and alters both the composition and technological properties of the milk. It leads to increased blood capillary permeability in the udder, allowing ions such as sodium, chloride and calcium to pass into the milk at elevated levels, thereby altering its ionic concentrations. This disruption of tight junctions between mammary epithelial cells facilitates the leakage of these ions from the bloodstream into the milk. Consequently, sodium and chloride levels rise, while potassium concentrations decrease as the mammary gland attempts to maintain osmotic balance. These ionic shifts contribute to elevated electrical conductivity and pH levels in the milk serving as indicators of mastitis. Change in the ionic concentration in mastitis milk causes an increase in electric conductivity above its normal value of approximately 4.6 mS/cm (Hogeveen & Ouweltjes, 2002; Huang et al., 2022).

The California Mastitis Test (CMT), the cow-side test is a highly effective tool for early detection and management of subclinical mastitis; however, its utility diminishes in cases of acute clinical mastitis. The CMT qualitatively assesses the deoxyribonucleic acid (DNA) concentration in milk secretions. Since DNA is predominantly present in white blood cells (WBCs), the test indirectly measures WBC counts. The CMT reagent lyses these cells, releasing DNA that forms a gel-like structure when react with the reagent. The degree of gel formation correlates with the WBC concentration, providing an estimate of the inflammatory response in the udder (George et al., 2008; Abdulkhader et al., 2022).

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In dairy cattle, somatic cell count (SCC) serves as a reliable indicator of subclinical mastitis, making it a crucial parameter for assessing milk quality, hygiene standards, and the effectiveness of mastitis control measures (Bergonier et al., 2003; Persson & Olofsson, 2011). The somatic cells, primarily composed of white blood cells (leukocytes), will be migrated to the mammary gland during both clinical and subclinical infections. This migration is a key component of the udder's inflammatory response to bacterial pathogens. An elevated somatic cell count indicates the presence of inflammation in udder (mastitis), which in turn affects the milk yield and quality, making SCC a critical indicator of health status in dairy farming. Milk with an elevated somatic cell concentration can be harmful to human health. It also contains less casein and results in lower cheese yields. It has elevated levels of enzymes that affect protein quality, alter fatty acid composition and influence lactose, ion and mineral concentrations as well as increases the pH of raw milk. These enzymes contribute to the development of off-flavors and odors. Therefore, the somatic cell count in raw milk plays a critical role in determining shelf life, flavor and especially cheese yield (Kumari et al., 2025).

As mastitis develops in dairy cattle, the mammary gland initiates an inflammatory response, leading to increased permeability of blood capillaries. This alteration allows an influx of immune cells such as neutrophils and macrophages and in severe cases, the presence of blood cells into the milk, which significantly elevate somatic cell counts. The capacitance of immune and blood cells is higher than that of healthy milk; therefore, an increase in immune cells or blood in the milk leads to higher capacitance in infected milk. The more severe the infection, the greater impact on the capacitance of milk is observed (Michael & Shoreview, 2013).

Numerous diagnostic tools for mastitis have been developed over time, some of which are still in use in the dairy industry today. However, detection of mastitis by traditional method is time consuming and laborious. Other methods are also not as efficient as they give false interpretation and not suitable for farmers. Further, no systematic study has been carried out to relate the mastitis with electrochemical properties of milk owing to the non availability of requisite data on above parameters. Therefore, present study was undertaken to see the effect of SCC on the dielectric properties *viz.* dielectric constant (ε') and dielectric loss factor (ε'') that could be correlated with the detection of mastitis in dairy cattle.

MATERIALS AND METHODS

Milk samples

This study was conducted for a period of three months (May–July 2022) on 100 lactating Hardhenu cows maintained in the cattle yard at Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. Early morning milk samples were collected from apparently healthy cows during their scheduled lactation periods. Before collecting the milk samples, the udder and teats were cleaned using a clean towel. For each quarter, the first few streams of milk (foremilk) were discarded to minimize contamination. Subsequently, the mid stream milk was aseptically collected from each quarter into a sterile bottle. The collected samples were transported to the laboratory within 20 minutes to ensure their integrity. Each sample was divided into two portions, one for analysis of dielectric properties and the other for determining the subclinical mastitis status of the animals through somatic cells.

