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Research Article

Production of Pre-ripened Provolone Cheese and Quality Characterization Using Blends of Whole Milk

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ABSTRACT

Cheese is a product made from the curd obtained from milk by coagulating the casein with the help of rennet in the presence of lactic acid produced by added starter culture. The study was aimed to evaluate the yield and quality of pre-ripened provolone cheese prepared from different blends of cow, doe, ewe and camel whole milk. Pre-ripened provolone cheeses were made from blends contained ratio between (60-80)%, (10-30)%, (10-30)% and (0-20)% for cow, doe, ewe and camel milk, respectively using standard procedure. The whole milk and its pre-ripened provolone cheeses were subjected to physicochemical analyses. The physicochemical analyses, bioactive compounds, microbial quality and consumer acceptability of the pre-ripened provolone cheese were analyzed. The blending proportion of different milk had significant ($p<0.05$) effect on the physiochemical property, mineral, bioactive and sensory quality of pre-ripened provolone cheese. The physiochemical property of whole milk for manufacture of pre-ripened provolone cheeses were in the range between (10.56 to 15.08)% for total solids, (3.45 to 5.20)% for fat and (3 to 4.19)% for crude protein. The chemical composition of pre-ripened provolone cheese prepared from different blended milk ranged from (47.32- 67.05)% for total solids, (24.26-36.81)% for fat, (17.78-26.30)% for crude protein, (1.09-3.49)% for total ash, and (0.75-2.98)% for lactose; ascorbic acid (0.49 to 3.08) mg/kg and total polyphenols (1.00 to 17.50) mg GAE/g. The fat, protein and total solids recovery of pre-ripened provolone cheeses ranged from 64.87% to 95.39%, 54.58% to 84.67% and 41.35% to 59.92% respectively. The yield of pre-ripened provolone cheese ranged from 9.22% to 13.47%. Total bacteria count was found to be the predominant micro flora of pre-ripened provolone cheeses and reached 5.24 cfu/g in the control cheese. The entire consumer acceptability of the pre-ripened provolone cheese was in acceptable range. In conclusion, the pre-ripened provolone cheese prepared from T12 (60% cow, 10% doe and 30% ewe) milk gave better cheese yield and had auspicious results in nutritional qualities comparable with that of control cheese and other cheese samples.

Keywords: Blends of whole milk; Cheese yield; Pre-ripened provolone cheese; Quality characterization; Total Solids (TS); Titratable Acidity (TA)

INTRODUCTION

Cheese is one of the most widely consumed fermented dairy products with a growing consumer demand. Cheese is a fresh or matured product obtained by the drainage of liquid after the coagulation of milk, cream, skimmed or partly skimmed milk, butter milk or a combination them [1]. Provolone is a typical semi-hard drawn-curd cheese and it became popular around the end of the 19th century when it began to be produced in the southern regions of Italy [2].

Cheese is a nutrient-dense food, the precise nutritional composition and it is a popular food, a good source of nutrients and is generally considered as part of a healthy diet. The type of milk gives the cheese different nutritional and organoleptic properties.

In Ethiopia, milk is produced and marketed to consumer without being pasteurized. About 98% of the annual milk produced by subsistence farmers who live in rural areas where dairy processing in the country is basically limited to smallholder level and hygienic qualities of products are generally poor [3]. Milk and milk products

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form part of the diet for many Ethiopians. They consume dairy products either as fresh milk or in fermented or soured form estimated that 68% of the total milk produced is used for human consumption in the form of fresh milk, butter, yogurt and cheese while the rest is given to calves and wasted in the process [4].

Cheese can be manufactured from different types of milk. In regions where fresh milk is scarce, cheese has been successfully made from recombined anhydrous milk fat and reconstituted skim milk powder. However, it can produce cheese from cows, ewes, does and camel's milk and their combination [5]. For instance, ewe milk is considered more appropriate than cow milk for the production of good quality cheese [1]. Provolone is one of the major cheeses and it was become popular around the end of the 19th century when it began to be produced in the southern regions of Italy [1].

Given the high potential for dairy development and the ongoing policy reforms and technological interventions, success similar to that realized in the neighboring Kenya under a very similar production environment is expected in Ethiopia. In this experimental thesis Provolone type cheese will be prepared from blends of animal-based milk for local consumption and also appropriate parameters will be identified for best quality. Therefore, the aim of the present study was thus to utilize the different milk types, notably cow, doe, ewe and camel milk for manufacture of pre-ripened provolone cheese. With this aim there is a need to study the effect of different blending of milk on the Physiochemical, bioactive component microbial characteristics and sensory quality of pre-ripened provolone cheese.

MATERIALS AND METHODS

Sample collection and transportation

A total of 70 liters of fresh milk was collected for Cheese making. Four types of raw milk samples (cow, doe, ewe and camel) were used for cheese making. Camel milk and Cow milk were collected from Bulbula village and Hawassa town respectivelty. Fresh doe and ewe milk were collected from Langano area and Kofele particularly from Ashoka (A village 15 km from Kofele). The samples were immediately placed in ice box and transported to Hawassa University Food Science laboratory. Then after the milk sample was stored for 4°C until the cheese was prepared.

Experimental design and treatments

The blending formulation of cow, doe, ewe and camel milk is presented (Table 1). Design expert 7.0 software was used for the blending of each milk to make Provolone cheese. The experimental design was Completely Randomized Design (CRD) for physicochemical properties, bioactive compounds and microbial load and Randomized Complete Block Design (RCBD) for the sensory analysis. In this experiment, the different milks were first collected from the available source. The different milks were then blended for pre-ripened provolone cheese manufacturing. The blended whole milk samples were analyzed and then cheese was developed.

Table 1: Blend formulations of cow, doe, ewe and camel milk for cheese preparation.

Run order	Cow milk (%)	Doe milk (%)	Ewe milk (%)	Camel milk (%)
1	80.00	10.00	10.00	0.00
2	75.00	10.00	10.00	5.00
3	70.00	10.00	20.00	0.00
4	70.00	15.00	10.00	5.00
5	70.00	10.00	15.00	5.00
6	65.00	10.00	15.00	10.00
7	65.00	15.00	10.00	10.00
8	65.00	20.00	10.00	5.00
9	65.00	10.00	10.00	15.00
10	60.00	10.00	10.00	20.00
11	60.00	15.00	15.00	10.00
12	60.00	10.00	30.00	0.00
13	60.00	10.00	20.00	10.00
14	60.00	30.00	10.00	0.00
15	60.00	20.00	20.00	0.00
16	60.00	20.00	10.00	10.00
Control	100.00	0.00	0.00	0.00

Manufacture of pre-ripened provolone cheese

Process of pre-ripened provolone cheese was conducted according to the method as described by Codex Standard presented in Figure 1 [6]. Cow milk was the experimental control. The blended milk samples were pasteurized at 63°C for 30 minutes in batch pasteurizer. Then the milk was cooled to 37°C in order to add starter culture (*S.thermophilus+L.bulgaricus*) at 2% w/v and kept for 20 minutes. About 1.5 g/100 L, milk rennet was added and kept for 40 minutes. After that, the curd was cut and cooked at 42°C for 40 minutes. The desired pH of the curd was pH 5.5 for stretch it. Then the cheese was stretched and molded in molding tube and it was put for overnight on working table. Then the cheese was removed from the mold and immersed in to brine (20%) for 5 hours.

Analysis methods

Characterization of milk: The whole cow milk and different blends of milk obtained by the blend proportion were characterized in terms of pH, acidity, total solids, lactose, protein, fat and total minerals (ash), using methodology of Richardson and AOAC [7,8].

Characterization of pre-ripened cheese: Each cheese samples was determined for total solids, fat, protein, ash and lactose according to the method described by Richardson et al [7]. Cheese yield was calculated as a weight of cheese divided by weight of milk expressed as a percentage [7]. Recoveries of components (protein, fat and total solid) was calculated as the component in the cheese divided by the original weight of the component in the milk expressed as percentage as suggested by Mehaia [9].

