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RESEARCH ARTICLE

Seasonal variation in composition, physicochemical properties and microbial load of raw milk: A comparative study between organized and unorganized dairy farms

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Abstract: Raw milk is a highly perishable agricultural product that plays an important role in the dairy industry and human nutrition. The present study investigated the seasonal variation in the composition, physicochemical properties, and microbial load of cow raw milk in Guwahati, Assam. A total of 144 raw milk samples were collected from organized and unorganized dairy farms in different seasons. In comparison to unorganized farms, organized farms had significantly higher (P<0.01) levels of fat, SNF, protein, lactose, and ash. In comparison to summer milk, winter milk had higher (P<0.05) levels of fat, SNF, protein, and ash except for the lactose content which was lower (P<0.05) in winter. The pH of raw milk was also higher (P<0.01) in the winter. Furthermore, organized farms had a higher (P<0.01) raw milk specific gravity than unorganized farms. Both room and refrigeration storage temperatures caused significantly higher (P<0.01) total viable count (TVC) and coliform count in milk during the summer. In conclusion, the findings of this study illustrated the dynamic nature of raw milk quality in various seasons and farm types. These variations have implications for raw milk quality and safety, emphasizing the need to implement appropriate management practices in dairy farms to maintain high-quality and safe milk throughout the year.

Keywords: Coliform, Milk protein, Seasons, Specific gravity, Subtropical region

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Introduction

Seasonal variation in the composition, physicochemical properties and microbial load of cow raw milk is an essential consideration for ensuring food safety and quality, particularly in the context of dairy farms operating in tropical regions. Temperature, humidity, and rainfall can affect the health of dairy cattle, the quality of forage, and the incidence of infections, all of which influence milk composition and microbial load (Oliver and Page 2016; Sahaet al. 2024). The microbial load of raw milk not only shortens its shelf life but also is potentially hazardous to consumers' health if ingested without proper processing and treatment (Terefe and Walelegne, 2024). Additionally, milk's physicochemical attributes such as its acidity, fat content, and total solids, are very important in deciding whether it is suitable to make other dairy products (Coulon et al. 1998; Yasmin et al. 2012; Mohsinet al. 2024). The nutrient content (Fat, protein, lactose, vitamins, and minerals) of milk is frequently highly affected by seasonal changes in environmental factors such as temperature, humidity, and forage availability (Vélez-Terranova et al. 2023). Additionally, factors such as environmental factors, animal health, and milking hygiene practices influence the microbial load of raw milk (Lakew et al. 2019; Terefe and Walelegne, 2024). Therefore, to address the challenges posed by climatic conditions in subtropical areas, understanding seasonal variations in milk quality is crucial.

To our best knowledge, no studies have been conducted on seasonal variation in composition, physicochemical properties and microbial load of raw milk in the subtropical eastern Himalayan region. This comparative study is aimed at developing targeted policies and interventions that can improve dairy farm practices, ensuring safer and higher-quality milk for consumers. Therefore, the main objective of this study was to find out how cow milk differed from organized and unorganized farm management in terms of nutritional composition and other physicochemical properties throughout different seasons.

Materials and Methods

Location and collection of milk samples

The experiment was carried out in the College of Veterinary Science, Assam Agriculture University (AAU), Khanapara, Guwahati, India. A total of 144 samples were collected, with 36 samples each from organized and unorganized farms during both winter and summer seasons, respectively. Raw milk samples were collected from the region of Khanapara and nearby regions. Organized farms had Sahiwal, crossbred of Holstein Friesian and Jersey cows, while unorganized farms predominantly had local indigenous breeds and crossbred of Jersey cows. Milk samples were collected from 3 to 4 cows, mixed with a sterile plunger, and stored in sterile containers. Samples were collected during summer (May to July) and winter (December to February) and transported in iceboxes at 2–8°C to preserve quality. Upon arrival at the laboratory, they were promptly processed and stored under appropriate conditions until analysis.

Analysis of milk sample for composition

Immediately after collection, the milk samples were brought to the laboratory and Quick judging of raw cow's milk, immediately on receipt, was done in an Ultrasonic Milk Analyser (Master Classic, Bengaluru, India). Fat, solids not fat (SNF), Protein, Lactose, and Ash of the raw milk were estimated.

