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Research article

The effect of season and agro-ecology on physicochemical properties of cow's raw milk in Central and North-Western Zone of Tigray, Ethiopia

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ABSTRACT

Milk at a normal state has unique physical, chemical, and biological characteristics that are used as quality indicators. The quality of milk is influenced by many factors and the components of milk are very sensitive indicators for milk production practices. Season and agro-ecologies have influences on physicochemical properties of cow milk. Therefore, this research aimed to investigate the effect of season and agro-ecology on the physicochemical properties of cow's raw milk in Central and North-Western Zone of Tigray. The study was conducted in Aksum, Shire-Indaselassie, and Sheraro administrative towns. A two-stage sampling method was used to select both the study area and respondents. All towns in the Central and North-Western zones of Tigray were categorized into three agro-ecologies, and sample towns were purposively selected from each category based on accessibility and milk production potential. Sample respondents were then randomly selected from each agro-ecology. Agro-ecology categorization was based on altitude, temperature, and feeding practices, while seasons were classified according to rainfall and temperature. A total of 210 samples, comprising 35 samples from dairy farms and 35 samples from cafeterias within each agro-ecology, were analyzed to assess the physicochemical properties of cow milk across two different seasons. Physicochemical analysis including determination of fat, crude protein, solids not fat, density, lactose, salt, water added and freezing point was conducted in the laboratory of Mekelle University using Lacto scan, Bulgaria. The overall mean values of milk fat, solids-not-fat (SNF), protein, and lactose were 3.72 ± 0.12 %, 6.72 ± 0.17 %, 2.6 ± 0.08 %, and 3.72 ± 0.09 %, respectively. Specific gravity, salt content, water added, and freezing point exhibited mean values of 1.026 ± 0.001 g/ml, 0.53 ± 0.01 %, 19.33 ± 1.8 %, and −0.42 ± 0.01°C, respectively. Significant differences ($p < 0.05$) were observed in protein, solids-not-fat, lactose, and salt content among agro-ecologies, seasons, and sources of milk. Correlation analysis revealed positive correlations between specific gravity and solids-not-fat, protein, lactose, and total salt, while negative correlations were observed with water added and freezing point ($p \leq 0.01$). In conclusion, the physical and chemical properties of cow's raw milk are influenced by agro-ecology, season, and the source of milk. Notably, milk adulteration, particularly with the addition of water, was more prevalent among cafeteria owners. Given the absence of established

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milk quality standards in Ethiopian conditions, it is imperative for the government to introduce quality standard measures, rules, and regulations to effectively control milk quality.

1. Introduction

Ethiopia has the largest cattle population in Africa and ranks fifth globally, following Brazil, India, China, and the USA [1]. Female cattle make up approximately 54.68 % of Ethiopia's total cattle population, serving as the primary source of milk production [2]. In 2018, global milk production reached an estimated 843 million tons, with cows' milk accounting for 541 million tons [3]. Cow milk production in Ethiopia is estimated to be 3.32 billion litres annually, contributing around 91 % of the total milk output from dairy cows and camels nationwide [2]. In Tigray, milk production exceeds 207.8 million litres annually, with the Central and North Western zones contributing 17.7 and 73.3 million litres, respectively [2] (see Fig. 1).

Cow milk is widely recognized as one of nature's most complete foods [4] and it is a highly nutritional food [5] with abundant protein, minerals, particularly calcium and phosphorus that improves overall dietary quality [6,7]. It has a major contribution to food security as it consists of essential nutrients and mitigates malnutrition [8]. The components of milk such as proteins, fat, lactose, vitamins and minerals are necessary to nurture infants and consequently, they contribute to its growth [9]. Additionally, milk serves as a significant source of calcium, with an average content of 1150 mg/L in most developed countries [10]. Calcium not only aids in bone formation and strength but also contributes to cholesterol regulation, weight management, and blood pressure control, underscoring its importance in maintaining overall health and well-being [11].

The nutritional and economic significance of milk are associated with its solids content. The higher solids content is indicative of superior nutritional value in milk [12]. Nowadays, there is a growing emphasis on maximizing the fat and protein content of milk and dairy industry producers are incentivized to maximize the fat and protein content of milk to meet consumer preferences and industry standards [13]. Both the volume and composition of milk are pivotal factors in determining its quality and the price paid to producers [14]. Protein content, in particular, serves as a primary quality benchmark in many countries, influencing milk payment to producers. While some regions prioritize protein content, others base pricing on fat and solids-non-fat composition [15]. Therefore, selecting specific milk components as criteria for quality determination has become economically advantageous [16]. By understanding and optimizing the factors that contribute to milk quality, producers can enhance both the nutritional value of their products and their economic returns. The composition and physicochemical properties of milk are of great importance to the dairy industry [17] Milk in its natural state possesses unique physical, chemical, and biological characteristics [18], which serve as key quality indicators [19]. The physicochemical properties of cow milk is affected by season due to temperature, rainfall, nutrition/feed quality [20,21], and health of the animal [22–24]. Adulteration and overall management practice of producers are also factors affecting physicochemical