Bioactive compounds: Total polyphenols: The total polyphenol content in the cheese samples were determined by the method of Anesini et al [10]. 1 ml Folin Ciocalteu reagent (diluted ten times) was added and the mixture was left for 5 min and then

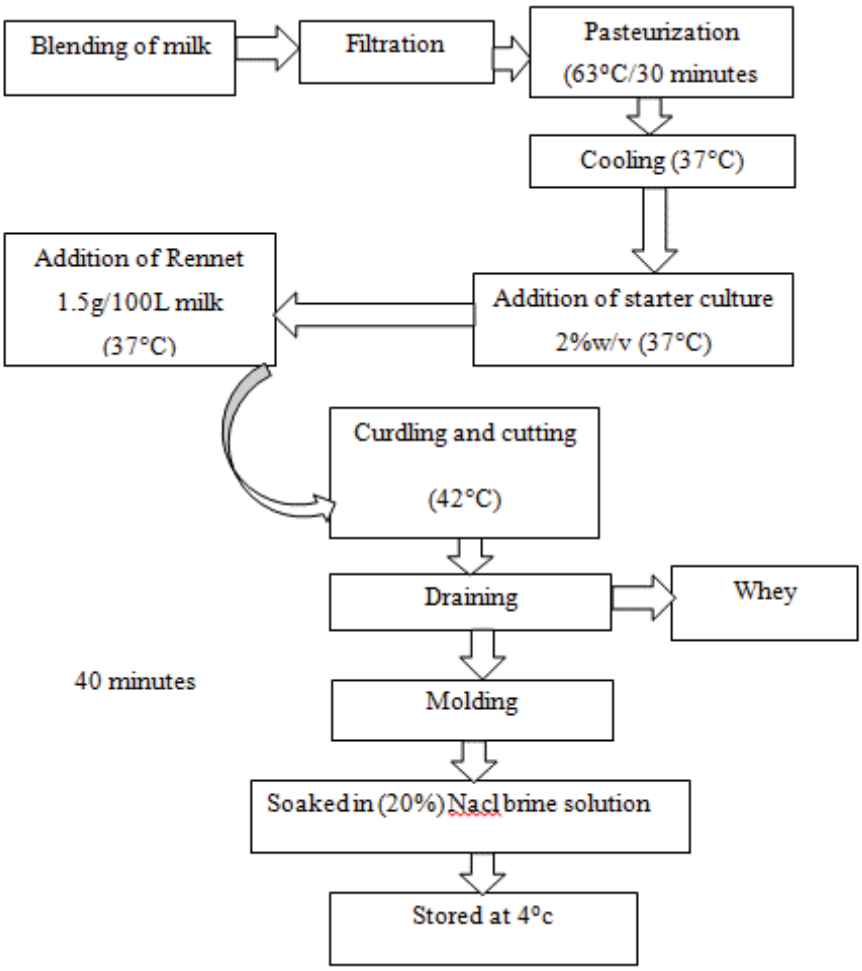

Figure 1: Flow diagram illustrating pre-ripened provolone cheese preparation.

1 ml (75 g/L) of sodium carbonate was added. The absorbance of the resulting blue color was measured at 765 nm with a UV-visible spectrophotometer (JENWAY, 63000, UK) after incubation for 90 min at room temperature.

Ascorbic acid: Ascorbic acid was determined according to the method of Nweze et al [11]. The equivalence point of the titration determined using a starch indicator. 20 mL of filtrate sample was added into a 125 mL Erlenmeyer flask. 25 mL of distilled water and 1 mL of starch indicator solution was added. Then the sample titrated with standardized iodine solution.

Microbiological quality of cheese: Microbiological analysis (Total Bacteria Count, Total coliform count and Yeast and mold) of the pre-ripened provolone cheeses was conducted by the method of International dairy federation by using spread plate technique [12].

Consumer acceptability: Sensory acceptability of the cheeses was evaluated according to the method of International dairy federation using 5-point hedonic scale [12]. Twenty panelists were selected for evaluating the sample. The analysis was conducted in duplicate with 5-point hedonic scale (1=dislike extremely, 2=dislike moderately, 3=neither like nor dislike, 4=like moderately and 5=like extremely).

Statistical Data Analysis

The data was subjected to one factor of variance (ANOVA) used SAS (Statistical Analysis System, version 9; SAS Institute, 2001). All the samples were analyzed in duplicates and Duncan's multiple-range test for mean comparison with at significance level of 5% was used.

RESULTS AND DISCUSSION

Physiochemical property of whole blended milk and provolone cheese

Physiochemical property of whole blended milk: The physicochemical properties of different blended milk in comparison with those of cow milk were presented (Table 2). The results show that pH value of various milk samples varied from 6.40 ± 0.01 to 6.61 ± 0.01. Maximum pH was found in case of cow milk (6.62), while results presented that pH value of T_{16} (60% cow, 20% doe, 10% ewe and 10% camel) milk was significantly ($p<0.0001$) lower than cow milk sample.

The higher pH of raw cow milk is due to the transportation handling system and the location of milk that was collected. Raw cow milk was collected from Hawassa town and other milk samples were transported from the area far from Hawssa and this could result the decrement of pH due to long transportation time. The lower pH in T_{16} may be due to the production of acid resulting from bacterial growth and multiplication in the milk samples and lower pH of milk could be because of milk composition and amount of normal flora in the milk during transportation and storage [1]. The pH of milk samples used for the development of

pre-ripened provolone cheeses in the current study were within the normal range [13,14]. The pH value found in cow milk was in agreement with the findings of Kanwal et al and Hanna A [15,16]. The pH of control and blended milk in the current study was fit for coagulation and cheese production. The pH values between 5 and 7 are said to generally best for coagulation of milk [17].

The titratable acidity of milk blended from (60% cow, 20% doe, 10% ewe and 10% camel) milk (T_{16}) was significantly ($p<0.0001$) higher than the titratable acidity values of 100% cow milk (control) and other treatments except T_{13} and T_{14}. The cow milk was recorded lower titratable acidity value than other milk types used for pre-ripened provolone cheese preparation. The lower titratable acidity in cow milk may be due to the higher amount of pH. The low pH has been found to increase acidity which is due to increased lactic acid bacteria [18].

In this study, the titratable acidity of blended milk was increased with the decrement of cow milk. This may be due to the production of lactic acid bacteria in the doe, ewe and camel milk during transportation. The titratable acidity of cow milk in the current study is similar with the finding of Enb et al. [19]. However the titratable acidity of blended milk is higher the titratable acidity value reported by Rasheed et al. [1]. This might be due to bacterial growth and multiplication during transportation before cheese preparation.

Results illustrated that total solid content of cow milk and blended milk measured between (10.56 ± 0.10)% to (15.08 ± 0.25)%. Statistical analysis showed a significant ($p<0.0001$) difference of the total solid content due to the blending proportion of milk. The total solid content of control (100% cow) milk was 12.66 which is significantly differ from T_{12} and T_{10} which have the highest and lowest value of total solids content. The blended milk prepared from T_{12} (60% cow, 10% doe and 30% ewe) milk had significantly ($p<0.0001$) higher (15.08%) total solids content than the control and other samples. While the lowest total solids in T_{10} (60% cow, 10% doe, 10% ewe and 20% camel) milk which was significantly ($p<0.0001$) lower (10.56%) than the other treatments. The higher total solids of T_{12} is because high proportion of ewe milk causes increment of total solids of blended milk and the high composition the milk compared to the other treatments. Rasheed et al reported that total solids content was increasing as the amount of ewe milk incorporated in the blended milk was increasing [1]. The TS (Total Solid) (14.50 ± 0.32) content of blended camel milk found by Amenu and Deeth was higher compared to the finding of the current study [20]. The total solids content of cow milk in the current study which is slightly comparable with the result of Gemechu et al. (2015) who found total solid in milk from Shashemene town (12.87 ± 0.11). This variation might be due to different species and environmental condition. Different values of total solid content of raw milk samples have been reported by different scholars. The variation could be due to difference in breed, feeding and managing practices which have important effects on milk composition and quality [1, 21].

Results presented that fat content of (60% cow, 10% doe and 30% ewe) milk (T_{12}) was significantly ($p<0.0001$) higher than other milk samples while minimum fat content was observed in T_{10} (60% cow, 10% doe, 10% ewe and 20% camel) milk. In the current study the fat content of cow (control) milk (4.60) was not significantly differ from other treatments except T_3, T_7, T_9, T_8 T_{10}, and T_{12}. This result was comparable with the finding of Hanna [16]. On the other hand, Rasheed et al observed that the lower fat content of cow milk compared to the current study [1]. In the present study, fat content of different blended milk samples was lower than the finding of Pandya et al who observed that fat content of different types of milk varied from 3.7% to 7.90% [21]. This difference might be due the type of breed in which the milk produced.