Physicochemical assessment of collected milk samples

The following physicochemical assessment of collected raw milk samples was done during the experimental periods. The pH of milk was determined by using a digital pH meter Model 780 (Metrohm, Switzerland). Tritrable acidity was determined by following the standard method described by Artherton and Newlander (1977). The specific gravity and freezing point raw of milk samples were determined using an Ultrasonic Milk Analyser (Master Classic, Bengaluru, India).

Microbiological assessment

The total viable count (TVC) and the coliform count was done as per the method described by Harrigan and McCance (1976).

Statistical analysis

Statistical analysis was performed using SPSS for the collected data. An analysis of variance was conducted using the general univariate linear model (GLM) and the Scheffe test was used to compare least-square means of significant effects. Significant levels were set at P<0.05. To address the wide distribution variation of microbial data, a logarithmic conversion was applied to the TVC and coliform count values (Log_{10}) to achieve normalization. The following linear model was utilized in the statistical analysis:

$$Y_{ijk} = \mu + a_i + b_i + (ab)_{ij} + e_{ijk}$$

Where,

μ = overall mean

a_i = effect of ith farms (i=1,2; organized or unorganized)

b_i = effect of jth seasons (j=1,2; summer or winter)

$(ab)_{ij}$ = interaction between ith farms and jth seasons

e_{ijk} = Random error of observation or residual effect 4 NID $(0, \sigma^2_e)$

Results and Discussion

Milk composition

The interaction between season × farm did not have a significant impact on the average fat percentage of raw milk. However, both season (P<0.01) and farm (P<0.05) individually affected the fat percentage of raw milk (Table 1). Specifically, within the farms, the organized farm had a higher fat percentage in raw milk compared to the unorganized farm. Additionally, during the winter season, the fat percentage was greater than during the summer season. The SNF percentage was affected by the farm (P<0.01). Among the different farms, the organized farm had a higher SNF percentage in raw milk than the unorganized farm (Table 1). The percentage of protein in raw milk was influenced by multiple factors, including the farm (P<0.01), the season (P<0.05), and the season x farm interaction (P<0.05). A higher protein percentage was observed in raw milk from the organized farm compared to the unorganized farm. Further, when considering the influence of the season, the percentage of protein was higher during the winter than during the summer. The fat percentage of raw milk was also influenced by season (P<0.05) and farm (P<0.01). Among the different farms, the organized farm displayed a higher lactose percentage in raw milk compared to the unorganized farm. Furthermore, during the winter season, the lactose percentage was observed to be lower than during the summer season. The proportion of ash percentage in raw milk remained unaltered by both the season and the interaction between season × farm. However, the ash percentage was significantly impacted by the farm (P<0.01). When comparing the various farms, the organized farm demonstrated a greater ash percentage in raw milk in contrast to the unorganized farm.

Studies have demonstrated that milk contains more fat and SNF in the winter than in the summer (Arora and Bhojak 2013; Lakew et al. 2019; Ramadaniet al. 2024). A similar result was observed in the present study. Variations in factors such as temperature, humidity, hygiene, and stress levels in different seasons as well as different types of organized and unorganized farms can affect the cow's overall health and milk production, subsequently influencing the fat and SNF content (Vélez-Terranova et al. 2023). Protein composition in milk also exhibits seasonal changes (Arora and Bhojak 2013; Bokharaeianet al. 2023). Multiple factors contribute to the variation in protein contents of milk in different farms, including, the cleanliness of cows, the stage of lactation, milk somatic cell count, nutritional factors, and genetic variants of casein (Coulon et al. 1998). Furthermore, seasonal variations impact the lactose content of milk (Yasmin et al. 2012). Studies have revealed that lactose concentrations tend to be higher in milk during the summer months (Arora and Bhojak 2013; Richardset al. 2023). Genetic differences in breeds, different feeding strategies, general management, and environmental conditions might account for this variation in lactose content. Minerals in milk, which contribute to its ash content, are directly related to the diet of the cow (Sirinayakeet al. 2023). In organized farms, high-quality forage is usually available and mineral-rich feed additives and supplements may be administered to the cows, which may result in the intake of more minerals, which can increase milk ash content.

