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Relationship between milk stability and physico-chemical characteristics of raw milk of different breeds during storage at ambient temperature

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Abstract

This study investigated the relationship between milk stability and physico-chemical characteristics of raw milk of four cow breeds in Sri Lanka: Sahiwal, Friesian Cross, Jersey Cross, and Australian Friesian Sahiwal. This study examined the changes in electrical conductivity (EC), pH, turbidity, and total soluble solids (TSS%) during an 8 h storage period at ambient temperature (28±1 ºC) and their correlation with the alcohol test results as a platform test. Results indicated that EC did not significantly change in the raw milk samples of Sahiwal, Friesian cross, or Australian Friesian Sahiwal breeds. Milk pH of all samples decreased with the storage period; however, significant ($P<0.05$) reductions of pH were observed in the Jersey cross and Australian Friesian Sahiwal breeds, compared to their initial pH of the raw milk samples 6.10±0.2 and 6.36±0.2, respectively. The turbidity of raw milk samples of the four cow breeds was significantly increased with the storage period ($P<0.05$). Australian Friesian Sahiwal breeds showed the highest turbidity development (241.0±3.7 NTU) while Jersey Crossbreeds showed the significantly ($P<0.05$) lowest turbidity development of 191.2±11.3 NTU. Both Sahiwal and Jersey Cross breeds significantly decreased ($P<0.05$) TSS content from 9.35 to 7.10% and 9.25 to 7.25% respectively with the storage period. The chi-square results indicated that there is no significant association ($P>0.05$) between alcohol test and the milk samples obtained from the different breeds. The significant negative correlation of turbidity ($\rho>-0.8$; $P<0.05$) and TSS% ($\rho>-0.4$; $P<0.05$) showed with the alcohol test in all cow breeds. Binary logistic regression results showed that the significant correlation between alcohol test and milk turbidity (Wald $\chi^2 = 4.44$, $P<0.05$). These findings highlight measuring the turbidity of raw milk would be a critical parameter for assessing milk stability and development of an *in-situ* instrument to quantitatively detect raw milk turbidity instead of the qualitative alcohol test.

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1. Introduction

Cow's milk is the most consumed type of milk worldwide and it possesses numerous applications in the dairy industry (More, 2009; Bekere et al., 2022). However, the dairy industry faces a challenge in obtaining quality milk for manufacturing purposes due to their inherent variability in the composition and diverse properties of raw milk (Bekere et al., 2022).

Milk, a complex colloidal system, contains a myriad of elements crucial to its stability and composition. The interplay of water, milk fat, proteins, vitamins, and minerals, coupled with the equilibrium between soluble and colloidal ionic compounds, plays a pivotal role in maintaining the stability of milk, especially during thermal processing (Holt, 2004; Yüksel & Erdem, 2005;

Burrow et al., 2019). The intricate balance of minerals such as phosphate (PO_4^{3-}), citrate ($HOC(CH_2CO_2H)_2$), chloride (Cl^-), sulphate (SO_4^{2-}), bicarbonate (HCO^-_3), sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) within the colloidal and liquid phases emphasizes the delicate balance essential for structural integrity of milk (Yüksel & Erdem, 2005; Burrow et al., 2019). The soluble and colloidal phases host different ions, with Na^+ and K^+ ions existing in a soluble state, while Ca^{2+}, Mg^{2+}, PO_4^{3-}, and citrate dominate the colloidal phase (Holt, 2004). This equilibrium between salts within casein micelles and the soluble phase becomes paramount during thermal processing, where any disruption can lead to protein destabilization and, subsequently, milk clotting (Pierre & Brule, 1981). Acidification of milk further complicates this equilibrium, as it solubilizes salts from the colloidal phase, destabilizing casein micelles and promoting protein coagulation (Zhao & Corroding, 2016a).

Turbidity which is measured in Nephelometric turbidity units (NTU) is a measure of cloudiness in a liquid. In milk, this cloudiness arises from protein coagulation, particularly due to the denaturation of the albumin and whey protein component (Rakib et al., 2014). This denaturation leads to the formation of aggregates and complexes that scatter light, causing an increase in turbidity. Detecting turbidity in fresh milk samples before processing becomes crucial, and various methods, including light reflection and infrared absorption, have been employed for this purpose (Banon & Hardy, 1991). Recent advancements propose novel techniques, such as utilizing Near Infrared digital cameras integrated with image processing technique, to accurately measure milk turbidity (Zhu et al., 2020).

Total soluble solids (TSS), encompassing minerals, proteins, lactose, and other dissolved components, provide a comprehensive measure of the dissolved solids in milk (Lee & Smith, 2019). This measurement, expressed as a percentage, is vital in the dairy industry for quality assurance and determining milk composition, nutritional content, and sensory properties (Lee & Smith, 2019). The quality of milk can also be assessed by measuring its pH and electric conductivity (EC), which can reveal bacterial growth and the existence of mastitis (Chakraborty et al., 2016).

Raw milk platform tests are initial, quick tests performed at dairy processing facilities to assess the quality and safety of raw milk upon arrival for processing. These tests help determine whether the milk meets basic quality standards before it undergoes processing. The alcohol test is a quick and reliable raw milk platform test method. In the quest for assessing the colloidal stability of milk, the alcohol test emerges as a platform test within the industry (Machado et al., 2017). This test relies on the coagulation of milk proteins induced by mixing equal parts of milk and 75% ethanol (v/v %) (Zhao & Corroding, 2016a). The addition of alcohol to milk lowers the dielectric constant of milk which results in an interaction between the charges on the casein surface, thus lowers the repulsive force and negative micelle charges (Rosa et al., 2020). Accordingly, alcohol neutralizes the negative charges of milk proteins, promoting coagulation (Rosa et al., 2020). Therefore, appearance of clots in the milk sample, observed qualitatively, serves as an indicator of milk quality (Mezdour et al., 2006). However, this method has its limitations, including subjectivity, lack of precision, qualitative nature, potential for false results, limited sensitivity, and complexity associated with milk composition in relation to the test (Chavez et al., 2004).

According to published literature there is no quantitative and reliable method developed to test the raw milk stability as a platform test. Therefore, having a feasible method to quantify the outcomes of the alcohol test would be highly beneficial for the dairy industry. Hence, it is important to investigate the correlation between the results of alcohol test and quantitative physicochemical characteristics of raw milk. The aims of the present study are to investigate the changes in physico-chemical properties of milk such as EC, pH, turbidity and TSS% content during the storage and to assess the correlation between physico-chemical properties with the qualitative alcohol test results.