The control sample had protein content of 3.46, which is lower than the result founded by Amenu et al [20]. This difference might be due to different in breed and type of feeding. During this research work, maximum amount of protein was found in T_{12} (60% cow, 10% doe and 30% ewe) milk as compared to the rest of

Table 2: Physicochemical properties of whole blended milk. [a-h]: All values are presented in mean ± standard deviation; Values within the same column with different superscript letters are significantly ($p< 0.05$) different from each other; T_1-T_{16}: Treatment 1-Treatment 16.

Treatments	pH	Titratable Acidity (TA) (%)	Total Solid (TS) %	Fat %	Protein %
Control	6.62 ± 0.01^{a}	0.17 ± 0.01^{f}	12.66 ± 0.21^{de}	4.60 ± 0.29^{cd}	3.46 ± 0.03^{def}
T_1	6.58 ± 0.04^{ab}	0.19 ± 0.01^{ef}	12.91 ± 0.10^{cd}	4.80 ± 0.14^{bc}	3.57 ± 0.12^{c-f}
T_2	6.54 ± 0.01^{bcd}	0.19 ± 0.00^{ef}	12.44 ± 0.08^{def}	4.55 ± 0.07^{cde}	3.39 ± 0.12^{ef}
T_3	6.57 ± 0.03^{abc}	0.19 ± 0.00^{ef}	14.03 ± 0.12^{b}	5.00 ± 0.14^{ab}	3.94 ± 0.09^{ab}
T_4	6.50 ± 0.01^{cde}	0.18 ± 0.00^{ef}	12.23 ± 0.28^{efg}	4.55 ± 0.07^{cde}	3.47 ± 0.06^{def}
T_5	6.54 ± 0.04^{bcd}	0.17 ± 0.00^{f}	12.90 ± 0.14^{cd}	4.85 ± 0.07^{bc}	3.84 ± 0.08^{bc}
T_6	6.50 ± 0.01^{cde}	0.17 ± 0.00^{f}	12.04 ± 0.23^{fg}	4.55 ± 0.07^{cde}	3.48 ± 0.25^{def}
T_7	6.53 ± 0.00^{bcd}	0.17 ± 0.01^{f}	11.89 ± 0.13^{g}	4.10 ± 0.14^{f}	3.65 ± 0.25^{cde}
T_8	6.54 ± 0.02^{bcd}	0.20 ± 0.00^{de}	12.85 ± 0.16^{cd}	4.25 ± 0.21^{ef}	3.28 ± 0.03^{fg}
T_9	6.51 ± 0.01^{bcd}	0.20 ± 0.02^{de}	11.04 ± 0.08^{h}	3.70 ± 0.14^{g}	3.28 ± 0.07^{fg}
T_{10}	6.49 ± 0.07^{def}	0.20 ± 0.01^{def}	10.56 ± 0.10^{i}	3.45 ± 0.07^{g}	3.00 ± 0.03^{h}
T_{11}	6.44 ± 0.01^{efg}	0.21 ± 0.03^{cde}	12.10 ± 0.42^{fg}	4.60 ± 0.14^{cd}	3.05 ± 0.12^{gh}
T_{12}	6.47 ± 0.04^{d-g}	0.24 ± 0.01^{bcd}	15.08 ± 0.25^{a}	5.20 ± 0.14^{a}	4.19 ± 0.09^{a}
T_{13}	6.48 ± 0.03^{def}	0.25 ± 0.03^{abc}	12.82 ± 0.40^{cd}	4.60 ± 0.14^{cd}	3.63 ± 0.21^{cde}
T_{14}	6.42 ± 0.03^{fg}	0.26 ± 0.01^{ab}	13.16 ± 0.12^{c}	4.45 ± 0.07^{de}	3.53 ± 0.00^{def}
T_{15}	6.49 ± 0.03^{def}	0.23 ± 0.01^{bcd}	13.88 ± 0.11^{b}	4.85 ± 0.07^{bc}	3.72 ± 0.09^{bcd}
T_{16}	6.40 ± 0.01^{g}	0.29 ± 0.03^{a}	12.14 ± 0.06^{fg}	4.30 ± 0.14^{def}	3.39 ± 0.05^{ef}

milk samples. However, minimum protein content was observed in T_{10} (60% cow, 10% doe, 10% ewe and 20% camel) milk that resembled to the findings of Amenu et al [20] which was 2.9 ± 0.13. Protein content of different blends of milk found in this study was lower than reported by Zedan et al. [22]. This might be due to difference species and environmental condition.

Physiochemical property of pre-ripened provolone cheese: Table 3 shows the pH and titratable acidity values during storage period for the different pre-ripened provolone cheese types. Results indicated that the average pH value of pre-ripened provolone cheese prepared from cow (control) milk and different blends of milk samples varied from 5.14 ± 0.03 to 5.46 ± 0.06 (1st day) storage (Table 3). The pH value of pre-ripened provolone cheese prepared from (60% cow, 10% doe, 10% ewe and 20% camel) milk (T_{10}) observed in the present study was higher than the other treatments throughout the storage day (1st up to 3rd day). This might be due to high proportion of camel milk from other treatments. This observation is in agreement with pH values reported by Khan et al [23]. The lowest pH value (5.14 ± 0.03) was observed in cheese samples made from pure cow milk (control) in the present study.

The pH values observed between the 1st and 3rd days of storage cow milk cheese in the present study are in total agreement with the previous results of Mamo, who reported a value of 5.2 ± 0.21 in cow's milk cheese [24]. The pH value of control cheese made from pure cow milk is higher than the finding of Ashenafi who reported pH value ranging from 3.7 to 4.6 [25]. The pH values of cheese made from blended milk (T_1-T_{16}) are in agreement with the pH value range of (4.3-4.7) reported by O'Mahony [26]. During storage, the decrease in pH was most rapid in 100% cow milk and slowest in that of T_{10} milk. This difference in the rate of pH decrease resulted from higher amount of camel milk in T_{10} from other treatments. The presence lactoferrin; lactoperoxidase and immunoglobulin prevent the growth of lactic acid bacteria and the pH slightly decreased during storage compared with those in other milk [27]. It can be also seen that there was a decrease in the pH of all cheeses starting from the first to third day storage. Similar trend in the pH of Cheddar cheese made from cow milk was reported by Walstra et al. [14].

The result of Titratable Acidity (TA) during storage period for pre-ripened provolone cheeses prepared from cow (control) milk and different blends of milk were tabulated (Table 3). In the current study, the titratable acidity of pre-ripened provolone cheese manufactured from cow milk was higher than that of other cheese samples (T_1-T_{16}) throughout the storage period. This value was higher than the finding of Sulieman et al. [28], who reported a value of (0.59 ± 0.90)%. This difference may be due to the environmental condition and type of coagulant used. On the other hand the titratable acidity of pre-ripened provolone cheese prepared from (60% cow, 10% doe, 10% ewe and 20% camel) milk (T_{10}) was lower throughout the storage period compared to control and other samples. This might be the amount of lactose available in cheese. The shortage of lactose could thus reduce the activity of lactic acid bacteria and the acid production in the cheese [27]. Therefore the lower amount of acidity in cheese sample made from (60% cow, 10% doe, 10% ewe and 20% camel) milk (T_{10}) had high amount of camel milk than the other treatments and camel milk by nature had the property of antimicrobial activity and decrease the production of lactic acid bacteria which produce lactic acid in the cheese [27]. In the current study, the increased in acidity of all cheese samples during storage showed the activity of starters added during cheese manufacturing. The primary function of starters is the conversion of lactose and other sugars in milk to lactic and other acids [29]. The titratable acidity of the cheese samples was higher than the finding of Nada et al [30]. This difference may be due to the environmental condition in which the cheese sample stored; the method of cheese preparation and the type of milk used.

In the current study, control sample (100% cow) milk cheese had total solid content of 57.19 ± 0.71. In this study, as the amount of

Table 3: pH and Titratable Acidity (TA) of pre-ripened provolone cheese made from different blends of milk during storage at 4°C for 3 days. [a-g]: All values are means ±SD; Values with in the same column with different superscript are significantly ($p<0.05$) different; T_1 – T_{16}: Treatment 1 - Treatment 16.