California mastitis test and somatic cell count

California Mastitis Test (CMT) was performed using CMT reagent containing sodium lauryl sulphate with bromocresol purple (pH 7.0-7.5). The milk sample and CMT reagent were combined in equal parts in the paddle cups and mixed by gentle whirling of the paddle horizontally in a circular motion for about 10 seconds. A positive reaction was indicated by the immediate formation of a gel-like consistency, suggesting elevated somatic cell counts characteristic of subclinical mastitis (Abebe et al., 2016).

The somatic cell count in the milk was estimated by microscopic method using Newman-Lampert staining (Mammadova & Keskin, 2015). Animals having milk SCC below 2 lakhs/mL were categorized as healthy animals, whereas those with SCC between 2-5 lakhs/mL were considered as having subclinical mastitis. Animals with a milk somatic cell count exceeding 5 lakhs cells/mL were classified as having clinical mastitis (Alhussien & Dang, 2018).

Measurement of dielectric properties

The dielectric properties of raw milk were characterized by the dielectric constant (ε') and the dielectric loss factor (ε''). The ε' and ε'' spectra of raw milk, measured over a frequency range of 4 to 2954 MHz, were obtained using an open coaxial probe technique at 25°C within 6 hours after milking. The dielectric measurement system included planar R60 network analyzer and DAK-12 MSS probe. In this study, the dielectric spectra (ε′ and ε″) were recorded across 60 discrete frequency ranges for each sample. Prior to measurement, all samples were stirred using an electric stirrer for 1 minute to ensure uniformity. The dielectric constant and dielectric loss factor were calculated using the MATLAB-based Dielectric Probe Kit software, based on the reflection coefficient of the material in contact with the active tip of the probe.

Statistical analysis

The experimental data were analyzed using one-way ANOVA with the SAS 20 Enterprise software package to determine the mean, standard deviation (SD), and statistically significant differences among the means. A significance level of $p<0.05$ was used to define meaningful differences. Additionally, Pearson's correlation analysis was conducted at $p<0.01$ to evaluate the relationships among the defined characteristics.

RESULTS

Out of 100 milk samples screened, 27 animals were found to be positive for subclinical mastitis by CMT method. All animals that tested positive by the CMT were also showed exhibited elevated somatic cell counts. Specifically, 16 samples had SCC ranging from 2 to 5 lakh cells/mL, while 11 samples showed SCC exceeding 5 lakh cells/mL indicating clinical mastitis. The remaining 73 animals were CMT negative, with SCC below 2 lakh cells/mL suggesting healthy udder status.

The effect of frequency on dielectric constant (ε′) in healthy and mastitis milk samples over the frequency range of 4-2954 MHz at 25°C are shown in Table (1). The value of ε′ decreased significantly with increase in frequencies. When the frequency increased from 4 to 2954 MHz the value of ε′ decreased from 59.23±0.52 to 57.71±0.52 in healthy milk however in subclinical affected animals ε′ decreased from 67.67±0.53 to 67.25±0.30 and 66.78±0.54 to 65.12±0.55 in clinical affected animals. Dielectric constant of milk showed higher values in subclinical and clinical stages as compared to healthy animal with significant effect ($p<0.05$). The maximum value of ε′ was found at lower frequency range and minimum value at higher frequency range in healthy and mastitis animals (Fig. 1).