This study was conducted on four distinct cow breeds in Sri Lanka, namely Sahiwal, Friesian Cross, Jersey Cross, and Australian Friesian Sahiwal. These breeds are recognized as the predominant cow breeds in Sri Lanka (Premaratne et al., 2012; Samaraweera et al., 2020). The results of this study are in line with previous studies that have demonstrated, alcohol test as a platform test and the physicochemical qualities of milk from different cow breeds are correlated, and those attributes might vary with the seasons (Machado et al., 2017). Therefore, it is vital to examine the fluctuations in the physicochemical characteristics of raw milk from the studied cow breeds over the ambient storage period and their correlation with the results of the alcohol test as milk stability prior to processing. The findings of this study may provide unique insights to develop novel analytical techniques to be used in dairy industry and will ensure the quality of milk products with higher accuracy.

2. Materials and Methods

2.1 Sample Collection

In this study, raw milk samples were collected from four different dairy cow breeds (Sahiwal, Friesian Cross, Jersey Cross, and Australian Friesian Sahiwal) from the National Livestock Development Board (NLDB) farm in Kekanadura (5º 96' 28" N, 80º 61' 32" E), Sri Lanka. To ensure methodological consistency, milk samples were mechanically collected from healthy and young cows. From each breed, 5 cows having identical lactation periods were selected to get representative bulk milk samples. The collected milk samples were stored in sterilized stainless-steel containers kept under cold storage conditions (4 ºC), and immediately transported to the Food Technology Laboratory at the University College of Matara, Sri Lanka for further analysis. Thereafter, 250 ml of milk collected from each of the four breeds were stored for 8 hours at ambient temperature (28±1 ºC) to simulate the milk spoilage and denaturation process and to establish the relationship between the alcohol test and the physico-chemical parameters of raw milk of different cow breeds. Measuring Electrical Conductivity, pH, Turbidity, and Total Soluble Solids. At least 3 milk samples (not less than 20 ml for each replicate) from each breed were used for analytical tests. Sample collection and analysis commenced immediately after the raw milk reached the ambient temperature (28±1 ºC), which was considered as the initial value of measurements. The rest of the sample collection and analysis continued over 8 hours. Readings were consistently recorded in every hour throughout the test period. The physicochemical parameters of each milk sample, electric conductivity (EC), pH, and total soluble solids (TSS%), were determined using the standard methods. The EC, pH and TSS% of milk were determined using an electric conductivity meter (HANNA HI 5522, USA) a pH meter (OHAUS STARTER 2100, USA) and a refractometer (ATAGO DR-A, Japan) using the following methods, AOAC-973.40, AOAC-981.12 and AOAC 948.10 respectively (AOAC, 1984).

The turbidity of each milk sample was qualitatively analyzed by moderating the Ammonium sulphate $(NH_4)_2SO_4$ turbidity test method (Shende et al., 2024). According to the method, 4±0.1 g of $(NH_4)_2SO_4$ was placed in a conical flask, followed by adding 20±0.5 ml of raw milk at room temperature. The flask was agitated to ensure dissolution of the $(NH_4)_2SO_4$ and then allowed to stand for 5 min. After that the sample was filtered using ash-less filter paper (Whatman No. 52). Finally, the turbidity (NTU) of the filtrate (10 ml) was quantified using a turbidity meter (VELP TB1XX, USA).

2.2 Alcohol Test

The ion stability of raw milk was evaluated using the alcohol test (Chavez et al., 2004; Rosa et al., 2020). In his procedure, 5 ml of raw milk sample was accurately mixed with an equal amount of 75% Ethyl alcohol (v/v%). Then the coagulation of milk sample was visually inspected, and recorded the clotting time (Machado et al., 2017). This test was repeated every hour over 8 h of storage period under the ambient storage conditions (28±1ºC). The method was repeated in 4 different days to determine the inter-day variability of the alcohol test for the precision.

2.3 Statistical Analysis

All physicochemical characteristics data were presented as the means of triplicate measurements. The experimental data were analyzed using one-way ANOVA, and the Tukey's test at the statistical significance of $P \leq 0.05$. Subsequently, the alcohol test results were ranked using binary number system by denoting "0" for negative results and "1" for positive results for milk clotting. The chi-square (χ^2) test was used to determine the relationship between alcohol test results and breed type. The degree of relationship and strength of dependency of each parameter on the alcohol test were assessed using the Spearman and Kendall rank correlation tests. The Binary logistic regression method was used to determine the best fit model for identifying variables significantly correlated to the alcohol test. All the statistical computations were performed using IBM SPSS Statistics (Version 20).

3. Results and Discussion

This study designed to observe the changes in physico-chemical properties of raw milk in different cow breeds over the storage period and to determine the relationship between the physico-chemical properties and alcohol test results, provided noteworthy findings important for the dairy industry of Sri Lanka.

3.1 Electric Conductivity

The initial EC of milk drawn from Jersey cross breed was 4.61±0.01 mS cm^{-1} after one hour at ambient temperature. It was significantly increased to 4.74±0.06 mS cm^{-1} ($P < 0.05$) after 7 h of ambient storage (Figure 1). In contrast, there was no significant difference in the EC values of the Sahiwal, Friesian cross, and Australian Friesian Sahiwal breeds that remained relatively stable.

The observed changes in conductivity of Jersey crossbreed milk indicated an elevated level of ionic discharge (Figure 1). This may be caused by increased microbial activity or metabolic alterations (Thakur, et al., 2019). Ionic composition and electric conductivity (EC) of milk can be greatly impacted by metabolic changes, especially by microbial growth and activity (Holt, 2004). Lactic acid bacteria (LAB), such as *Lactobacillus* and *Streptococcus* species, ferment lactose to produce lactic acid as part of their metabolic activities (Cremonesi et al., 2018). This lowers the pH of milk and increases the amount of free hydrogen ions (H^+) and other dissociated ions in the milk. The EC rises as a result of this ion release (Zhao & Corredig, 2016a).

Furthermore, the proliferation of microorganisms leads to the generation of diverse metabolic by-products, such as sodium (Na^+), potassium (K^+), and ammonium ions (NH_4^+), which subsequently increases the total ion concentration in milk (Holt, 2004).