Treatment	1st day		2nd day		3rd day	
	pH	TA	pH	TA	pH	TA
Control	5.14 ± 0.03^{e}	0.65 ± 0.02^{a}	4.79 ± 0.08^{d}	0.74 ± 0.02^{a}	4.09 ± 0.05^{g}	0.87 ± 0.02^{a}
T_1	5.31 ± 0.03^{bcd}	0.55 ± 0.04^{b}	4.83 ± 0.08^{cd}	0.67 ± 0.05^{abc}	4.20 ± 0.03^{efg}	0.84 ± 0.03^{abc}
T_2	5.30 ± 0.03^{bcd}	0.56 ± 0.01^{b}	4.94 ± 0.04^{bcd}	0.70 ± 0.03^{ab}	4.27 ± 0.01^{ef}	0.84 ± 0.02^{abc}
T_3	5.27 ± 0.01^{bcd}	0.57 ± 0.01^{ab}	4.97 ± 0.03^{bcd}	0.65 ± 0.06^{abc}	4.28 ± 0.02^{ef}	0.79 ± 0.02^{c}
T_4	5.27 ± 0.01^{bcd}	0.53 ± 0.08^{bc}	4.97 ± 0.03^{bcd}	0.65 ± 0.11^{abc}	4.23 ± 0.11^{efg}	0.85 ± 0.02^{ab}
T_5	5.31 ± 0.01^{bcd}	0.53 ± 0.08^{bc}	5.00 ± 0.01^{a-d}	0.64 ± 0.11^{abc}	4.23 ± 0.14^{efg}	0.81 ± 0.03^{abc}
T_6	5.34 ± 0.01^{abc}	0.48 ± 0.02^{bcd}	5.08 ± 0.21^{ab}	0.55 ± 0.01^{cd}	4.53 ± 0.04^{bc}	0.63 ± 0.01^{e}
T_7	5.26 ± 0.02^{b-e}	0.43 ± 0.04^{d}	5.09 ± 0.18^{ab}	0.55 ± 0.05^{cd}	4.43 ± 0.08^{cd}	0.64 ± 0.04^{e}
T_8	5.28 ± 0.06^{bcd}	0.53 ± 0.06^{bc}	4.97 ± 0.03^{bcd}	0.66 ± 0.09^{abc}	4.34 ± 0.06^{de}	0.81 ± 0.01^{abc}
T_9	5.39 ± 0.01^{ab}	0.45 ± 0.01^{cd}	5.06 ± 0.06^{abc}	0.48 ± 0.03^{d}	4.85 ± 0.06^{a}	0.60 ± 0.01^{e}
T_{10}	5.46 ± 0.06^{a}	0.40 ± 0.01^{d}	5.22 ± 0.11^{a}	0.47 ± 0.06^{d}	4.91 ± 0.08^{a}	0.58 ± 0.01^{e}
T_{11}	5.33 ± 0.14^{bcd}	0.44 ± 0.01^{cd}	5.04 ± 0.13^{abc}	0.54 ± 0.01^{cd}	4.63 ± 0.08^{b}	0.63 ± 0.05^{e}
T_{12}	5.21 ± 0.02^{de}	0.55 ± 0.03^{b}	4.94 ± 0.03^{bcd}	0.65 ± 0.02^{abc}	4.13 ± 0.05^{fg}	0.80 ± 0.01^{bc}
T_{13}	5.32 ± 0.08^{bcd}	0.42 ± 0.03^{d}	5.09 ± 0.03^{ab}	0.56 ± 0.12^{bcd}	4.66 ± 0.02^{b}	0.69 ± 0.03^{d}
T_{14}	5.22 ± 0.06^{cde}	0.53 ± 0.03^{bc}	5.04 ± 0.01^{abc}	0.74 ± 0.01^{a}	4.65 ± 0.02^{b}	0.83 ± 0.01^{abc}
T_{15}	5.20 ± 0.05^{de}	0.53 ± 0.02^{bc}	5.04 ± 0.01^{abc}	0.74 ± 0.01^{a}	4.66 ± 0.03^{b}	0.86 ± 0.03^{a}
T_{16}	5.32 ± 0.06^{bcd}	0.44 ± 0.03^{cd}	5.12 ± 0.13^{ab}	0.52 ± 0.06^{cd}	4.61 ± 0.02^{b}	0.63 ± 0.01^{e}

Table 4: Chemical composition of pre-ripened provolone cheese made from different blends of whole milk. [a-j]:All values are means ±SD; Values with in the same column with different superscript are significantly ($p < 0.05$) different; T_1 – T_{16}: Treatment 1 - Treatment 16.

Treatment	Parameters				
	Fat %	Protein %	Total solids %	Ash %	Lactose %
Control	29.75 ± 1.51^{e}	23.38 ± 0.45^{cde}	57.2 ± 0.71^{cde}	1.09 ± 0.10^{f}	2.98 ± 0.33^{a}
T_1	29.79 ± 0.10^{e}	23.75 ± 0.45^{bcd}	58.17 ± 0.57^{cd}	2.17 ± 0.34^{de}	2.46 ± 0.18^{abc}
T_2	28.30 ± 0.53^{efg}	22.60 ± 0.14^{e}	55.93 ± 0.45^{de}	2.53 ± 0.11^{b-e}	2.50 ± 0.04^{abc}
T_3	34.28 ± 0.89^{b}	24.22 ± 0.49^{bc}	62.38 ± 1.34^{b}	2.76 ± 0.23^{bc}	1.12 ± 0.66^{bc}
T_4	27.70 ± 0.44^{fgh}	22.65 ± 0.17^{e}	55.86 ± 0.99^{de}	2.74 ± 0.18^{bcd}	2.82 ± 0.56^{ab}
T_5	29.80 ± 0.52^{e}	22.50 ± 0.40^{e}	57.57 ± 1.04^{cde}	2.48 ± 0.10^{cde}	2.79 ± 0.02^{ab}
T_6	27.32 ± 0.63^{fgh}	20.51 ± 0.62^{f}	52.35 ± 1.88^{f}	3.08 ± 0.04^{ab}	1.44 ± 0.59^{abcd}
T_7	26.56 ± 0.08^{hi}	20.55 ± 0.07^{f}	51.14 ± 0.49^{f}	2.76 ± 0.28^{abc}	1.33 ± 0.27^{a-d}
T_8	27.01 ± 0.22^{gh}	21.01 ± 0.13^{f}	52.8 ± 1.21^{f}	2.57 ± 0.05^{b-e}	2.21 ± 1.51^{a-d}
T_9	25.13 ± 0.38^{ij}	18.72 ± 0.49^{g}	48.00 ± 0.05^{g}	3.40 ± 0.12^{a}	0.75 ± 0.29^{d}
T_{10}	24.26 ± 0.81^{j}	17.78 ± 0.93^{h}	47.32 ± 0.59^{g}	3.49 ± 0.07^{a}	1.79 ± 0.78^{a-d}
T_{11}	31.35 ± 0.68^{d}	20.73 ± 0.26^{f}	56.29 ± 0.87^{de}	3.00 ± 0.16^{abc}	1.21 ± 0.08^{bcd}
T_{12}	36.81 ± 0.40^{a}	26.30 ± 0.57^{a}	67.06 ± 1.06^{a}	2.68 ± 0.62^{b-e}	1.27 ± 0.28^{bcd}
T_{13}	29.41 ± 1.21^{e}	22.97 ± 0.14^{de}	56.17 ± 1.19^{de}	2.92 ± 0.16^{abc}	0.87 ± 0.04^{cd}
T_{14}	32.20 ± 0.31^{cd}	23.42 ± 0.22^{cde}	59.17 ± 0.71^{c}	2.13 ± 0.35^{e}	1.42 ± 0.271^{abc}
T_{15}	33.37 ± 0.47^{bc}	24.49 ± 0.06^{b}	62.30 ± 1.04^{b}	2.46 ± 0.29^{cde}	1.98 ± 1.27^{a-d}
T_{16}	28.85 ± 0.92^{ef}	21.46 ± 0.50^{f}	54.79 ± 0.64^{e}	2.94 ± 0.06^{abc}	2.54 ± 1.11^{abc}

Table 5: Component recovery and yield of pre-ripened provolone cheese samples. [a-j]: All values are presented in mean ± standard deviation; Values within the same column with different superscript letters are significantly ($p<0.05$) different from each other; T_1-T_{16}: Treatment 1-Treatment 16