Table 1. Somatic Cell Count (SCC), dielectric constant (ε′) and the dielectric loss factor (ε″) of healthy and mastitis milk samples at different frequencies

Milk samples	Mean SCC(10^3) ±SD	Permittivity	Frequency (MHz) 4-804	804-1604	1604-2404	> 2404
Healthy	106±97[a]	ε′	59.23±0.52	57.55±0.45	57.23±0.45	57.71±0.52
		ε″	36.65±1.46	10.93±0.12	9.62±0.33	11.46±0.38
Sub-clinical	384±264[b]	ε′	67.67±0.53	67.46±0.12	67.25±0.17	67.25±0.30
		ε″	43.72±2.95	13.27±0.10	12.83±0.18	16.17±0.31
Clinical	957±858[c]	ε′	66.78±0.54	65.64±0.93	65.77±0.52	65.12±0.55
		ε″	47.43±3.73	14.74±0.17	13.91±0.14	16.28±0.25

Superscripts within column differ significantly from each other ($p<0.05$)

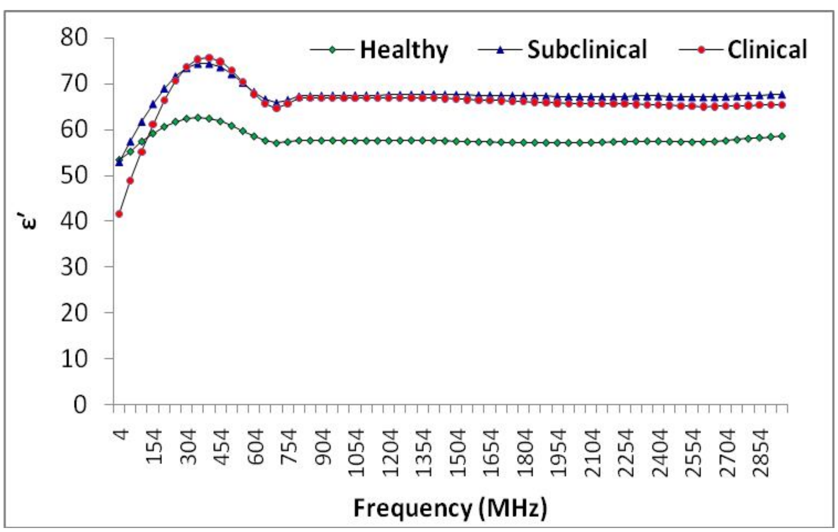

Fig. 1. Effect of frequency on ε′ in milk samples of healthy and mastitis animals over the frequency range from 4 to 2954 MHz at 25°C

Table (1) shows the effect of frequency on dielectric loss factor (ε″) in healthy and mastitis milk samples over the frequency range of 4-2954 MHz at 25°C. The value of ε″ decreased linearly with increasing frequency except the frequency range of >2404 MHz. When the frequency increased from 4 to 2954 MHz the value of ε″ decreased from 36.65±1.46 to 11.46±0.38 in healthy animals however in subclinical affected animals ε″ decreased from 43.72±2.95 to 16.17±0.31 while in clinical affected animals the value decreased from 47.43±3.73 to 16.28±0.25. The higher values of ε″ was observed at clinical stage as compared to subclinical and healthy stage of animals over the whole investigated frequency range of 4 to 2954 MHz with significance ($p<0.01$) (Fig. 2).