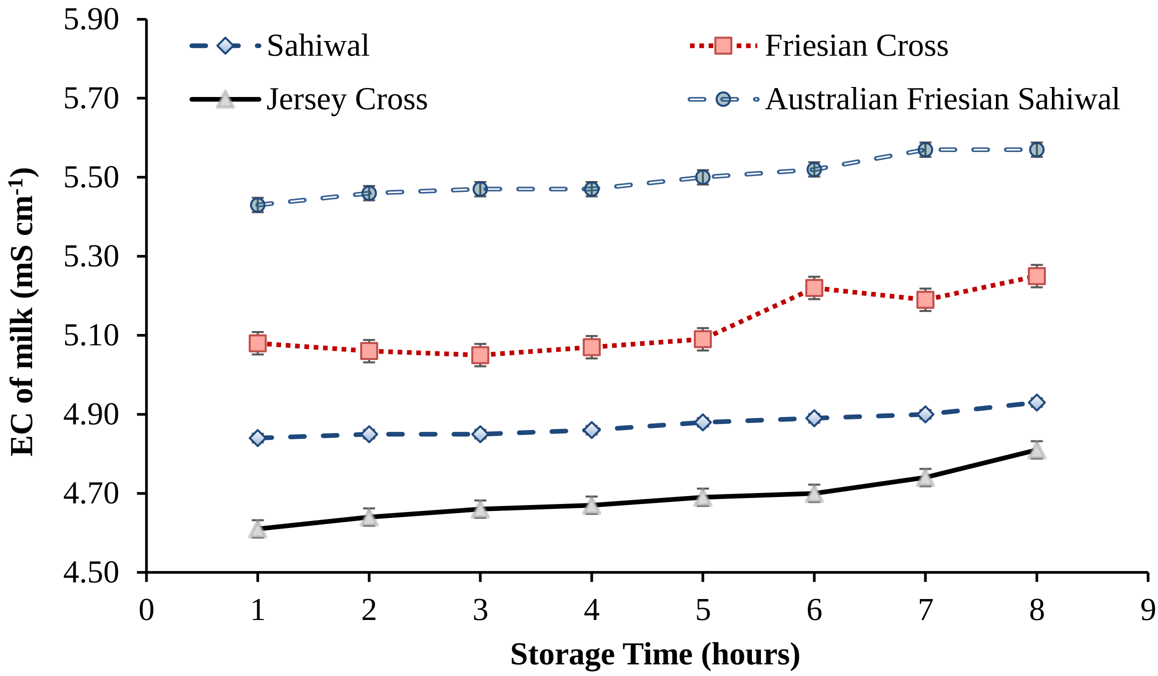

Figure 1: Change in milk EC of Sahiwal, Friesian Cross, Jersey Cross and Australian Friesian Sahiwal cow breeds during storage period of 8 h at ambient temperature. Vertical lines indicate mean±SE (n = 3).

Previous research have demonstrated that the ionic by-products of microbial metabolism caused by microbial proliferation in milk kept at room temperature, result in a detectable rise in EC (Cremonesi et al., 2018). Also, the colloidal stability of the milk is greatly impacted by microbial activities, which eventually cause the EC values to rise even more due to changes in casein micelle structures and mineral balances (Pierre & Brule, 1981). The production of extracellular enzymes and ion-releasing metabolic products by known milk contaminants, *Escherichia coli* and *Staphylococcus aureus* also accelerates the ionic imbalance and raises the EC of milk during extended storage (Zhao & Corredig, 2016b; Thakur et al., 2019). The consistent stability of EC in milk samples from Sahiwal, Friesian cross, and Australian Friesian Sahiwal breeds suggests that these breeds may be less susceptible to changing the milk EC during prolonged storage conditions (Thakur et al., 2019).

3.2 Milk pH

Among the four cow breeds, the Sahiwal exhibited the highest pH value of 6.7±0.05 at the initial milk samples (Figure 2). The milk acidity of all samples decreased with the storage period. Significant ($P<0.05$) reductions in pH were observed in the Jersey cross and Australian Friesian Sahiwal breeds, compared to their initial pH of the raw milk samples 6.10±0.2 and 6.36±0.2, respectively. This pH reduction is more significant within the 1-4 h after harvesting the milk samples. Microbial activity, particularly lactic acid production, plays a significant role in decreased pH during storage period (Eleya et al., 1995; Kimoto-Nira et al., 2012). This phenomenon may contribute to the gradual decline in the pH values of milk samples, which may be due to the breed-specific microbial populations such as *Lactococcus lactis* and *Streptococcus uberis* in Friesian Cross breeds (Cremonesi et al., 2018). pH of Friesian Cross breed did not exhibit a significant reduction during the 8 h study period, which suggests that milk from Friesian Cross breed is having lesser acidity development during the ambient storage. This result aligns with a previous study which showed that milk from Jersey breed coagulated faster than Friesian cross milk (Auldist et al., 2004). The observed variations in pH changes of milk among the different species of cow breeds indicate that there is multifactorial interaction such as genetic factors, microbial dynamics, and environmental factors (Ikonen et al., 2004; Vallas et al., 2010). However, pH reduction observed within 8 h could be due to demographic variations, temperature conditions and initial microbial count of milk samples (Troch et al., 2017).

3.3 Milk Turbidity

The investigation of turbidity changes in raw milk samples of the four cow breeds were significantly increased with storage period ($P<0.05$). The turbidity development of one hour stored milk samples of different cow breeds were in the range of 71.06-91.33 NTU (Figure 3). Australian Friesian Sahiwal breeds showed the highest turbidity development of 241.00□3.73 NTU after 8 h while Jersey Cross breed showed the significantly ($P<0.05$) lowest turbidity development of 191.15±11.34 NTU during the storage period (Figure 3).