Treatments	Fat recovery (%)	Protein recovery (%)	TS recovery (%)	Yield (%)
Control	71.96 ± 0.56^{d-g}	75.17 ± 0.68^{b}	50.27 ± 0.14^{efg}	11.13 ± 0.04^{fg}
T_1	70.99 ± 0.24^{d-g}	74.96 ± 3.54^{b}	50.68 ± 0.08^{def}	11.25 ± 0.07^{efg}
T_2	68.91 ± 2.57^{fgh}	73.81 ± 2.45^{b}	49.79 ± 0.22^{efg}	11.08 ± 0.04^{fg}
T_3	82.10 ± 0.93^{c}	73.59 ± 0.54^{b}	53.26 ± 1.07^{c}	11.98 ± 0.11^{c}
T_4	69.85 ± 1.11^{efg}	73.31 ± 1.82^{b}	52.45 ± 0.53^{cd}	11.48 ± 0.18^{de}
T_5	70.50 ± 0.89^{d-g}	67.31 ± 1.20^{cd}	51.20 ± 0.43^{de}	11.48 ± 0.17^{de}
T_6	67.70 ± 2.40^{gh}	66.54 ± 2.92^{cd}	49.02 ± 0.67^{fg}	11.28 ± 0.03^{ef}
T_7	72.75 ± 2.52^{def}	63.29 ± 4.68^{d}	48.53 ± 0.09^{g}	11.23 ± 0.04^{efg}
T_8	71.28 ± 4.58^{d-g}	71.68 ± 0.75^{bc}	46.02 ± 0.18^{h}	11.20 ± 0.07^{efg}
T_9	68.27 ± 0.62^{fgh}	57.35 ± 1.92^{e}	43.70 ± 1.0^{i}	10.05 ± 0.14^{i}
T_{10}	64.87 ± 3.06^{h}	54.58 ± 1.43^{e}	41.35 ± 2.33^{j}	9.22 ± 0.32^{j}
T_{11}	74.45 ± 0.53^{c}	74.38 ± 0.86^{b}	50.82 ± 0.17^{def}	10.93 ± 0.18^{gh}
T_{12}	95.39 ± 0.99^{a}	84.67 ± 0.47^{a}	59.92 ± 1.49^{a}	13.47 ± 0.11^{a}
T_{13}	74.31 ± 1.90^{de}	73.70 ± 4.97^{b}	50.93 ± 0.27^{def}	11.62 ± 0.18^{d}
T_{14}	82.14 ± 1.53^{c}	75.32 ± 1.75^{b}	51.04 ± 0.39^{def}	11.35 ± 0.14^{def}
T_{15}	87.38 ± 1.41^{b}	83.75 ± 0.32^{a}	57.00 ± 1.12^{b}	12.70 ± 0.14^{b}
T_{16}	71.96 ± 0.31^{d-g}	66.25 ± 0.47^{cd}	49.34 ± 0.95^{efg}	10.73 ± 0.04^{d}

camel milk for samples mixed with other milk type's increased, the total solid content was decreased following the naturally abundant water available in the camel milk [31]. Based on their total solid content the cheese sample coded T_{12} had higher total solid content and the lowest moisture content implies best quality because lower moisture content of cheese helps the cheese to have longer shelf life. According to Adegoke et al higher moisture could favor the growth and proliferation of microorganisms and thus reduces the shelf life of cheese [32].

Statistical analysis showed significant ($p<0.0001$) influence of different blends of milk on protein content of pre-ripened provolone cheese. The present results are in accordance to those reported by Oluwayemisi et al [33]. The protein content found for T_{10} cheese was lower than that reported by Derar et al who found (24.86 ± 4.1)% and higher than the value reported by Nada et al who found (16.12 ± 0.71)%. T_{12} (60% cow, 10% doe and 30% ewe) pre-ripened provolone cheese had the highest protein content than the control and other samples because ewe milk had high amount of protein [30, 34]. The ewe milk has the ability to recover high protein in cheese making [35, 36]. The protein values discovered in this study were higher than those reported by earlier researchers on cheese and these values are 5.33% and 12.86%, but lower than the findings of Fasakin et al who reported 44.5% [36-38].

Results illustrated that fat content of pre-ripened provolone cheese

in the range of (24.26 ± 0.81)% and (36.81 ± 0.40)%. Pre-ripened provolone cheese prepared from (60% cow, 10% doe and 30% ewe milk) (T_{12}) had significantly ($p<0.0001$) higher fat contents (36.81%) than the other treatments, while the cheese made from T_{10} had the least fat content. There was an increase in fat content of cheese with the composition of sheep milk mixed in each milk samples. According to Rasheed et al, the presence of high amount ewe milk during cheese preparation affects the fat content of cheese [1]. The lower fat content observed in T_{10} indicates that, cheese can be stored over a longer period without developing rancid flavors [32]. The current results are in accordance with the findings of Khan et al, who observed (21.4 to 23.6)% fat content [39]. Similar result reported regarding fat content of cheese made from cow milk [40]. The differences in fat content of cheese might be due to losses of fat with whey during cheese preparation [41].

From the blended pre-ripened provolone cheese, higher ash content (3.49 ± 0.07), (3.40 ± 0.12) and (3.08 ± 0.04) found in samples T_{10}, T_9 and T_6 respectively. On the other hand, the cheese prepared from cow milk had significantly ($p<0.0001$) lower than the other cheese samples. The ash content of cheese was higher when the proportion of camel milk increased. In this study, the ash content of the cheese is higher than the result reported by Oladipo et al as they observed lower content [42]. This might be influenced by the strength of the brine solution used during cheese preparation. The average ash content of cheese recorded in the present study for cow milk cheeses were in line with the observations of Zedan et al [22]. They found 1.17 % ash content in cow milk cheese. The ash content of the pre ripened provolone cheese samples analyzed in the present study is also higher than the ash content (1.16%) of Ayib reported by Kassa [40]. This might be the difference in cheese preparation and milk types.

Lactose content of pre ripened provolone cheese samples presented in Table 4. Sample coded T_9 has contained the lowest lactose content (0.75 ± 0.029) than other cheeses. The control has contained remarkably higher lactose content (2.98 ± 0.33) than other blended provolone cheeses. Fermented dairy products have been reported to be more nutritious than the milk from which they are made [43]. The higher nutritional value of these products has been attributed to the increased production or availability of certain nutrients and to the pre-hydrolysis of the major milk components by lactic starter cultures, rendering them more digestible.

Table 6: Bioactive compounds of pre-ripened provolone cheese made from different blends of milk. [a-i]: All values are presented in mean ± standard deviation; Values within the same column with different superscript letters are significantly ($p< 0.05$) different from each other; T_1-T_{16}: Treatment 1-Treatment 16

Treatments	Ascorbic acid (mg/kg)	Total Polyphenols (mg GAE/g)
Control	0.49 ± 0.06^{i}	1.00 ± 0.28^{h}
T_1	1.23 ± 0.13^{h}	4.80 ± 0.28^{g}
T_2	1.50 ± 0.13^{g}	5.10 ± 0.14^{g}
T_3	1.98 ± 0.07^{f}	5.67 ± 0.38^{g}
T_4	2.03 ± 0.00^{ef}	5.34 ± 0.09^{g}
T_5	2.16 ± 0.06^{ef}	6.10 ± 1.56^{g}
T_6	2.24 ± 0.19^{ed}	10.50 ± 0.42^{de}
T_7	2.20 ± 0.13^{ef}	9.24 ± 0.141^{ef}
T_8	2.16 ± 0.18^{ef}	8.20 ± 0.47^{f}
T_9	2.73 ± 0.13^{b}	15.30 ± 1.84^{b}
T_{10}	3.08 ± 0.12^{a}	17.50 ± 0.71^{a}
T_{11}	2.47 ± 0.12^{cd}	14.07 ± 0.57^{b}
T_{12}	2.69 ± 0.06^{bc}	12.34 ± 0.76^{c}
T_{13}	2.51 ± 0.06^{bc}	14.44 ± 0.15^{b}
T_{14}	2.03 ± 0.25^{ef}	10.54 ± 0.37^{de}
T_{15}	1.98 ± 0.06^{f}	11.37 ± 0.24^{cd}
T_{16}	2.07 ± 0.05^{ef}	13.84 ± 0.62^{b}

Cheese yield and component recovery

The recovery for different treatments from different blends of milk ranged from (64.87 to 95.39)% for fat, (54.58 to 84.67)% for protein, and (41.35 to 59.92)% for total solid (Table 5). Pre-ripened provolone cheese prepared from (60% cow, 10% doe and 30% ewe) milk had significantly ($p<0.0001$) higher total solids recovery and fat recovery than the other cheese samples and the lowest component recovery was for T_{10} (60% cow, 10% doe, 10% ewe and 20% camel) milk. The additions of camel milk to cow, goat and sheep milk decreased the total recovery of protein. T_{10} had significantly ($p<0.0001$) lower total solids recovery than the other cheese including the control cheese. On the other hand, recovery of protein (54.58%) is lower in T_{10} than other cheese treatment.