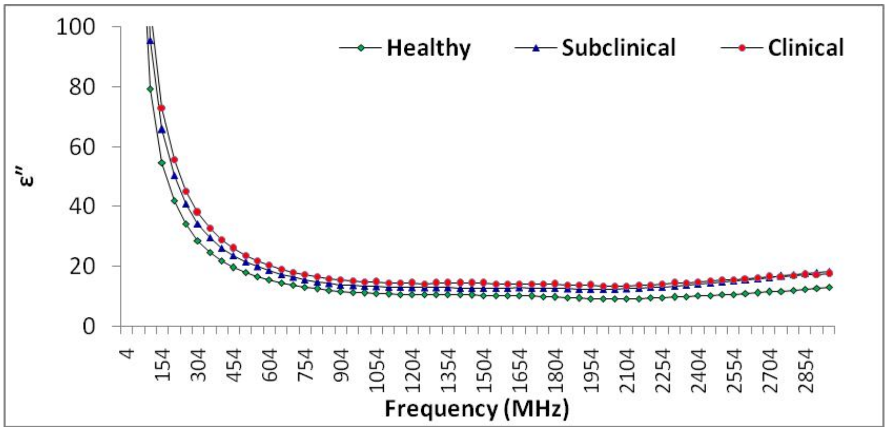

Fig. 2. Effect of frequency on ε″ in milk samples of healthy and mastitis animals over the frequency range from 4 to 2954 MHz at 25°C

A significant positive correlation ($p<0.01$) was observed between somatic cell counts (SCC), dielectric constant (ε′), and dielectric loss factor (ε″) by Pearson's correlation analysis. SCC demonstrated a strong positive association with both ε′ and ε″, indicating that as somatic cell levels increase, the dielectric properties of the milk are notably affected ($r = 0.279$ and 0.99).

DISCUSSION

In the current study, 27% of animals were found to have subclinical mastitis based on California mastitis test. Senthilkumar et al. (2020) reported that 24 out of 120 milk samples tested positive for subclinical mastitis using the CMT, while the remaining 96 samples were deemed normal. In our study, a similar correlation was observed between CMT positive samples and elevated somatic cell counts. All the CMT positive samples were having a higher somatic cell count above 2 lakh/mL. This finding aligns with previous research by Hoque et al. (2015), and Kasikci et al. (2012), who also reported a significant correlation between SCC and CMT, with higher CMT scores associated with elevated SCC levels. Additionally, Supriya et al. (2010) found that 35% of apparently healthy lactating cows had SCCs exceeding 5 lakh cells/mL. Vashisth et al. (2021) reported that 26.66% of crossbred cows in Haryana tested positive for mastitis by CMT and 23.33% had SCCs greater than 5 lakh cells/mL.

The presence of bound water and salts causes the dielectric constant to increase with temperature at low frequencies. Similar trends were observed in studies involving liquid whey protein mixtures and cheese sauces, where dielectric constants also rose with temperature at low frequencies (Kumari et al., 2025). Our findings are consistent with those of Michael and Shoreview (2013), who reported that the capacitance of immune and blood cells is higher than that of healthy milk. Consequently, an increase in immune cells or blood in milk leads to higher capacitance in infected samples.

In comparison to other studies that have examined the dielectric properties of milk (Coronel et al., 2003; Zhu et al., 2014; Zhu et al., 2015; Munoz et al., 2018) the results of this work are consistent and further support the relationship between somatic cell count and changes in dielectric behavior. These findings reinforce the potential of dielectric measurements as a valuable tool for assessing milk quality and detecting mastitis.

Somatic cells serve as a crucial indicator of udder health reflecting the presence of mastitis and directly influencing both milk yield and quality. The current study indicated that SCC exhibited a positive correlation between dielectric constant and dielectric loss factor of cow's milk which is a promising finding that can be used for the early detection of mastitis among bovines. Dielectric constant was high at lower frequencies and low at higher frequencies values. Dielectric loss factor could be an alternate technique for quick identification of the presence of mastitis in the dairy cattle. Its reliability can be further enhanced when combined with other quality indicators, providing a more comprehensive assessment of milk quality.

Conflict of interest: Authors have no conflict of interest in this study.

Data availability statement: The data used to support the findings of this study are included within the article.

Author's contribution: IP, JM, VK: Sample collection, carried out the work, analysis and interpretation of the results; IP, SM, BS: Designed, supervised and monitored the work regularly; IP, JM: Corrected the manuscript and interpreted the data analysis.

Ethical approval: This research did not require ethical approval as per our institute's norms because the samples were collected during routine milking procedure only.

Use of artificial intelligence tools: During the preparation of this work, the authors did not use any such artificial intelligence tools. The authors reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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