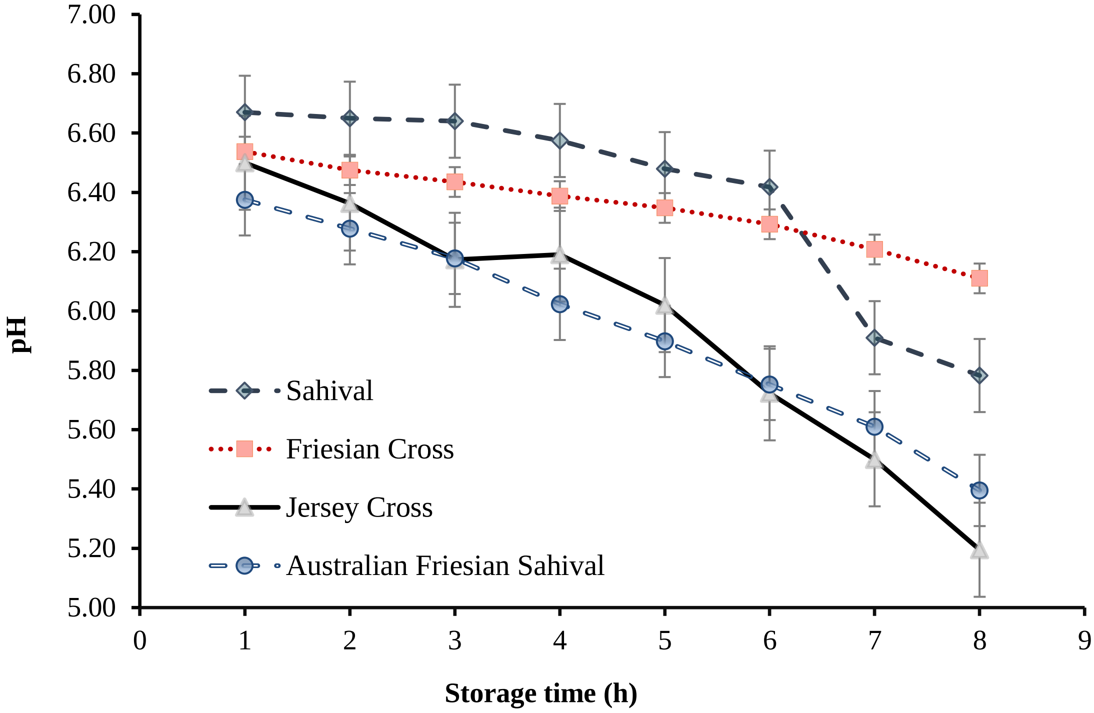

Figure 2: Changes in the pH of milk collected from Sahiwal, Friesian Cross, Jersey Cross and Australian Friesian Sahiwal cow breeds during storage. Vertical lines indicate mean±SE (n = 3)

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However, relative to the initial turbidity of stored milk samples, the turbidity values were approximately 2.4 to 3 times higher in the Australian Friesian Sahiwal and Jersey crossbreeds, respectively. The Friesian Cross breed exhibited significantly higher turbidity of 92.00±5.22 NTU after 2 h at ambient storage condition, whereas the Jersey Cross (85.00±4.95) breed showed relatively negligible variations of turbidity during the 2 h storage period ($P<0.05$). The turbidity changes among the different cow breeds may vary due to the nature of milk composition and other genetic factors (Eleya et al., 1995; Alexander & Dalgleish, 2005). The significant increase in turbidity, especially in the Australian-Friesian-Sahiwal breed should be further investigated.

Development of turbidity is known to be due to coagulated protein in milk (Scholl et al., 2014) and occurs when the repulsion among casein micelle is reduced during protein denaturation (Rakib et al., 2014). Turbidity in milk is influenced by the presence of suspended colloidal particles, reflecting the interaction of mineral complexes, whey proteins, and casein micelles in creating light-dispersing clusters (Culler et al., 2017). Consequently, turbidity can be influenced by formation of small aggregates resulting from the interaction of soluble whey proteins and ƙ-casein micelles (Alexander & Dalgleish, 2005). This interaction affected both heated and unheated casein micelles, thereby impacting milk turbidity (Alexander & Dalgleish, 2005). Additionally, bacterial activity emerged as a potential contributor to changes in milk turbidity (Zhang et al., 2019). As bacteria proliferate, the release of extracellular polymeric substances may lead to the formation of biofilms and aggregates in milk colloids finally causing the overall turbidity of milk (Zhang et al., 2019).

However, there is no direct technique available to quantitatively evaluate the turbidity of milk due to its opaque nature (Scholl et al., 2014). The Ammonium sulphate turbidity test method is used to qualitatively assess the turbidity of sterilized milk (Rakib et al., 2014). This procedure involves two sequential steps: first, milk is mixed with ammonium sulphate and then the resulting mixture is subjected to filtration to improve the clarity of the milk. Subsequently, the resulting sample is then heated to develop turbidity through protein denaturation. In this experiment, an innovative technique was applied to quantitatively measure turbidity. Thus, the ammonium sulphate turbidity test method was performed without heating to obtain a transparent sample and then quantification was done by the digital turbidity meter.

3.4 Total soluble solids content

The Friesian Cross breed initially showed the highest levels of TSS content of 10.00±0.28% and gradually decreased to 9.20 ± 0.42% after 4 h of storage (Table 1). This suggests that the composition of Friesian Cross breed milk may be less likely to fluctuate during the 1st 4 hours of storage period (Table 1). The Sahiwal breed significantly decreased ($P<0.05$) TSS content ranging from 9.35±0.21% to 7.10±0.14% during the study period of 8 h (Table 1).