Further increase in the camel milk proportion exhibited a downward trend in the TS recovery. The TS recovery was lower in camel milk cheese followed by cow milk cheese and was relatively high in cow milk cheese as reported by Hanna [16]. When the blending proportion of sheep milk increased with cow, doe and camel milk, the recovery of total solids and fat increased. Similar results being higher recovery of protein and fat for ewe milk cheese [16]. In the current study, the addition of sheep milk reduced the ratio of milk total solids retained in the whey and increases the total solids recovery of cheese. This supported the previous report that the recovery rate of milk solids in the cheese is significantly increased; after enriching camel milk with ewe milk, recovery increased, respectively, instead of only 37% for the pure camel milk [44].

The yield of cheese was calculated for fresh cheese and results were tabulated (Table 5). The cheese prepared from T_{12} (60% cow, 10% doe and 30% ewe) milk had significantly ($p<0.0001$) higher yield (13.47%) than other cheese samples, while the cheese made from T_{10} (60% cow, 10% doe, 10% ewe and 20% camel) milk gave the lowest yield (9.22%). The control cheese had cheese yield of 11.13% and it is significantly differ from T_{12}.

The lower cheese yield in T_{10} is due to high amount of camel milk from the other treatments. The camel milk contains abnormally low milk solids and its cheese processing ability is poor due to differences in availability of β-casein and it has more large casein micelles than goat and cow milk. The low content in β-casein and its ratio to total proteins in addition to the lack of β-lactoglobulins are the main factors that limit cheese making performances and cheese yield from camel milk [43, 44]. Mixing the sheep milk with the other milk raised the yield of cheese treatments increased. The highest cheese yield of 30% sheep milk from the other treatments is due to the higher TS of milk for the cheese preparation and higher recovery of components as compared with the other blends of milk. It is a very important parameter: the higher the recovered percentage of solids, the greater is the amount of cheese obtained

and therefore gains in economic terms [45]. The higher Feta cheese yield was obtained from milk that has higher total solids content [35]. The yield of cheese in this study considerably deviates from the findings of Hühn et al who reported (8-10)% for cheese made from cow milk [46].

Bioactive compounds of pre-ripened provolone cheese

Ascorbic acid: The ascorbic acid content of pre-ripened provolone cheese made from (60% cow, 10% doe, 10% ewe and 20% camel) milk (T_{10}) scored significantly ($p<0.0001$) higher (3.08 ± 0.12) content than the control and other treatments (Table 6). The ascorbic acid content of cow milk pre-ripened provolone cheese was 0.49 ± 0.06. This value is significantly lower than the other cheeses samples. Ascorbic acid decreased with decreasing blend proportion of camel milk. This result indicated that camel milk could be a good source of ascorbic acid. In the current study, pre-ripened provolone cheese made from higher proportion of camel milk from other treatments had high ascorbic acid content. This is due to the fact the feed of camel milk is differ from other species and camel consume different herbs and plants, which have high content of ascorbic acid [47]. Ascorbic acid plays a major part in the medicinal reputation of camel milk [48]. In the current studies, the ascorbic acid content of pre-ripened provolone cheese was analyzed. However, the other finding did the fresh milk of different milks. In the current study, the ascorbic acid content of cheese is lower than other works that did on fresh milk. This difference might be due to the treatment of heat. The ascorbic acid is highly unstable, especially with temperature change [49].

Total polyphenols: Total polyphenols content of pre ripened provolone cheese samples were in the range between 1.00 ± 0.28 to17.50 ± 0.71. The cheese prepared from (60% cow, 10% doe, 10% ewe and 20% camel) milk scored significantly ($p<0.0001$) higher total polyphenol (17.50 ± 0.71) content than the cheese made from other treatments, while the cheese made from 100% cow milk (control) had lower amount total polyphenols. It was observed that the bioactive compounds of pre-ripened provolone cheese were decreasing with decreasing blend proportion of camel milk.

The difference in total polyphenols of the cheese in the current study could be due to the different milk types and feeding systems. The presence of phenolic compounds in the milk and later in the cheese is a result of their transfer from plant to milk. According to Hilario et al, pasture plants are rich and significant source of bioactive components and they can transfer into the milk and cheese [50]. In the current study the total polyphenols content of the cheese samples is contradict with the finding of Levkov et al [51]. This is due to the processing method during cheese preparation. It was found that salting negatively influenced the total polyphenol concentration by hiding the approach of the phenolic compounds to react with Folin reagent [52]. In the current finding total polyphenol was observed in all pre-ripened provolone cheese samples. This is because the presence of phenolic compounds in the pre-ripened provolone cheese might be attributed to the pasture, animal metabolism and amino acid catabolism or microbial activity [53].

Microbial quality of pre-ripened provolone cheese

The main microbiological group in the cheese after preparation was Total Bacteria Count (TBC). The initial mean (day 1 storage) of TBC counts for the pre-ripened provolone cheese samples were in the range between 2.26 log cfu/g for T_{10} and 3.63 log cfu/g for control (Table 7). This microbial group showed an increase during storage time and reached values in the range of (3.14 to 5.24) log cfu/g (3rd day storage). The cheese prepared from cow milk had high TBC during storage (1st up to 3rd) days. This is due to reduction in pH, which has an inhibitory effect on the growth of

Table 7: Microbial load of pre-ripened provolone cheese made from different blends of milk during storage at 4°C for 3 days. $^{a-h}$: All values are presented in mean ± standard deviation; Values within the same column with different superscript letters are significantly ($p<0.05$) different from each other; T_1-T_{16}: Treatment 1-Treatment 16

Treatments	Total Bacteria Count			Total coliform count		
	1st day	2nd day	3rd day	1st day	2nd day	3rd day
Control	3.63 ± 0.50^{a}	4.70 ± 0.08^{a}	5.24 ± 0.09^{a}	3.42 ± 0.07^{a}	2.76 ± 0.06^{a}	2.27 ± 0.07^{bcd}
T_1	3.26 ± 0.32^{ab}	3.70 ± 0.16^{de}	4.14 ± 0.15^{ef}	3.34 ± 0.06^{a}	2.75 ± 0.18^{a}	2.62 ± 0.07^{a}
T_2	3.19 ± 0.01^{ab}	3.98 ± 0.11^{b-e}	4.70 ± 0.19^{a-e}	3.20 ± 0.27^{ab}	2.73 ± 0.35^{a}	2.40 ± 0.30^{abc}
T_3	3.12 ± 0.13^{ab}	4.09 ± 0.04^{bcd}	4.78 ± 0.17^{a-d}	3.07 ± 0.07^{abc}	2.71 ± 0.08^{ab}	2.54 ± 0.06^{ab}
T_4	3.28 ± 0.37^{ab}	3.96 ± 0.34^{b-e}	4.55 ± 0.30^{cde}	2.98 ± 0.16^{bcd}	2.55 ± 0.05^{abc}	2.03 ± 0.37^{d}
T_5	3.22 ± 0.06^{ab}	3.84 ± 0.13^{de}	4.29 ± 0.27^{def}	2.88 ± 0.06^{b-e}	2.46 ± 0.05^{a-d}	2.08 ± 0.06^{cd}
T_6	3.16 ± 0.30^{ab}	3.88 ± 0.14^{cde}	4.56 ± 0.16^{cde}	2.86 ± 0.09^{b-e}	2.37 ± 0.17^{a-d}	ND
T_7	3.07 ± 0.11^{ab}	4.08 ± 0.37^{bcd}	4.88 ± 0.49^{a-d}	2.88 ± 0.08^{b-e}	2.35 ± 0.23^{a-d}	ND
T_8	3.58 ± 0.16^{a}	4.39 ± 0.28^{ab}	4.90 ± 0.37^{abc}	2.77 ± 0.20^{cde}	2.56 ± 0.08^{abc}	2.38 ± 0.11^{abc}
T_9	2.44 ± 0.08^{cd}	3.08 ± 0.02^{f}	3.42 ± 0.08^{gh}	2.56 ± 0.07^{ef}	2.18 ± 0.06^{cd}	ND
T_{10}	2.26 ± 0.32^{d}	2.70 ± 0.16^{g}	3.14 ± 0.15^{h}	2.39 ± 0.11^{f}	2.03 ± 0.08^{d}	ND
T_{11}	2.88 ± 0.19^{bc}	4.0 ± 0.11 $^{b-e}$	4.87 ± 0.33^{a-d}	2.83 ± 0.23^{b-e}	2.25 ± 0.33^{bcd}	ND
T_{12}	3.17 ± 0.16^{ab}	4.29 ± 0.02^{bc}	5.15 ± 0.20^{ab}	2.93 ± 0.16^{b-e}	2.64 ± 0.13^{abc}	2.13 ± 0.16^{cd}
T_{13}	2.90 ± 0.30^{bc}	3.88 ± 0.03^{de}	5.12 ± 0.06^{cde}	2.66 ± 0.16^{def}	2.34 ± 0.13^{a-d}	ND
T_{14}	3.19 ± 0.28^{ab}	4.03 ± 0.07^{b-e}	4.65 ± 0.02^{b-e}	2.91 ± 0.28^{b-e}	2.53 ± 0.34^{abc}	2.11 ± 0.28^{cd}
T_{15}	3.30 ± 0.09^{ab}	3.61 ± 0.05^{e}	3.89 ± 0.04^{fg}	2.91 ± 0.09^{b-e}	2.57 ± 0.11^{abc}	2.01 ± 0.09^{d}
T_{16}	2.82 ± 0.08^{bc}	3.73 ± 0.18^{de}	4.36 ± 0.31^{dc-f}	2.91 ± 0.16^{b-e}	2.32 ± 0.13^{a-d}	ND