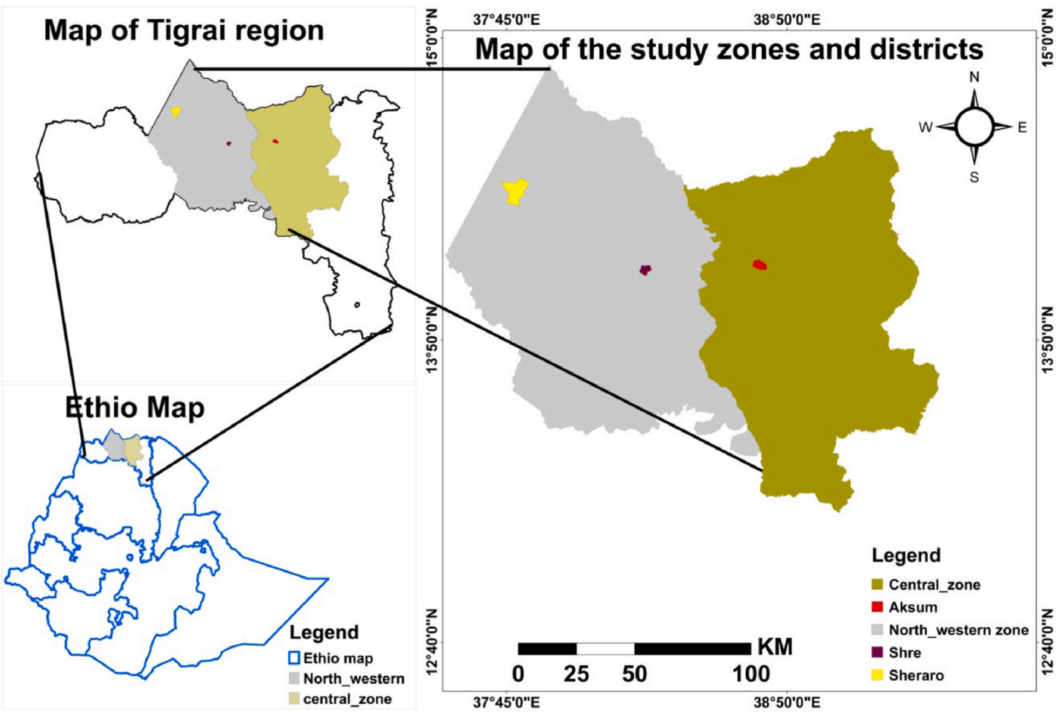

Fig. 1. Map of study areas.

properties of milk [24].The components of milk are very sensitive indicators for detecting adulteration or issues related to nutrition and cow health [25].

In Ethiopia, the practice of adulterating raw milk, particularly by adding water and removing cream is common among smallholder milk producers, vendors, and cafeterias [26]. Such illegal practice may contribute to affect milk quality and reduce the standard of milk composition. The determination of milk adulteration has paramount importance to define economic losses for the smallholder dairy producers and processing units [27,28].

Despite some studies reporting on the chemical composition of raw milk, research specifically examining the effects of season and agro-ecology on the physicochemical properties of cow milk in the Tigray regional state is lacking. In response to this gap, the government of the Tigray regional state has commissioned Aksum University to conduct research in this area. The aim of this research is to investigate how season and agro-ecology impact the physicochemical properties of cow's raw milk and milk production practices in small-scale dairy farms and cafeterias in the Central and North-Western Zones of Tigray. Due to financial constraints and security concerns, the study's scope is limited to three districts across three agro-ecologies.

2. Methodology

2.1. Descriptions of the study area

The study was conducted across three districts in the Central and North-Western Zones of the Tigray region, namely Aksum, Shire-Indaselassie, and Sheraro Figure [1]. Each district represents a distinct agro-ecology, encompassing highland, midland, and lowland regions, respectively. The classification of these regions into highland, midland, and lowland categories in the Tigray region was primarily based on agro-ecological factors, including altitude, temperature, and feeding practices.

According to previous studies [29,30], the agro-ecological zones in Tigray were classified using the Koppen-Geiger agro-climatic classifications. The lowland areas, characterized as hot semiarid regions, are situated at elevations between 500 and 1500 m above sea level (m.a.s.l), with annual temperatures ranging from 20.1 to 42.0 °C. The midland zones exhibit tropical savannah climates and are located at elevations between 1500 and 2000 m.a.s.l, with annual temperature ranges of 17.2–28.4 °C and the highland regions represent sub-tropical highlands, situated at elevations between 2000 and 2500 m.a.s.l, with annual temperature ranges of 16.2–26.2 °C. The agro-ecosystem and socioeconomic characteristics of the areas can vary significantly across agro-ecological zones (AEZ). In the context of the Tigray region, there are notable differences in infrastructure between AEZ. For instance, the lowland areas are often characterized as relatively inaccessible, semi-arid, low rainfall and sparsely populated compared to other zones. In the Ethiopian context, particularly in the study area, spring is typically characterized by hot and dry conditions, while summer is characterized by cooler temperatures and rainy weather. These seasonal variations have significant implications for agricultural practices, water availability, and overall livelihoods in the region.