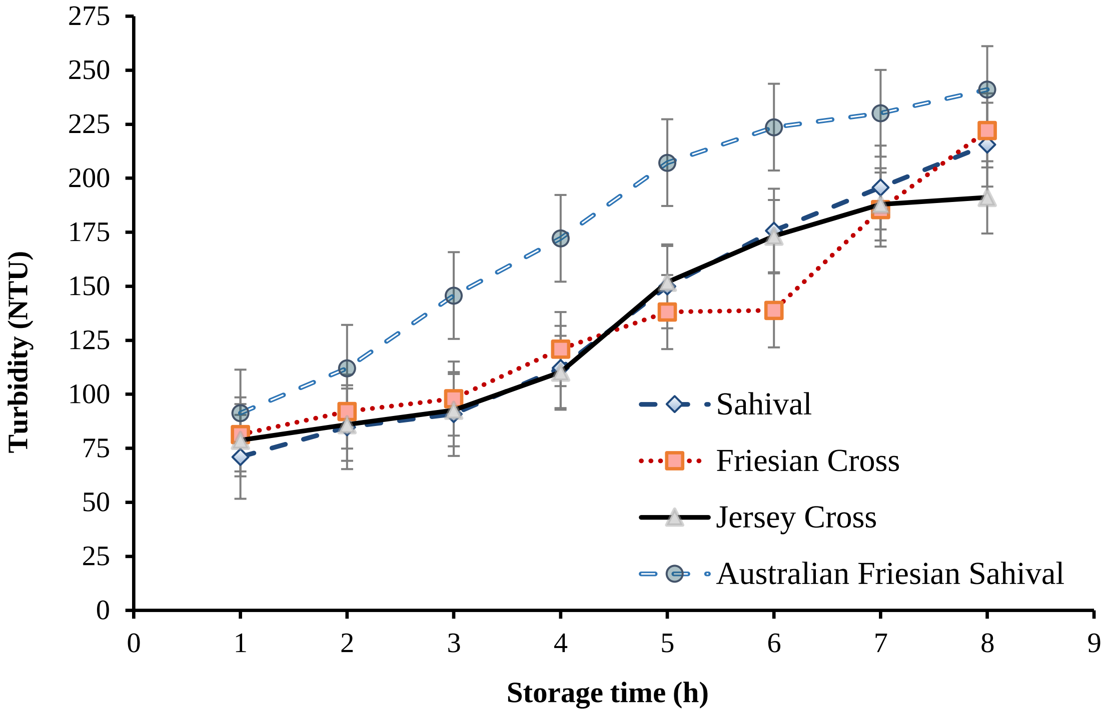

Figure 3: Changes in turbidity of milk across Sahiwal, Friesian Cross, Jersey Cross and Australian Friesian Sahiwal cow breeds during storage period of 8 h at ambient temperature. Vertical lines indicate mean±SE (n = 3)

Table 1: Changes in total soluble solid content of different cow breeds with storage period.

Storage Time (hours)	**Total Soluble Solid content of milk (%)**			
	Sahiwal breed	Friesian Cross breed	Jersey Cross bread	Australian Friesian Sahiwal bread
1	9.35 ± 0.21[a*]	10.00 ± 0.28[a]	9.25 ± 0.50[a]	9.45 ± 0.35[a]
2	9.05 ± 0.35[ab]	9.85 ± 0.35[a]	9.00 ± 0.42[ab]	9.35 ± 0.35[ab]
3	8.40 ± 0.28[bc]	9.60 ± 0.57[a]	8.65 ± 0.07[bc]	9.15 ± 0.35[ab]
4	8.10 ± 0.57[bcd]	9.20 ± 0.42[a]	8.30 ± 0.14[bcd]	8.80 ± 0.14[ab]
5	7.75 ± 0.21[cd]	8.95 ± 0.50[ab]	8.10 ± 0.00[cde]	8.55 ± 0.21[ab]
6	7.40 ± 0.14[cd]	8.80 ± 0.42[ab]	7.75 ± 0.21[cde]	8.40 ± 0.28[bc]
7	7.20 ± 0.14[d]	8.65 ± 0.21[b]	7.45 ± 0.07[de]	8.20 ± 0.28[bc]
8	7.10 ± 0.14[d]	8.50 ± 0.28[b]	7.25 ± 0.21[ef]	7.85 ± 0.35[c]

*Mean (±SE) followed by a common letter within a column are not significantly different (P>0.05).

Similarly, Jersey Cross breed showed a decreased TSS from 9.25±0.50% to 7.25±0.21%, by exhibiting a significant reduction (P<0.05) in TSS content of milk.

Previous studies show gradual changes in milk composition, observed as a general decreasing trend, from the first to the eighth hour, may be due to enzymatic and microbial activity (Miglior et al., 2006; Zajác et al., 2015). Bacterial proliferation and indigenous lipases catalyze the hydrolysis of triacylglycerides, resulting in the breakdown of free fatty acids (Miglior et al., 2006; El-Salam & El-Shibiny, 2019). This process can cause the degradation of fats, accompanying a reduction in TSS.

Table 2: Qualitative alcohol test results of milk samples obtained from four different cow breeds over the storage period of 8 hours during four different days.

Storage Time (hours)	Binary alcohol test results															
	Sahiwal breed				Friesian Cross breed				Jersey Cross breed				Australian Friesian Sahiwal breed			
	1d*	2d	3d	4d	1d	2d	3d	4d	1d	2d	3d	4d	1d	2d	3d	4d
1	0[†]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
3	0	1[$]	0	1	0	1	0	0	1	0	0	0	0	1	0	0
4	1	1	0	1	1	1	1	0	1	1	0	1	0	1	0	0
5	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

[†]0= Negative results for the alcohol test; [$]1= Positive results for the alcohol test; *1d, 2d, 3d and 4d are four different days of analysis

Breed-specific increased ranges of TSS observed initially may reflect enhanced natural proliferation of milk ingredients like lactose, proteins and lipids, which lead to a higher TSS% (Miglior et al., 2006). The findings align with the previously reported variations in the composition of the Friesian Cross breed milk, which has a higher concentration of fat and protein compared to other types of dairy breeds (Miglior et al., 2006; Zajác et al., 2015) . This composition not only affects its nutritional value, but it may also make it more susceptible to enzymatic and microbial alterations over time. The other possible factors for the gradual decline of TSS content among different cow breeds may be related to the genetic factors and differences in milk composition (Omar et al., 2024).

Further, breed-specific characteristics such as types of breeds, lactation stage, and geographic region (Kędzierska-Matysek et al., 2011) play a pivotal role in TSS of milk. Previous studies have shown that, TSS content of raw milk can be affected by some other factors such as fluctuation in whey protein content (Šlosárková et al., 2021). Additionally, the management and handling of colostrum in dairy cows can impact the microbiological quality of milk, which may result in contamination and alterations in TSS levels (Cummins et al., 2016; Šlosárková et al., 2021). Further, application of processing procedures to milk, such as pasteurization and homogenization, can modify the characteristics and arrangement of dietary components in milk, particularly the amounts of total soluble solids (Pestana, 2015).

3.5 Alcohol test

Consequently, the alcohol test was performed on all milk samples obtained from the four different cow breeds, over the storage period of 8 h. The χ^2 test was performed to determine the association between alcohol test results and the different types of cow breeds (Table 2). Results indicated that there is no significant association ($P>0.05$) between alcohol test and the milk samples obtained from the different breed types.

The lack of a significant association between alcohol test results and breed types suggested that alcohol test results are not affected by the types of the cow breeds and, with the repeated test results. Therefore, the results confirm that the alcohol test consistently indicates the stability of raw milk samples, regardless of the differences in cow breeds. Previous research has highlighted various factors that influenced ion stability of milk, including lactose content, ash percentage, and α-lactalbumin levels (Fagnani et al., 2018). A recent study noted a positive correlation between performances of milk stability with the alcohol test results (Rosa et al., 2020). The present study showed no breed-specific association with alcohol test results. That may indicate the factors influencing milk stability in an alcohol test could not be individually determined by the types of breeds. Therefore, further investigations should be done to identify the effect of multiple factors such as parity, seasonal variations and geographical effects on breed-specific association with alcohol test results.