Physicochemical properties

The density of raw milk, as indicated by its specific gravity, did not demonstrate any significant variation due to the season and the interaction between season × farm (Table 1). However, the specific gravity was found to be influenced by the farm (P<0.01). Notably, among the farms examined, the organized farm exhibited a higher specific gravity of raw milk compared to the unorganized

Table 1: Seasonal and farm-specific variations in the nutritional composition and physicochemical properties (mean ± SE) of raw milk (n=144)

Parameters	Season (S)	Farm (F)		Season mean	F value		
		Organized	Unorganized		Season	Farm	S × F
Fat (%)	Winter	4.40±0.12^{Aa}	3.92±0.28^{Ab}	4.16±0.32^{A}	F=18.526**	F=7.5023*	F=0.0096^{NS}
	Summer	3.65±0.08^{B}	3.20±0.13^{B}	3.43±0.10^{B}			
	Farm mean	4.03±0.13^{a}	3.56±0.18^{b}	-			
SNF (%)	Winter	9.40±0.10^{a}	8.97±0.26^{b}	9.18±0.15	F=4.273^{NS}	F=17.917**	F=2.8003^{NS}
	Summer	9.33±0.04^{a}	8.33±0.18^{b}	8.83±0.18			
	Farm mean	9.37±0.05^{a}	8.65±0.18^{b}				
Protein (%)	Winter	3.58±0.03	3.55±0.12^{A}	3.57±0.06^{A}	F=5.3476*	F=9.037**	F=6.471*
	Summer	3.60±0.03^{a}	3.20±0.07^{Bb}	3.4±0.07^{B}			
	Farm mean	3.60±0.02^{a}	3.38±0.08^{b}				
Lactose (%)	Winter	5.33±0.03^{Aa}	4.81±0.10^{Ab}	5.08±0.09^{A}	F=5.615*	F=17.575**	F=2.126^{NS}
	Summer	5.42±0.06^{B}	5.17±0.14^{B}	5.30±0.08^{B}			
	Farm mean	5.38±0.04^{a}	4.99±0.10^{b}				
Ash (%)	Winter	0.77±0.02	0.70±0.03	0.73±0.02	F=0.152^{NS}	F=12.273**	F=0.152^{NS}
	Summer	0.77±0.02^{a}	0.68±0.02^{b}	0.73±0.02			
	Farm mean	0.77±0.01^{a}	0.69±0.01^{b}				
Specific gravity	Winter	1.0333±0.0041	1.0321±0.0094	1.0327±0.0052	F=3.982^{NS}	F=10.418**	F=1.619^{NS}
	Summer	1.0329±0.0033	1.0301±0.0062	1.0315±0.0054			
	Farm mean	1.0331±0.0026^{a}	1.0311±0.0062^{b}	-			
Freezing point depression (^{o}C)	Winter	0.64±0.01	0.60±0.03	0.62±0.01	F=1.844^{NS}	F=1.306^{NS}	F=2.443^{NS}
	Summer	0.64±0.00	0.55±0.02	0.59±0.01			
	Farm mean	0.64±0.00	0.58±0.02	-			
pH	Winter	7.06±0.05^{A}	6.88±0.06^{A}	6.97±0.04^{A}	F=58.962**	F=1.939^{NS}	F=0.0105*
	Summer	6.75±0.02^{B}	6.57±0.02^{B}	6.66±0.03^{B}			
	Farm mean	6.90±0.05	6.73±0.06	-			
Titratable Acidity (%)	Winter	0.16±0.01^{A}	0.15±0.01^{A}	0.16±0.01^{A}	F=162.598**	F=0.0063^{NS}	F=0.4489^{NS}
	Summer	0.18±0.02^{B}	0.17±0.01^{B}	0.18±0.01^{B}			
	Farm mean	0.17±0.01	0.16±0.00	-			

Row-wise (a, b) and column-wise (A, B) means with different superscripts differ significantly
**Significant= P<0.01, *Significant= P<0.05, NS= non-significant

farm. The cryoscopic test, measuring the freezing point depression (!) of raw milk, did not exhibit any significant alterations due to the season, farm, and the interaction between season × farm. Nonetheless, the pH was observed to be impacted by the season (P<0.01) and the interaction between season × farm (P<0.01). Within the different seasons, a higher raw milk pH was observed during the winter season compared to the summer season. The titratable acidity was found to be influenced by the season (P<0.05). When comparing different seasons, a lower titratable acidity percentage of raw milk was observed during the winter season in contrast to the summer season (Table 1). As a result of changes in diet and nutrient composition of fodder during different seasons, the fermentation process in the rumen may differ, subsequently impacting the pH of the milk (Ponnampalamet al. 2024). Furthermore, environmental factors like temperature and humidity also affect the pH of raw milk (Estremadoyroet al. 2024); which has also been similarly observed in our study. Microbial activity in milk increases during warmer seasons, potentially affecting pH levels. The titratable Acidity (%) of raw milk has been observed to be higher in the winter than in the summer. Different forage availability and composition throughout the year can impact the microbial activity in the cows' rumen, subsequently affecting the production of volatile fatty acids (Butler et al. 2008), which in turn influence the titratable acidity of milk.