Aksum, the administrative town of the Central Zone of Tigray, lies approximately 963 km north of Addis Ababa, Ethiopia, and 202 km from Mekelle, the capital city of the Tigray region. Its geographical coordinates are 14°7′15″N latitude and 38°43′40″E longitude, with an average elevation of 2331 m above sea level (m.a.s.l). The town is classified as sub-tropical highland agro-ecology with annual temperature ranges from 17.1 to 22.1 °C. Aksum has a total population of 66,818 individuals, consisting of 32,402 males and 34,416 females [31].

Shire-Indaselassie, the administrative town of the North Western Zone of Tigray, situates approximately 63 km away to the west from Aksum at a geographical coordination of 14° 6′ 6.60″N latitude and 38° 16′ 46.83″E longitude with an average elevation of 1953 m.a.s.l. This town is classified as a midland (tropical savannah climates) with annual temperature ranges from 18.4 to 26.5°C. It has 70,753 (34,161 male and 36,592 female) human population.

Sheraro town is found in the North Western Zone of Tigray located at a distance of 95 km from Shire-Indaselassie to the West in a geographical coordination of 14° 24′ 0.00″N latitude and 37° 55′ 60.00″E longitude with an altitude of 1246 m.a.s.l. Sheraro is classified as lowland (hot semiarid agro-ecology) with annual temperature ranges from 20.6 to 37.2°C. Human population of the town is estimated to be 25,539 (12,752 male and 12,787 female) [31].

The total cattle population of Central and North Western zones were 725,700 and 1,954,132 of which 323,488 and 1,041,463 were dairy cattle respectively [32].

2.2. Sampling method

A two-stage sampling method, comprising purposive and random sampling, was employed to select the study area and respondents, respectively. In the initial stage, three towns representing different agro-ecologies (highland, midland, and lowland) were purposively selected based on criteria such as milk production potential, accessibility, and population density. These districts were chosen due to the presence of a large number of small-scale dairy farms relative to other areas. According to data from the Bureau of Agriculture and Rural Development of the Tigray region [33], Aksum, Shire-Indaselassie, and Sheraro were identified as towns with substantial dairy farm activity. Specifically, Aksum had 344 dairy farms with an average of 5 dairy cows per farm, Shire-Indaselassie had 332 dairy farms with an average of 4.85 cows per farm, and Sheraro had 304 dairy farms with an average of 4.55 cows per farm.

In the second stage, a stratified random sampling [34] approach was employed to select samples from both small-scale dairy farms and cafeterias. Two strata were created based on this stratified random sampling process. Based on [35] approximately 10 % of the total number of dairy farms in each agro-ecology was included in the sample. Samples were collected during both the summer/rainy and spring/dry seasons to examine the physicochemical properties of cow's raw milk.

2.3. Sample size determination

To calculate the sample size for this study, the formula provided by Ref. [35] was utilized:

$n = Z^2SD^2/e^2$. Where n is the sample size, Z is the estimated standard deviation at a 95 % confidence interval (CI), which corresponds to the point of the normal distribution at the level of significance (Z = 1.96). SD is the estimated standard deviation, set at 0.15 or 15 % and e is the estimated error, set at 5 %

Therefore, the sample size 'n' was calculated as:

$n = \frac{((1.96)^2 * (0.15)^2}{(0.05)^2} = 34.6$, approximately n = 35 samples per each district.

Based on the above formula, 35 samples from dairy farms in each agro-ecology (3 × 35 = 105) and another 35 samples from cafeterias in each agro-ecology (3 × 35 = 105); totally 210 samples were used for the analysis of physicochemical properties of cow milk in two season (summer and spring).

2.4. Data collection methods

The data collection process involved the utilization of a semi-structured questionnaire administered through interviews with individuals engaged in various aspects of dairy farming, including farm management, milking, and dairy product marketing. The questionnaire was designed to cover a range of parameters relevant to dairy farming, such as milk production levels, water sources, watering frequency, feed sources, and any constraints faced by dairy farms.

In addition to the questionnaire, a checklist was prepared to ensure comprehensive data collection. Based on this checklist, focus group discussions were conducted with dairy farm owners and development agents within each agro-ecology. These discussions provided an opportunity to gather additional insights and information on topics such as feed availability, feeding practices, animal health, milk production, and handling practices, particularly in relation to both dry and rainy seasons.

By employing a combination of semi-structured interviews, checklists, and focus group discussions, the data collection process aimed to capture a holistic understanding of the dairy farming practices and challenges within the study area. This comprehensive approach facilitated the gathering of diverse perspectives and valuable insights from key stakeholders involved in the dairy industry.

2.5. Milk sample collection

The laboratory-based investigation in this research project was focused on assessing the quality of raw milk samples. To conduct this analysis, a total of 105 samples of cow milk were collected from dairy farms in the morning, immediately after milking Holstein Friesian (HF) dairy cows. These samples were collected using 500 ml sterilized glass bottles to ensure proper containment and preservation. In addition to the samples collected from dairy farms, another set of 105 milk samples were obtained from cafeterias. This comprehensive approach allowed for a comparison between milk sourced directly from farms and milk served in cafeterias. Overall, a total of 210 milk samples were gathered for analysis of physicochemical properties. These samples were collected over both the dry and rainy seasons, providing insights into any potential seasonal variations in milk quality. By conducting laboratory analyses on these samples, the research aimed to assess various physicochemical properties of the milk, thereby contributing to a better understanding of the overall quality and characteristics of cow milk sourced from both farms and cafeterias within the study area.