3.6 Spearman's Rho rank correlation

There was a strong negative correlation between turbidity and alcohol test results of milk samples obtained from each cow breed (Table 3); Sahiwal ($\rho = -0.803$; $P<0.05$), Friesian Cross ($\rho = -0.851$; $P<0.05$), Jersey Cross ($\rho = -0.825$; $P<0.05$), and Australian Friesian Sahiwal ($\rho = -0.851$; $P<0.05$). The strong relationship between protein coagulation and milk clarity revealed by the substantial negative correlation between the alcohol test and turbidity.

Table 3: Spearman's Rho rank correlations between the alcohol test with the milk electrical conductivity (EC), turbidity, pH, and total soluble solids (TSS) tests.

Cow breed	Milk parameters	Spearman's rho Correlation (ρ) value	P value
Sahiwal	EC	0.124	0.500
	pH	−0.307	0.088
	Turbidity	−0.803*	0.001*
	TSS	−0.694*	0.001*
Friesian Cross	EC	0.081	0.822
	pH	−0.376*	0.034*
	Turbidity	−0.851*	0.001*
	TSS	−0.555*	0.001*
Jersey Cross	EC	0.315	0.080
	pH	−0.444*	0.011*
	Turbidity	−0.825*	0.001*
	TSS	−0.555*	0.001*
Australian Friesian Sahiwal	EC	0.238	0.190
	pH	-0.570*	0.001*
	Turbidity	-0.851*	0.001*
	TSS	-0.444*	0.022*

*Statistically significant of correlations ($P<0.05$)

This suggests that higher turbidity corresponds to a decrease in ion stability in raw milk (Czerniewicz et al., 2006). O'Connell et al. (2003) showed that the ion instability of cow's milk is predominantly associated with the denaturation of whey proteins, and it reduces the dissolvability in water phase (Genkina & Koltysheva, 2001). Hence, the significant association observed between turbidity and the alcohol test indicates impaired ion stability in raw milk.

The TSS% content also showed a significant negative correlation with the alcohol test in all cow breeds, suggesting a possible inverse relationship. The TSS level of Sahiwal breed showed a moderately significant correlation with the alcohol test ($\rho = -0.694$, $P<0.01$). The TSS content decreased with time as a result of milk protein coagulation caused by ion instability (Hsieh & Pan, 2012; Ramasubramanian et al., 2012). Therefore, the alcohol test indicates an inverse relationship with TSS.

The correlation between pH and the alcohol test differed across all the cow breeds. Both Friesian Cross and Jersey Cross showed significantly but week negative correlations (Friesian Cross; $\rho = -0.376$, $P = 0.034$ and Jersey Cross; $\rho = -0.444$, $P = 0.011$) between the pH and the alcohol test result (Table 3). However, the Australian Friesian Sahiwal exhibited a moderately negative relationship ($\rho = -0.570$, $P = 0.001$) between pH and the alcohol test results. Milk is a colloidal suspension with a pH close to neutral (Zhao & Corredig, 2016b). The stability of the casein micelles can be impacted due to the changes of raw milk pH range, rendering them more susceptible to denaturation and coagulation when alcohol is introduced (Huppertz, 2022). Additionally, the buffering capacity of the milk, which is a measure of its resistance to variations in pH, can have an impact on the results of the alcohol test (Salaün et al., 2005). Therefore, variations in the relationship between pH and alcohol test results could be attributed to the varying buffering capacities across different breeds (Salaün et al., 2005). In addition, breed-specific variables including feed intake, genetics, and metabolic processes, along with environmental stress, can have an impact on this complex relationship (Salaün et al., 2005).

3.7 Binary logistic regression

According to the findings of the Spearman's Rho rank correlation analysis, TSS and turbidity showed a significant correlation with the alcohol test results of all milk samples obtained from 4 different cow breeds. Binary logistic regression results showed that the significant correlation between alcohol test and turbidity (Wald $\chi^2 = 4.44$, $P=0.035$) (Table 4). However, none of the other physico-chemical variables such as EC, pH and TSS showed significant correlation with alcohol test ($P>0.05$).

Table 4: Significance of model effects in binary logistic regression

Variable	Wald χ^2 Value	P value
TSS	2.990	0.084
Turbidity	4.441	0.035*

*Statistical significance ($P<0.05$)

The observed significant correlation between turbidity and alcohol test results in the binary logistic regression analysis indicates that turbidity can be used as a representative variable to predict alcohol test results in stability of raw milk (Anema, 2023). Therefore, turbidity can be used as a reliable parameter to represent milk protein denaturation in relation to the ion instability of raw milk. Based on the outcome of this study, the conventional alcohol test in the dairy industry as a platform test can be replaced with a more precise instrumental application based on the turbidity measurement. Thus, it is beneficial to enhance the precision and reliability of alcohol test results. This may help in preventing the acceptance of low-quality raw milk for thermal processing such as production of pasteurized or sterilized milk products.

4. Conclusion

The research highlights the critical role of turbidity in evaluating the stability of raw milk and its correlation with alcohol test results. Turbidity of raw milk was identified as a significant predictor of alcohol test outcomes, through the results of Spearman's Rho rank correlation and binary logistic regression. Thus, it is a reliable parameter to assess the milk protein denaturation related to ion instability. This study demonstrates that turbidity measurements of raw milk can quantitatively enhance the precision and reliability of qualitative alcohol tests used in the dairy industry. This reduces the risk of processing low-quality milk into pasteurized or sterilized dairy products by ensuring that only high-quality milk is subjected to further thermal processing, safeguarding consumer health and product standards.

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This study was conducted at the Division of Applied and Natural Sciences, University College of Matara, Meddawatta, Matara, Sri Lanka.

Declaration of Competing Interest

The authors reported no potential conflict of interest.

References

Alexander, M., Dalgleish, G.D. (2005) Interactions between denatured milk serum proteins and casein micelles studied by diffusing wave spectroscopy, *Langmuir* 21 (24): 11380-11386. https://doi.org/10.1021/la0519958

Anema, S. G. (2023) The turbidity of heated milk in relation to particle size and protein distributions between the casein micelles and the serum phase, *International Dairy Journal* 147: e105771. https://doi.org/10.1016/j.idairyj.2023.105771

AOAC. (1984) Official Methods of Analysis (14th ed.), *Association of Official Analytical Chemists*, Arlington, VA.