some natural micro flora other than total count bacteria [25]. Pre-ripened provolone cheese made from T_{10} (60% cow, 10% doe, 10% ewe and 20% camel milk) had low TBC from other cheese samples during storage day. This might be due to high proportion of camel milk from the other treatments and there is low production of lactic acid bacteria in this treatment. This result is comparable with the findings of Mohamed et al [49]. The total bacteria count of provolone cheese samples observed in this study is lower than the corresponding value reported by Ashenafi [25]. This might be associated to its low pH and low moisture content of cheese samples. On the other hand, the TBC of the current cheeses were higher than that cheddar cheese [54]. This is might be due to the type of milk used for cheese preparation, the flora in raw milk, the processing conditions and contamination after heat treatment affect the microbiological quality of cheese product [55].

The means of Total Coliform Count (TCC) count for provolone cheeses at 1st day were in the range between (2.39 to 4.42) log cfu/g. Means of TCC for all treatments of cheese except T_9 and T_{10} cheese were high during the first day and then gradually decreased at day 2 and 3 (Table 7). This might be due to the lack of proper handling and hence contamination by microorganisms during storage [56]. The TCC of cheese samples made from cow milk (control) has the highest coliform count at the first day and decreased to 2.27 log cfu/g on the 3rd day. The lower coliform count was observed T_9 and T_{10} throughout the storage day. In the current study the cheese samples prepared from high proportion of camel milk from other treatments had low coliform during storage. In all cheese types that have 10%, 15% and 20% camel milk the TCC at third day was not detected. This may be because of camel milk was reported to have an antimicrobial effect against Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium* [57]. The TCC obtained in this study was lower than reported by Yigrem et al which were 5.7 log cfu/ml and 6.14 log cfu/ml for Ethiopian unpasteurized traditional fermented products, Ayib [58]. According to international standards, soft cheese should not contain more than 100 cfu/ml coliforms bacteria [59]. However in the current study high coliform counts was observed. This might be due to production of milk and cheese under poor conditions. In general the counts of TCC continuously decreased from the first day of storage to the final (3rd day) of storage. The various metabolites excreted by Lactic Acid Bacteria (LAB) and the decrease in pH as a result of their high acidifying capability may partially explain the reduction and disappearance of the total coliform [59].

The Yeast and Mold Count (YMC) of pre-ripened provolone cheese made from different blends of milk was presented (Table 8). The means of YMC for cheeses made using different blends of milk was in the range between (1.72 to 2.75) log cfu/g and (2.42 to 3.59) log cfu/g at beginning and third day of storage respectively. The maximum number of YMC was found to be 2.75 log cfu/g (T_{13}) and minimum 1.72 log cfu/g (T_8) at first day. This number increased maximum to 3.59 log cfu/g (T_{10}) during three days storage period. The highest YMC was obtained throughout storage time (1st up to 3rd day). The acceptable standard count of yeast and mold forming bacteria was <10,000sfu/ml [24]. The results of the present study showed that the pre ripened provolone cheese made from pure cow milk and different blends of cow, doe, ewe and camel milk, contained yeast and mold below the standard acceptable level. The YMC of treated cheese were higher than control cheese. Similarly, the yeast and mold count observed in the current study was higher than that reported by Mamo, which 1.79 log cfu/ml for cheese is made from pasteurized milk within the same storage time [24]. This indicates that processing and storage area exposes cheese for recontamination and care should be taken to safe guard the consumer. Other reason that might increase the YMC load in a processed milk product could be the presence of spores in the milk. Spores are formed when the microbes undergo unfavorable conditions. Later when the milk is heat treated, the

Table 8: Yeast and mold count of pre-ripened provolone cheese made from different blends of milk during storage at 4°C for 3 days. [a-f]: All values are presented in mean ± standard deviation; Values within the same column with different superscript letters are significantly ($p< 0.05$) different from each other; T_1-T_{16}: Treatment 1-Treatment 16

Treatments	YMC Log10cfu/g		
	1st day	2nd day	3rd day
Control	2.27 ± 0.07^{bcd}	2.66 ± 0.16^{bcd}	2.97 ± 0.07^{b-e}
T_1	2.62 ± 0.07^{ab}	2.85 ± 0.04^{bc}	3.32 ± 0.07^{abc}
T_2	2.40 ± 0.30^{a-d}	2.73 ± 0.35^{bc}	3.10 ± 0.30^{a}e
T_3	2.54 ± 0.06^{abc}	2.76 ± 0.06^{bc}	3.24 ± 0.06^{a-d}
T_4	2.03 ± 0.37^{de}	2.34 ± 0.34^{cd}	2.73 ± 0.37^{ef}
T_5	2.08 ± 0.06^{cde}	2.41 ± 0.18^{bcd}	2.78 ± 0.06^{def}
T_6	2.14 ± 0.30^{cde}	2.47 ± 0.17^{bcd}	2.84 ± 0.30^{c-e}
T_7	2.24 ± 0.11^{bcd}	2.55 ± 0.23^{bcd}	2.94 ± 0.11^{cde}
T_8	1.72 ± 0.16^{e}	2.06 ± 0.06^{d}	2.42 ± 0.16^{f}
T_9	2.12 ± 0.01^{cde}	2.48 ± 0.06^{bcd}	2.82 ± 0.01^{def}
T_{10}	2.74 ± 0.18^{a}	3.48 ± 0.78^{a}	3.59 ± 0.39^{a}
T_{11}	2.43 ± 0.19^{a-d}	2.70 ± 0.25^{bcd}	3.13 ± 0.19^{a-e}
T_{12}	2.13 ± 0.16^{cde}	2.49 ± 0.08^{bcd}	2.83 ± 0.16^{c-e}
T_{13}	2.75 ± 0.18^{a}	3.07 ± 0.16^{ab}	3.45 ± 0.18^{ab}
T_{14}	2.11 ± 0.28^{cde}	2.53 ± 0.34^{bcd}	2.81 ± 0.28^{def}
T_{15}	2.01 ± 0.09^{de}	2.37 ± 0.03^{cd}	2.71 ± 0.09^{ef}
T_{16}	2.43 ± 0.16^{a-d}	2.77 ± 0.06^{bc}	3.13 ± 0.16^{a-e}

Table 9: Sensory characteristics of pre ripened provolone cheese made from different blends of whole milk. [a-i]: All Values are mean± standard deviation; values with the same column with different superscript are significantly (p≤0.05) different; T_1-T_{16}: Treatment 1-Treatment 16