The milk samples collected from both farms and cafeterias were consolidated into two large sterilized stainless steel containers, with one container designated for the pooled samples from farms and the other for those from cafeterias. Upon thorough mixing within each container, representative working samples were extracted using sterilized glass bottles. These working samples were meticulously labelled to ensure proper identification and then promptly transported to the Mekelle University Dairy laboratory within 8 h. To maintain the integrity of the samples during transport, an icebox set at 4 °C was utilized. This temperature-controlled environment helps to preserve the freshness and prevent any potential degradation of the milk samples. Upon arrival at the laboratory, the physicochemical properties of the cow milk were determined through various analytical procedures and tests. This method ensures the accuracy and reliability of the data obtained from the analysis of the milk samples.

2.6. Physicochemical analysis

The major chemical composition of cow milk determined to include the content of fat, lactose, solids not fat (SNF), protein, water added, salt, density, and freezing point. Milk compositions were determined using a calibrated Lacto scan (ultrasonic milk analyzer, Bulgaria). After recording the Lacto scan reading specific gravity was calculated using the formula; $SG = \frac{LC}{100} + 1$. Where, Lc = Lacto scan reading at a given temperature.

2.7. Data management and analysis

The data collected from the survey and laboratory analyses were entered in to MS Excel spreadsheets for organization and then analyzed using statistical software packages such as SPSS version 20 and JMP 5. Descriptive statistics such as means, frequency distributions, and percentages were employed to describe the survey data, providing a summary of the characteristics and trends observed in the dataset. Inferential statistics techniques were also utilized. One-way analysis of variance (ANOVA) was employed to compare agro-ecology as a parameters. Moreover, a T-test sample analysis was conducted to compare the means of physicochemical content of milk across two seasons and different milk sources.

This statistical test helps determine whether there are significant differences between the means of multiple groups. Pearson's correlation coefficient was utilized to explore the relationships between different physicochemical characteristics of the milk. This analysis helps identify any linear relationships between variables, providing insights into how changes in one variable may be associated with changes in another. Statistical significance was determined at the $\alpha = 0.05$ level, indicating that differences or relationships observed were considered significant if the probability of their occurrence by chance alone was less than 5 %. Overall, by employing a combination of descriptive and inferential statistical analyses, the research aimed to gain a comprehensive understanding of the factors influencing the physicochemical properties of cow milk and the relationships between these properties.

3. Results and discussion

3.1. Milk yield and herd structure

In Table 1, the herd structure and milk yield of dairy cattle in the study area are presented. The overall mean herd structure of dairy cattle includes lactating cows (2.65 ± 0.17), dry cows (1.87 ± 0.13), heifers (2.11 ± 0.14), and calves (2.22 ± 0.15). According to the survey results, there is a significant difference ($P < 0.05$) in the number of calves across different agro-ecologies. Specifically, there is a higher number of calves in the midland and lowland areas compared to the highland area. This observation may be attributed to the larger proportion of lactating cows being maintained in midland and lowland agro-ecologies, leading to more calves being born in these areas. Moreover, dairy producers in the highland areas cull male calves shortly after they reach one month of age. In contrast, producers in the lowland and midland areas keep male calves within the herd for breeding purposes, which leads to a larger number of calves. This finding provides valuable insight into the distribution of herd structure and milk yield across different agro-ecological zones, highlighting potential differences in management practices and productivity levels among these regions.

As noted in Table 1, the herd composition of lactating cows was found to be significantly higher in the midland and lowland areas compared to the highland area ($P < 0.05$). This observation may be attributed to the common practice of keeping lactating cows primarily for breeding purposes, resulting in a larger proportion of newly born animals in these regions. The overall average milk yield and lactation length in the current study were determined to be 13.38 ± 0.63 L and 8.53 ± 0.28 months, respectively. It was found that the average milk yield in the highland area was significantly higher than that in the lowland area ($P < 0.05$). This discrepancy in milk yield between highland and lowland areas may be influenced by various factors such as differences in environmental conditions, availability of forage and water, management practices, and breed characteristics. Similarly, the findings reported in Ref. [36] indicate that daily milk production in lowland areas was significantly lower ($P \leq 0.05$) compared to highland agro-ecology. Whereas the average number of lactating cows per household was significantly higher in midland (3.13 ± 0.27) and lowland (2.57 ± 0.29) areas compared to highland (2.21 ± 0.27) areas, despite the lower milk yield observed in lowland. This discrepancy in milk yield could be attributed to differences in management practices and environmental factors. Access to concentrate feed; clean water and health services are limited as the area is remote and far from the capital city.

In the present study, the overall herd composition per household included 2.65 ± 0.17 milking cows, 2.11 ± 0.14 heifers, and 2.22 ± 0.15 calves. The average milk yield per day in the study area was higher than that of Holstein Friesian (HF) and Borena crossbred cows in the Holeta research center and West Shewa, Oromia region [37,38]. However, the overall mean lactation length of milking cows was recorded at 8.53 months (Table 1), which is lower than the lactation length (11.40) reported for HF and Borena crossbreds in the Holeta research center [37]. This difference in lactation length may be attributed to variations in the blood level of dairy cows, overall management practices, and feeding practices. Moreover, according to Ref. [39], the season of the year had significant effects on milk production of dairy cattle. This suggests that seasonal variations may also contribute to differences in milk production levels observed across different regions and time periods.