Aschaffenburg, R. (1950) A simple turbidity test for sterilized milk, *International Journal of Dairy Technology* 3 (4): 236-237. https://doi.org/10.1111/j.1471-0307.1950.tb00747.x

Auldist, M. J., Johnston, K. A., White, N. J., Fitzsimons, W. P., Boland, M. J. (2004) A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows, *Journal of Dairy Research* 71 (1): 51–57. https://doi.org/10.1017/s0022029903006575

Banon, S., Hardy, J. (1991) Study of acid milk coagulation by an optical method using light reflection, *Journal of Dairy Research* 58 (1): 75-84. https://doi.org/10.1017/s0022029900033525

Bekere, H. Y., Utpal, D. M. A., Rahman, M., Al-Mujahidy, S. M. J., Dey, S. C., Abuhena, M., Harun, M. H. (2022) Exploration of the contents and features of milk from various natural sources, *European Journal of Medical Health Science* 4 (5): 173-183. https://doi.org/10.34104/ejmhs.022.01730183

Burrow, K., Young, W., Carne, A., McConnell, M., Bekhit, A. E. D. (2019) *Encyclopedia of Food Chemistry*, Elsevier Incorporation, Amsterdam, The Netherlands, 395 p.

Chavez, M. S., Negri, L. M., Taverna, M. A., Cuatrín, A. (2004) Bovine milk composition parameters affecting the ethanol stability, *Journal of Dairy Research* 71 (2): 201-206. https://doi.org/10.1017/s0022029904000172

Cremonesi, P., Ceccarani, C., Curone, G., Severgnini, M., Pollera, C., Bronzo, V., Riva, F., Addis, M., Filipe, J., Amadori, M., Trevisi, E., Vigo, D., Moroni, P., Castiglioni, B. (2018) Milk microbiome diversity and bacterial group prevalence in a comparison between healthy Holstein Friesian and Rendena cows, *Plos One* 13 (10): https://doi.org/10.1371/journal.pone.0205054

Culler, M. D., Saricay, Y., Harte, F. M. (2017) The effect of emulsifying salts on the turbidity of a diluted milk system with varying pH and protein concentration, *Journal of Dairy Science* 100 (6): 4241-4252. https://doi.org/10.3168/jds.2017-12549

Cummins, C., Lorenz, I., Kennedy, E. (2016) The effect of storage conditions over time on bovine colostral immunoglobulin G concentration, bacteria and pH, *Journal of Dairy Science* 99 (6): 4857-4863. https://doi.org/10.3168/jds.2015-10276

Czerniewicz, M., Kruk, A., Kielczewska, K. (2006) Storage stability of raw milk subjected to vibration, *Polish Journal of Food and Nutrition Sciences* 15 (1): 65-70.

Eleya, M.M.O., Banon, S.D., Hardy, J. (1995) A comparative study of pH and temperature effects on the acidic coagulation of milks from cows, goats, and sheep, *Journal of Dairy Science* 78 (12): 2675-2682. https://doi.org/10.3168/jds.S0022-0302(95)76898-9

El-Salam, M.H., El-Shibiny, S. (2019) Reduction of Milk Protein Antigenicity by Enzymatic Hydrolysis and Fermentation, A Review, *Food Reviews International* 37 (3): 276 - 295. https://doi.org/10.1080/87559129.2019.1701010

Fagnani, R., Araújo, J.P.A.D., Botaro, B.G. (2018) Field findings about milk ethanol stability: a first report of interrelationship between α-lactalbumin and lactose, *Journal of the science of food and agriculture* 98 (7): 2787-2792. https://doi.org/10.1002/jsfa.8775

Genkina, N. K., Koltysheva, G. I. (2001) Effect of thermolability of milk proteins on ion distribution in skimmed milk-water-salt, two-phase system, *Applied Biochemistry and Microbiology* 37: 327-330. https://doi.org/10.1023/A:1010205907485

Holt, C. (2004) An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk, *European Biophysics Journal* 33 (5): 421-434. https://doi.org/10.1007/s00249-003-0377-9

Hsieh, J. F., Pan, P. H. (2012) Proteomic profiling of the coagulation of milk proteins induced by chymosin, *Journal of Agricultural and Food Chemistry* 60 (8): 2039-2045. https://doi.org/10.1021/jf204582g

Huppertz, T. (2022) Influence of Ethanol on the Acid-Induced Flocculation of Casein Micelles, *Dairy* 3 (3): 693-699. https://doi.org/10.3390/dairy3030047

Ikonen, T., Morri, S., Tyrisevä, A.M., Ruottinen, O., Ojala, M. (2004) Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk, *Journal of Dairy Science* 87 (2): 458-67. https://doi.org/10.3168/jds.S0022-0302(04)73185-9

Kędzierska-Matysek, M., Litwińczuk, Z., Florek, M., Barłowska, J. (2011) The effects of breed and other factors on the composition and freezing point of cow's milk in Poland, *International Journal of Dairy Technology* 64 (3): 336-342. https://doi.org/10.1111/j.1471-0307.2011.00682.x

Kimoto-Nira, H., Aoki, R., Mizumachi, K., Sasaki, K., Naito, H., Sawada, T., Suzuki, C. (2012) Interaction between Lactococcus lactis and Lactococcus raffinolactis during growth in milk: development of a new starter culture, *Journal of Dairy Science* 95 (4): 2176-2185. https://doi.org/10.3168/jds.2011-4824

Lee, J., and Smith, A. M. (2019). Influence of pH on milk protein denaturation in Jersey crossbred cows. Journal of Dairy Science, 100(4), 3183-3191.

Machado, S. C., Fischer, V., Stumpf, M., Stivanin, S. C. B. (2017) Seasonal variation, method of determination of bovine milk stability, and its relation with physical, chemical, and sanitary characteristics of raw milk, *Brazilian Journal of Animal Science* 46 (4): 340-347. https://doi.org/10.1590/s1806-92902017000400010

Mezdour, S., Brulé, G., Korolczuk, J. (2006) Physicochemical analysis of casein solubility in water-ethanol solutions, *Lait* 86 (6): 435-452. https://doi.org/10.1051/lait:2006022

Miglior, F., Sewalem, A., Jamrozik, J., Lefebvre, D.M., Moore, R.K. (2006) Analysis of milk urea nitrogen and lactose and their effect on longevity in Canadian dairy cattle, *Journal of Dairy Science* 89 (12): 4886-4894. https://doi.org/10.3168/jds.S0022-0302(06)72537-1

More, S. J. (2009) Global trends in milk quality: implications for the Irish dairy industry, *Irish Veterinary Journal* 62 (4): 1-10. https://doi.org/10.1186/2046-0481-62-S4-S5

O'Connell, J.E., Fox, P.F. (2003) *Advanced Dairy Chemistry-1 Proteins*, Springer, Boston, MA, 879-945 p.