Microbial load

The total viable count (TVC) of raw milk on the day of collection (day 0 at room temperature) remained unaffected by the interaction between season × farm. However, it was influenced by both the season (P<0.01) and the farm (P<0.01). On the other hand, the TVC of raw milk on day 1 (at refrigeration temperature) was only influenced by the season (P<0.01). During both day 0 and day 1, a lower TVC of raw milk was observed during the winter season in comparison to the summer season (Table 2). Furthermore, among the different farms, the organized farm displayed a lower TVC of raw milk on the day of collection (day 0) when compared to the unorganized farm (Table 2). The coliform count of raw milk on day 0 (at room temperature) and day 1 (under refrigeration) exhibited no significant influence from the farm and the interaction between season × farm. However, the season had a significant (P<0.01) impact on the coliform count. It was observed that coliform counts in raw milk were lower during the winter season compared to the summer season, both on day 0 and day 1 (Table 2). The microbial load of milk is influenced by a variety of factors, such as healths, controlled feeding and housing conditions, and hygiene practices used during the milking process, storage conditions, and the overall microbial environment (Terefe and Walelegne, 2024). The warmer temperatures create an ideal condition for the rapid growth of bacteria, coliforms and other microorganisms present in the milk (Oliver and Page 2016; Sahaet al. 2024), leading to an increase in the total viable count as well as coliform count.

Table 2: Seasonal and farm-specific variations in microbial load (mean ± SE) of milk at different storage temperatures (n=144)

Parameters	Days	Seasons (S)	Farm (F) Organized	Unorganized	Season mean	F value Season	Farm	S × F
TVC (log10) cfu/ml	Day 0 (Room temp.)	Winter	5.03 ± 0.07^{Aa}	4.66 ± 0.10^{b}	4.84 ± 0.08^{A}	F=8.9096**	F=36.247**	F=3.5329^{NS}
		Summer	5.46 ± 0.10^{Ba}	4.75 ± 0.09^{b}	5.11 ± 0.12^{B}			
		Farm mean	5.25 ± 0.09^{a}	4.70 ± 0.05^{b}	-			
	Day 1 (Refg. Temp.)	Winter	4.10±0.31	3.91 ± 0.22^{A}	4.00 ± 0.18^{A}	F=9.7826**	F=0.7137^{NS}	F=0.0171^{NS}
		Summer	4.67±0.05	4.53 ± 0.03^{B}	4.60 ± 0.03^{B}			
		Farm mean	4.38±0.17	4.22±0.14	-			
Coliform (log10) cfu/ml	Day 0 (Room temp.)	Winter	3.07 ± 0.23^{A}	2.53 ± 0.26^{A}	2.80 ± 0.18^{A}	F=48.4895**	F=2.2046^{NS}	F=0.2392^{NS}
		Summer	4.85 ± 0.36^{B}	4.58 ± 0.23^{B}	4.71 ± 0.21^{B}			
		Farm mean	3.96±0.33	3.55±0.35	-			
	Day 1 (Refg. Temp)	Winter	2.20 ± 0.28^{A}	2.06 ± 0.22^{A}	2.13 ± 0.17^{A}	F=97.9951**	F=3.6907^{NS}	F=0.3138^{NS}
		Summer	4.67 ± 0.39^{B}	4.53 ± 0.31^{B}	4.60 ± 0.16^{B}			
		Farm mean	3.43±0.43	3.36±0.38	-			

Row-wise (a, b) and column-wise (A, B) means with different superscripts differ significantly
**Significant= P<0.01, *Significant= P<0.05, NS= non-significant

Conclusion

The findings of this study indicate significant differences in microbial load, physicochemical properties, and nutritional composition of raw milk between seasons and farm types. These variations have implications for raw milk quality and safety, emphasizing the need to implement appropriate management practices in dairy farms to maintain high-quality and safe milk throughout the year. Policymakers can use this information to promote the adoption of better farm management techniques, provide training for dairy farmers, and implement regulations that standardize milk production processes across both organized and unorganized farms. Further research and intervention are needed to enhance quality control in dairy farms in the Eastern Himalayan regions.

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