3.2. Physicochemical properties of Cow's raw milk

3.2.1. Physicochemical property of milk in dairy farms

The physicochemical property of cow's raw milk in different agro-ecology (highland, midland, and lowland) is depicted in Table 2.

Table 1
Milk yield and herd structure of dairy cattle in the study area.

Parameter	Agro-ecology (N = 35 for each agro-ecology)			Overall (mean ± SE)	P-value
	Highland (mean ± SE)	Midland (mean ± SE)	Lowland (mean ± SE)		
MY/day/cow (litre)	14.93 ± 1.30^{a}	13.21 ± 0.80^{ab}	10.93 ± 0.82^{b}	13.38 ± 0.63	0.049
Lactation length (month)	8.56 ± 0.58	8.17 ± 0.26	9.14 ± 0.57	8.53 ± 0.28	0.436
Lactating cows (head)	2.21 ± 0.27^{b}	3.13 ± 0.27^{a}	2.57 ± 0.29^{ab}	2.65 ± 0.17	0.046
Dry cows (head)	1.42 ± 0.16^{b}	2.26 ± 0.21^{a}	1.75 ± 0.28^{ab}	1.87 ± 0.13	0.011
Heifers (head)	2.04 ± 0.28	2.15 ± 0.17	2.17 ± 0.39	2.11 ± 0.14	0.935
Calves (head)	1.75 ± 0.24^{b}	2.57 ± 0.23^{b}	2.24 ± 0.28^{ab}	2.22 ± 0.15	0.0405

MY = milk yield, SE = standard error.
Different superscripts across rows are significant at ($P < 0.05$).

Mostly the composition of the milk changes markedly with agro-ecology, season, and source of milk samples (be it from dairy farms or cafeterias). The average value of milk fat content from dairy farms in the study area was 4.22 ± 0.1 %. In this study, it was noted that milk fat content did not significantly differ among different agro-ecologies, but there was a slightly higher fat content observed in lowland areas. Conversely, solid not fat and lactose contents were significantly higher in highland areas compared to lowland areas. Additionally, the milk protein content was found to be higher ($p < 0.05$) in highland regions compared to midland and lowland areas. One potential explanation for these differences in milk composition is the availability and quality of feed for dairy cows in different agro-ecologies. For instance, highland areas may have better access to concentrate feeds, which could contribute to higher protein content in the milk. On the other hand, lowland areas may face challenges such as remoteness, minimal infrastructure, and scarcity of green forages, especially during dry seasons. These factors can influence the feeding strategies adopted for dairy cows, thereby affecting the nutritional content of the milk they produce. Overall, the findings suggest that feeding strategies play a crucial role in determining the variation in milk composition across different agro-ecologies. Milk composition is determined by feeding (nurture), with a differing balance for each component [40]. Additionally, Parmar et al. [41] stated that, among other factors, the most important parameter affecting milk composition is diet/feed type. Understanding these factors is important for dairy farmers and policymakers to optimize milk production and quality in various geographic regions [42]. Moreover, environmental temperature can also affect the composition of milk. For instance, when the temperature increased from 6.2 °C to 31.3 °C, significant decreases were observed in milk protein, fat, solids-not-fat (SNF), and somatic cell count, by approximately 4.09 %, 5.75 %, 1.31 %, and 16.8 %, respectively [43].

The overall mean value of SNF, protein, and lactose content was 7.2 ± 0.16, 2.86 ± 0.1 and 4.01 ± 0.06, respectively. The protein and SNF content of cow milk in the present finding was lower than the finding of [44]. The results of protein and lactose in this study were notably lower compared to previous findings, with protein ranging from 3.28 % to 3.53 % and lactose from 4.38 % to 4.59 % [12,20,45,46]. Additionally, a study [21], reported a protein content of 3.55 %, which surpasses the current results. The variations observed could be attributed to differences in feedstuffs, breed, and ecological conditions between tropical and temperate regions. Research by Ref. [14] suggests that temperate forage grasses typically exhibit higher neutral detergent fiber (NDF) levels in spring and contain a higher concentration of soluble carbohydrates compared to tropical forage grasses. Moreover, milk composition can vary among countries [47] due to discrepancies in breed and climatic conditions [17,48]. In the study area, the primary feed sources were crop residue such as straw and by product of sorghum kernel *(gurdi)* and hay as roughage, along with wheat bran as concentrate feed. This particular feed composition likely contributed to the low protein and SNF (solids-not-fat) contents observed in the milk.

The specific gravity of cow's raw milk varied significantly ($P < 0.05$) across different geographic regions, with the highest value recorded in highland areas (1.032 ± 0.0 g/ml) and the lowest in lowland areas (1.029 ± 0.0 g/ml). The overall mean specific gravity of cow's raw milk in the study area was 1.03 ± 0.0 g/ml. This finding contrasts slightly with the average specific gravity of cow milk (1.032) reported by Ref. [49].