Omar, A. I., Khan, M. Y. A., Su, X., Dhakal, A., Hossain, S., Razu, M. T., Si, J., Pauciullo, A., Faruque, M.O., Zhang, Y. (2024) Factors affecting the milk production traits and lactation curve of the indigenous river buffalo populations in Bangladesh, *Animals* 14 (8): 1248-1263. https://doi.org/10.3390/ani14081248

Pestana, J.M., Gennari, A., Monteiro, B., Lehn, D., Souza, C.F.V.D. (2015) Effects of pasteurization and ultra-high temperature processes on proximate composition and fatty acid profile in bovine milk, *American Journal of Food Technology* 10 (6): 265-272. https://doi.org/10.3923/ajft.2015.265.272

Pierre, A., Brule, G. (1981) Mineral and protein equilibria between the colloidal and soluble phases of milk at low temperature, *Journal of Dairy Research* 48 (3): 417-428. https://doi.org/10.1017/S0022029900021890

Premaratne, S., Kollalpitiya, K. M. P. M. B., Peiris, B. L. (2012) Reproductive and productive performance of up-country exotic dairy cow breeds of Sri Lanka, *Tropical Agricultural Research* 23 (4): 319-326. https://doi.org/10.4038/tar.v23i4.4867

Rakib, M. R. H., Habib, R., Hassan, M. N., Imam, M. F. (2014) Development of turbidity index as field method to detect mixing of heated milk with raw milk, *Bangladesh Journal of Animal Science* 43 (2): https://doi.org/10.3329/bjas.v43i2.20708

Ramasubramanian, L.R., D'Arcy, B. R., Deeth, H. C. (2012) Heat-induced coagulation of whole milk by high levels of calcium chloride, *International Journal of Dairy Technology* 65 (2): 183-190. https://doi.org/10.1111/j.1471-0307.2012.00823.x

Rosa, P.P., Ávila, B., Angelo, I.D.V., Silva, P.M., Chesini, R.G., Mota, G., Sedrez, P.A., Fernandes, T.A., Bugoni, M., Roll, V.F.B. (2020) Factors that affect the thermal stability of bovine milk and the use of alcohol test in the milk industry – a review, *Nucleus Animalium* 12 (2): 15–46. https://doi.org/10.3738/21751463.3734

Salaün, F., Mietton, B., Gaucheron, F. (2005) Buffering capacity of dairy products, *International Dairy Journal* 15 (2): 95-109. https://doi.org/10.1016/j.idairyj.2004.06.007

Samaraweera, A. M., Boerner, V., Cyril, H. W., van der Werf, J., Hermesch, S. (2020) Genetic parameters for milk yield in imported Jersey and Jersey-Friesian cows using daily milk records in Sri Lanka, *Asian-Australasian Journal of Animal Sciences* 33 (11): 1741-1754. https://doi.org/10.5713/ajas.19.0798

Scholl, P. F., Farris, S. M., Mossoba, M. M. (2014) Rapid turbidimetric detection of milk powder adulteration with plant proteins. *Journal of*

Agricultural and Food Chemistry 62 (7): 1498-1505. https://doi.org/10.1021/jf405617f

Shende, S., Gupta, C., Veena, N., Ranveer, S., Sawale, P. (2024) *Analytical Methods for Milk and Milk Products*. Apple Academic Press, Florida, USA, 410 p.

Šlosárková, S., Pechová, A., Staněk, S., Fleischer, P., Zouharová, M., Nejedlá, E. (2021) Microbial contamination of harvested colostrum on Czech dairy farms, *Journal of Dairy Science* 104 (10): 11047-11058. https://doi.org/10.3168/jds.2020-19949

Thakur, R., Gupta, R.K., Mahajan, P.K. (2019) Statistical investigations to milk yield data of Jersey and Holstein Friesian cross breeds, *International Journal of Current Microbiology and Applied Sciences* 8 (03): 2056–2065. https://doi.org/10.20546/ijcmas.2019.803.245

Troch, T., Lefébure, É., Baeten, V., Colinet, F.G., Gengler, N., Sindic, M. (2017). Cow milk coagulation: process description, variation factors and evaluation methodologies. a review, *Biotechnology, Agronomy and Society and Environment* 21 (4): 276-287. https://doi.org/10.25518/1780-4507.13692

Vallas, M., Bovenhuis, H., Kaart, T., Pärna, K., Kiiman, H., Pärna, E. (2010) Genetic parameters for milk coagulation properties in Estonian Holstein cows, *Journal of Dairy Science* 93 (8): 3789-3796. https://doi.org/10.3168/jds.2009-2435

Yüksel, Z., Erdem, Y. K. (2005) The influence of main milk components on the hydrophobic interactions of milk protein system in the course of heat treatment, *Journal of Food Engineering* 67 (3): 301-308. https://doi.org/10.1016/j.jfoodeng.2004.04.032

Zajác, P., Čapla, J., Vietoris, V., Zubrická, S., Čurlej, J. (2015) Effects of storage on the major constituents of raw milk, *Potravinarstvo* 9 (1): 375-381. https://doi.org/10.5219/518

Zhao, Z., Corredig, M. (2016a) Influence of sodium chloride on the colloidal and rennet coagulation properties of concentrated casein micelle suspensions, *Journal of Dairy Science* 99 (8): 6036-6045. https://doi.org/10.3168/jds.2015-10622

Zhao, Z., Corredig, M. (2016b) Colloidal properties of casein micelles suspensions as a function of pH during concentration by osmotic stressing, *Food Hydrocolloids* 60 (1): 445-452. https://doi.org/10.1016/j.foodhyd.2016.04.01630**:**113-127. https://doi.org/10.1080/02705060.2014.960901

Yatigammana, S., Perera, M. (2017) Distribution of *Cylindrospermopsis raciborskii* (cyanobacteria) in Sri Lanka, *Ceylon Journal of Science* 46**:**65-80. http://doi.org/10.4038/cjs.v46i3.7444

Zhang, H.-H., Zhang, X.-K., Si R.-R., Shen, S.-C., Liang T.-T., Fan, T.-T., . . . Han B.-N. (2020). Chemical and biological study of novel aplysiatoxin derivatives from the marine cyanobacterium *Lyngbya* sp., *Toxins* 12(11): 733. https://doi.org/10.3390/toxins12110733