The total salt content of cow's raw milk was 0.60 %, 0.61 %, and 0.52 % in highland, midland, and lowland, respectively, and significantly lower ($p < 0.05$) salt content of cow milk was found in lowland agro-ecology. Salt and lactose are the decisive factors for the freezing point of milk [48]. The overall mean freezing point of cow's raw milk in the study area was −0.45°C. This was not in the range of freezing point of raw milk stated by Refs. [50–53]. The deviation from the specified range of −0.53 to −0.55 °C suggests abnormal milk composition, possibly indicating adulteration [50]. Discrepancies between these results and those of other studies may be attributed to variations in environmental conditions, breeds of cows, and types of feed provided to the animals. The freezing point was found to be statistically different ($p < 0.05$) across different agro-ecologies. The overall mean water content of cow milk in the study area was 14.9 ± 1.80 %, with significantly higher water content detected in lowland areas ($p < 0.05$). This discrepancy could be due to differences in the types of feed provided to the animals, and it is possible that some farm owners adulterate the milk by adding water to increase its volume. Indeed, previous studies [54] have reported that more than 95 % of examined samples were adulterated by the addition of water.

In the Tigray region, specifically in the study area, the summer season corresponds to the rainy season, while spring is the dry season. Table 3 displays the physicochemical properties of cow's raw milk by season. The average fat content of cow's raw milk in summer and spring was 4.5 ± 0.13 and 3.93 ± 0.08, respectively, with milk fat significantly ($P \leq 0.001$) higher in summer than in

Table 2
Physicochemical properties of cow milk from dairy farms by agro-ecology and season.

	Fat% M±SE	SNF% M ±SE	Protein% M ±SE	Lactose% M ±SE	SG% M ±SE	WA% M ±SE	FP% M ±SE	Salt% M ±SE
Agro-ecology (N = 35 samples in each agro-ecology and season)								
Highland	4.24 ± 0.02	7.37 ± 0.16^a	3.09 ± 0.21^a	4.22 ± 0.01^a	1.032 ± 0.0^a	12.35 ± 1.6^b	−0.47 ± 0.0^b	0.6 ± 0.0^a
Midland	4.06 ± 0.05	7.56 ± 0.02^a	2.98 ± 0.08^b	4.09 ± 0.04^a	1.031 ± 0.0^a	9.85 ± 1.0^b	−0.47 ± 0.0^b	0.61 ± 0.0^a
Lowland	4.35 ± 0.28	6.57 ± 0.39^b	2.53 ± 0.19^b	3.74 ± 0.14^b	1.029 ± 0.0^b	22.50 ± 4.1^a	−0.4 ± 0.02^a	0.52 ± 0.03^b
O. mean	4.22 ± 0.10	7.20 ± 0.16	2.86 ± 0.10	4.01 ± 0.06	1.03 ± 0.00	14.90 ± 1.80	−0.45 ± 0.01	0.58 ± 0.01
P-value	p ≤ 0.456	p ≤ 0.021	p ≤ 0.067	p ≤ 0.001	p ≤ 0.006	p ≤ 0.006	p ≤ 0.002	p ≤ 0.002
Season								
Summer	4.50 ± 0.19^a	6.72 ± 0.27^b	2.45±.1^b	3.96 ± 0.16	1.029 ± 0.0^b	18.68 ± 2.9^a	−0.43 ± 0.01	0.49 ± 0.03^b
Spring	3.93 ± 0.29^b	7.62 ± 0.01^a	3.28 ± 0.09^a	4.07 ± 0.06	1.032 ± 0.0^a	11.10 ± 1.4^b	−0.46 ± 0.01	0.58 ± 0.0^a
P-value	p ≤ 0.021	p ≤ 0.000	p ≤ 0.000	p ≤ 0.447	p ≤ 0.007	p ≤ 0.001	p ≤ 0.062	p ≤ 0.001

Different superscripts across + are significantly different at $P < 0.05$.
M = Mean, SE= Standard Error; SNF= Solid Not Fat; SG= Specific Gravity; WA= Water Added.
FP= Freezing Point; O.mean = Overall mean.

spring. Solid non-fat, protein, and salt contents of cow milk also varied with the season. Seasonality appeared to be a significant contributing factor to the variation in solid non-fat, protein, and salt content of raw milk, with significantly higher SNF, salt ($p < 0.05$), and protein contents ($p \leq 0.001$) found in spring. Seasonal variation was identified as one of the important factors altering milk composition, consistent with previous reports by Refs. [17,49,55–57].

Furthermore, seasonal variation in temperature, rainfall, feed availability, and feed utilization are reported as major factors affecting dairy cattle productivity and milk quality [58]. Milk composition is highly correlated with the availability of quality feed. During the summer/rainy season, green and nutritious feeds are available, leading to decreased supplementation of concentrate feed. Conversely, during the spring/dry season, farmers rely more heavily on low-quality roughages and increase supplementation with concentrate feed to compensate for nutrient deficits [59].

The lower composition of raw milk in summer, except for milk fat, may be attributed to the decreased availability of concentrate feed, with dairy cows mainly depending on green roughage feeds. Roughage feed typically has lower crude protein content compared to concentrate feed. Grasses, which are prevalent in summer, are rich in acetate, leading to higher fat content in milk as milk fat synthesis depends on the supply of acetate from the rumen [55]. Conversely, concentrate feeds are rich in propionate, resulting in milk that is higher in protein and SNF. With similar essence [60], reported milk fat content was severely reduced on low roughage feed, whereas lactose content was increased due to the change on pattern of rumen fermentation from high acetate on normal roughage to high propionate on high concentrate feed. This means differences in feed type may affect milk yield, composition and other qualities of the milk [57]. High concentrate feeding lowers the production of rumen acetate; as a result milk fat was lower [61]. However, protein and total digestible nutrients were highest with the concentrates compared to grasses [62]. Moreover, increased environmental temperatures can lead to decreased milk fat content [55], particularly in the dry season (mainly from March to May in the study area).

The mean specific gravity of milk samples collected in spring was 1.032 ± 0.0 g/ml, while in summer, it was 1.029 ± 0.0 g/ml. A significant difference ($p < 0.05$) in the specific gravity of cow milk was observed between the two seasons. This disparity could be attributed to the variation in milk composition (protein, SNF, salt, and lactose) between spring and summer seasons, as indicated in Table 2. Similarly [63], reported that the lactose concentration of cow milk increased with a higher dietary energy content of the feed provided to the cows. Additionally, according to Ref. [14], milk yield and lactose content were higher in spring.

3.2.2. Physicochemical property of milk in cafeterias

The milk collected from cafeterias exhibited the following composition: fat 3.22 ± 0.17, solids-not-fat (SNF) 6.26 ± 0.26, protein 2.33 ± 0.11, lactose 3.42 ± 0.14, and salt 0.49 ± 0.02 (Table 3). It was observed that the total content of milk composition in the lowland was significantly ($p < 0.05$) lower compared to milk collected from midland and highland regions.

The average specific gravity of cow milk collected from cafeterias was determined to be 1.024 ± 0.0. This finding falls below the normal range of specific gravity for cow's raw milk (1.028–1.033 g/ml) [64], and it is lower than the findings of other studies [20,45], which reported values of 1.04 g/ml and 1.028 g/ml, respectively. This variation could potentially be attributed to differences in feed, temperature, and breed of cows. However, the adulteration of milk with water is considered a significant factor in the study area. In the lowland agro-ecology, the addition of water during the boiling of milk by cafeteria owners is perceived as a common practice, as evidenced through direct observation and interviews with respondents. Moreover, the amount of water added in the lowland was notably higher (35.99 ± 2.85) compared to midland and highland agro-ecologies, with a significant difference ($p \leq 0.001$) observed. Additionally, the freezing point was also higher ($p < 0.05$) in the lowland compared to the highland agro-ecology.

As depicted in Table 4, the fat, protein, solids-not-fat (SNF), lactose, and salt content of cow's raw milk obtained from dairy farms were significantly ($p < 0.05$) higher compared to the raw milk collected from cafeterias. Additionally, the specific gravity of raw milk collected from dairy farms (1.03 ± 0.00 g/ml) was notably higher ($p \leq 0.000$) than the specific gravity of raw milk collected from cafeterias (1.022 ± 0.001 g/ml). This outcome suggests that cafeteria owners may be adding more water to the milk compared to small dairy farm owners, likely in an attempt to increase the quantity of milk and ultimately gain higher profits. This practice of adulterating milk with water is consistent with the findings of previous studies [65,66], which reported instances of raw milk being adulterated with water across various milk value chains. Furthermore, the value of water added and the freezing point were both higher ($p < 0.05$) in cafeterias compared to milk collected from dairy farms. This further supports the notion that adulteration with water is more prevalent in milk obtained from cafeterias than from dairy farms.

The result from the correlation analysis (Table 5) was also confirmed that freezing point and water added were positively associated

Table 3
Physicochemical properties of cow milk from cafeterias by agro-ecology.

	Fat% M±SE	SNF% M±SE	Protein% M±SE	Lactose% M±SE	SG% M±SE	WA% M±SE	FP% M±SE	Salt% M±SE
Agro-ecology (N = 35 samples in each agro-ecology)								
Highland	3.6 ± 0.14^{a}	7.27 ± 0.04^{a}	2.79 ± 0.06^{a}	3.93 ± 0.04^{a}	1.03 ± 0.0^{a}	11.44 ± 0.49^{c}	-0.45 ± 0.01^{b}	0.58 ± 0.01^{a}
Midland	3.55 ± 0.09^{a}	6.35 ± 0.41^{a}	2.32 ± 0.15^{b}	3.5 ± 0.23^{a}	1.025 ± 0.0^{b}	23.85 ± 5.35^{b}	-0.39 ± 0.03^{ab}	0.51 ± 0.03^{a}
Lowland	2.5 ± 0.4^{b}	5.16 ± 0.45^{b}	1.89 ± 0.17^{c}	2.84 ± 0.24^{b}	1.018 ± 0.0^{c}	35.99 ± 2.85^{a}	-0.33 ± 0.02^{a}	0.39 ± 0.04^{b}
O. mean	3.22 ± 0.17	6.26 ± 0.26	2.33 ± 0.11	3.42 ± 0.14	1.024 ± 0.0	23.76 ± 2.85	−0.39 ± 0.02	0.49 ± 0.02
P-value	$p \leq 0.008$	$p \leq 0.001$	$p \leq 0.0001$	$p \leq 0.003$	$p \leq 0.0001$	$p \leq 0.0001$	$p \leq 0.003$	$p \leq 0.002$

Different superscripts across columns are significantly different at $P < 0.05$.
M = Mean, SE= Standard Error; SNF= Solid Not Fat; SG= Specific Gravity; WA= Water Added.
FP= Freezing Point; O.mean = Overall mean.

Table 4
Physicochemical properties of cow milk by milk source (*t*-test analysis).

Milk composition	Cafeteria (N = 105)	Farm (N = 105)	Overall Mean ± SE	P-value
	Mean ± SE	Mean ± SE		
Fat	3.22 ± 0.25[b]	4.22 ± 0.14[a]	3.72 ± 0.12	p ≤ 0.002
SNF	6.26 ± 0.38[b]	7.17 ± 0.23[a]	6.72 ± 0.17	p ≤ 0.0056
Protein	2.33 ± 0.15[b]	2.86 ± 0.15[a]	2.6 ± 0.08	p ≤ 0.023
Lactose	3.42 ± 0.2[b]	4.01 ± 0.08[a]	3.72 ± 0.09	p ≤ 0.015
Specific gravity g/ml	1.022 ± 0.001[b]	1.03 ± 0.00[a]	1.026 ± 0.001	p ≤ 0.000
Water added	23.76 ± 2.85[a]	14.9 ± 1.66[b]	19.33 ± 1.8	p ≤ 0.012
Freezing point	−0.39 ± 0.02[a]	−0.45 ± 0.01[b]	−0.42 ± 0.01	p ≤ 0.036
Salt	0.49 ± 0.03[b]	0.58 ± 0.02[a]	0.53 ± 0.01	p ≤ 0.043

Different superscripts across rows are significantly different at P < 0.05.
SE = standard error mean.

with each other at (p ≤ 0.01) level of significance but negatively associated (p ≤ 0.01) with SNF, protein, lactose, specific gravity and salt content. Similarly [67], reported that freezing point was negatively associated with protein, SNF and density. These negative associations indicated that adulteration of milk with water. Specific gravity depends on the protein, SNF, and fat content of cow's raw milk; evidenced by the strong association (p ≤ 0.01) among each other except fat content. Solid not fat, protein, and lactose content were showed strong positive association (p ≤ 0.01) with specific gravity and with each other. This suggests that as the levels of fat, SNF, protein, and lactose in the milk increase, there is a corresponding increase in the specific gravity of the milk samples. This finding aligns with our understanding of milk composition and its relationship to specific gravity. Protein, lactose, and other dissolved solids contribute to the density of milk, and as their concentrations increase, the overall density or specific gravity of the milk also increases. Therefore, the observed correlation highlights the importance of these components in determining the physicochemical properties of milk.

The result obtained in this study is consistent with previous findings reported by Refs. [20,67,68] however; a negative correlation between protein and lactose was reported by Ref. [14]. This variation might be due to the difference in feed, feeding practice, disease, stage of lactation, milking frequency, udder health, heat stress and breed.

As exhibited in Table 5, milk fat was strongly and positively correlated with specific gravity but weakly correlated with all the other physicochemical parameters of cow milk. In line with this [69], reported that milk fat was positively but weakly associated with protein and lactose content of cow milk. Since dairy producers provide their animals with roughage and concentrate feeds with an inconsistent ratio across seasons.

4. Conclusion

From this study, it is likely to conclude that agro-ecology, season, and source of milk have significant effect on physicochemical properties of cow's raw milk. The adulteration of milk by the addition of water, particularly practiced by cafeteria owners across all agro-ecologies, poses a significant challenge to milk quality and consumer trust. In response to this issue, it is crucial for the bureau of agriculture, in collaboration with municipal administrations of respective towns, to establish legal entities and regulations aimed at combating milk adulteration. Overall, collaborative efforts between government agencies, milk producers, and cafeteria owners are essential to address the issue of milk adulteration and improve the overall quality and safety of milk supplied to consumers across all agro-ecologies.

CRediT authorship contribution statement

Alem Tadesse: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Dawit Gebremichael:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Birhane Hailay:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Fsahatsion Hailemariam:** Writing – original draft, Supervision, Software,

Table 5
Correlations among physicochemical properties of cow's raw milk.

Milk composition	Fat	SNF	Protein	Lactose	Specific gravity	Water added	Freezing point	Salt
Fat	1							
SNF	0.13	1						
Protein	0.186	0.930[a]	1					
Lactose	0.262	0.959[a]	0.864[a]	1				
Specific gravity	−0.116	0.905[a]	0.865[a]	0.938[a]	1			
Water added	−0.211	−0.965[a]	−0.890[a]	−0.924[a]	−0.871[a]	1		
Freezing point	−0.220	−0.958[a]	−0.880[a]	−0.952[a]	−0.871[a]	0.975[a]	1	
Salt	0.13	0.969[a]	0.883[a]	0.960[a]	0.863[a]	−0.946[a]	−0.966[a]	1

[a] Correlation is significant at the 0.01 level (2-tailed).

Methodology. **Hagos Hadgu:** Visualization, Supervision, Data curation. **Girmay Kalayu:** Resources, Methodology.

Data availability

No Data will be available on request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to grammar checking. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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