Treatments	Color	Taste	Flavor	Saltiness	Texture	Appearance	Overall acceptability
Control	4.3 ± 0.99^{a}	4.27 ± 0.78^{a}	4.23 ± 0.68^{a}	1.9 ± 1.12^{g}	3.9 ± 0.71^{abc}	4.33 ± 0.80^{a}	4.67 ± 0.55^{a}
T_1	4.23 ± 0.86^{a}	4.07 ± 0.64^{ab}	4.03 ± 0.85^{ab}	2.4 ± 1.00^{fg}	3.3 ± 1.15^{cd}	4.14 ± 0.78^{a}	4.4 ± 0.72^{ab}
T_2	3.8 ± 1.1^{ab}	3.97 ± 1.00^{ab}	3.9 ± 0.96^{abc}	2.53 ± 0.68^{ed}	3.37 ± 1.07^{bcd}	4.16 ± 0.79^{a}	4.14 ± 0.94^{b}
T_3	3.83 ± 1.05^{ab}	3.93 ± 1.11^{ab}	3.97 ± 0.61^{abc}	2.47 ± 0.86^{f}	3.97 ± 1.06^{ab}	4.17 ± 0.91^{a}	4.2 ± 0.71^{b}
T_4	3.8 ± 0.76^{ab}	3.67 ± 0.8^{bc}	3.57 ± 0.86^{abcd}	2.97 ± 1.38^{ef}	3.37 ± 1.06^{bcd}	4.03 ± 0.80^{a}	4.26 ± 0.69^{ab}
T_5	3.37 ± 1.27^{bc}	3.6 ± 0.67^{bc}	3.5 ± 0.82^{cd}	2.43 ± 1.10^{f}	3.03 ± 0.93^{d}	3.37 ± 1.22^{b}	3.13 ± 0.77^{d-g}
T_6	2.97 ± 1.10^{cd}	3.03 ± 1.10^{de}	3.37 ± 0.93^{d}	2.47 ± 1.20^{ef}	2.7 ± 1.47^{de}	2.93 ± 1.11^{bcd}	3.4 ± 0.93^{cde}
T_7	3.06 ± 0.87^{cd}	3.33 ± 1.12^{cd}	3.4 ± 0.77^{d}	3.13 ± 1.00^{de}	2.87 ± 1.20^{de}	3.2 ± 0.89^{bc}	2.97 ± 0.89^{efg}
T_8	2.67 ± 0.80^{cd}	2.87 ± 0.86^{def}	3.1 ± 0.55^{de}	2.5 ± 0.73^{f}	2.83 ± 1.46^{de}	2.93 ± 1.11^{bcd}	2.7 ± 1.06^{gh}
T_9	2.9 ± 1.21^{de}	2.37 ± 1.03^{f}	2.3 ± 0.99^{fg}	4.03 ± 1.19^{ab}	2.27 ± 1.01^{ef}	2.44 ± 1.10^{fd}	2.33 ± 0.99^{h}
T_{10}	1.83 ± 1.02^{g}	1.8 ± 1.10^{g}	2.2 ± 0.96^{g}	4.2 ± 1.00^{a}	2.1 ± 1.54^{f}	1.93 ± 1.17^{e}	1.63 ± 0.81^{i}
T_{11}	2.5 ± 0.97^{def}	2.93 ± 1.11^{def}	3.1 ± 0.88^{de}	3.53 ± 0.90^{b-e}	2.77 ± 1.52^{de}	2.96 ± 1.03^{bcd}	3.7 ± 0.60^{c}
T_{12}	2.5 ± 1.17^{def}	2.97 ± 1.13^{de}	2.87 ± 0.90^{e}	3.13 ± 1.07^{de}	4.5 ± 0.63^{a}	3.34 ± 0.80^{b}	3.3 ± 0.65^{d-g}
T_{13}	2.23 ± 0.97^{efg}	2.9 ± 0.71^{cde}	2.7 ± 0.95^{ef}	3.27 ± 0.91^{cde}	2.93 ± 1.20^{d}	2.8 ± 0.85^{bcd}	3.56 ± 0.97^{cd}
T_{14}	2.57 ± 0.97^{def}	2.67 ± 1.27^{ef}	2.83 ± 0.94^{e}	3.73 ± 0.91^{abc}	3.93 ± 0.78^{abc}	3.9 ± 0.76^{a}	3.34 ± 0.84^{cde}
T_{15}	2.57 ± 1.12^{def}	2.6 ± 1.04^{def}	2.67 ± 1.12^{efg}	3.2 ± 1.03^{cde}	3.93 ± 0.83^{abc}	2.7 ± 1.09^{cd}	3.53 ± 0.90^{cd}
T_{16}	1.9 ± 1.21^{fg}	2.87 ± 1.17^{cde}	2.67 ± 0.88^{efg}	3.67 ± 1.15^{a-d}	2.73 ± 0.83^{de}	3.17 ± 0.91^{bc}	2.87 ± 0.78^{fg}

spores flourish and increase the load of the microbes where also late blowing of cheese was observed due to such factor [14]. In general the yeasts and mold count found in the final day cheese ranged in the acceptable limit for consumption.

Consumer acceptability of pre-ripened provolone cheese

Blending proportion of milk has significant ($p<0.0001$) effect on the color of prepared pre-ripened provolone cheese. The color score of the different cheese samples ranged from 1.3 ± 1.66 to 4.3 ± 0.99. Pre-ripened Provolone cheese prepared from cow milk has higher mean color value than the other cheese samples. The color of the cow milk cheese in the current study closely related to the results reported by Pinto et al [60]. They observed that cow milk cheese is more acceptable in color as compared to the different sources of milk cheese. Sample coded T_{10} containing (20% camel milk) has scored the least among other cheeses in color. This might be due to deficiency of shine, which fat provides when present in minute amount. Chawla et al observed that low amount of fat results deficiency of shine and lower the quality of color [61].

The mean value for taste of pre-ripened provolone cheese samples were in the range of 1.8 ± 1.10 to 4.27 ± 0.78. Statistical analysis indicated that different blends of milk have significant ($p<0.05$) affect the taste of provolone cheese samples. In this study control (cow milk) cheese was preferable than other treatments and this result in agreements with the findings of Adedokun et al, as they found variation in taste score of cow milk cheese by using different coagulants [62]. In the contrary Bille et al reported that the Gouda cheese prepared from cow milk was less preferable by taste [63]. This is due to the processing and method of cheese preparation. T_9 and T_{10} samples had lower scores for taste than other samples. This might be due to component especially, fat found in the cheese. The fats play a vital role in defining the representative flavor and taste of cheese [61]. The flavor value of the cheese samples had ranged from 2.20 ± 0.96 to 4.23 ± 0.68. T_1 and control samples had higher scores for flavor than other samples. This could be due to the more amount of lactose available which could contribute to its flavor. T_{10} (60% cow, 10% doe, 10% ewe and 20% camel) milk had lower scores for flavor. This might be due to the moisture content of the cheese.

The saltiness value of cow milk cheese was lower compared to the blended milk cheese samples. This might be due to the type of feed available for animals. Sample coded with T_{10} had the higher value of saltiness. This is due to the high amount of camel milk from other treatments. The taste of camel milk is usually different from other animal species because camels are fed shrubs and herbs in the arid regions due to this the milk has salty taste [64]. In the current study, the saltiness content of cheese samples was in agreement with the finding of Drake et al, who reported saltiness of 2.1 to 3.6 [65]. On the other hand, Kanwal et al scored low value of saltiness compared to this study. This might be due to the amount of salt added in the cheese during brining [15].

Texture of cheese prepared from T_{12} (60% cow, 10% doe and 30% ewe) milk was extremely liked by the panel of judges as compared to rest of the cheese samples. Cheese made from T_3 (70% cow, 10% doe and 20% ewe) milk rank the second highest score and there were no significant differences from T_{12}. This could be attributed to the higher fat content in the milk, which in turn resulted in the smoothness and best texture of the cheese. Pinto et al. reported that the level of fat content of cheese could enhance the smoothness and texture of cheese [60]. Similarly Bylund reported that texture, flavor, mouth feel and consistency are predominantly influenced by the fat content of cheese [57]. T_{10} has lower value by texture from the other treatments; this may be due to the high content of pH and low moisture content cheese give less texture. The high pH cheeses are softer than more acid cheeses [24]. In this study, the texture of cheese prepared from different blends of milk was in agreement with Teshome et al [54].

The average mean values of pre-ripened provolone cheese samples varied from 2.39 to 3.42. The control cheese sample had the highest appearance value from the other cheese samples. However,

cheese made from (60% cow, 10% doe, 10% ewe and 20% camel) milk was significantly ($p<0.0001$) lower appearance value than other cheese samples. In this study, appearance scores were not in agreement with the findings of Drake et al [66]. This difference might be due to source of milk and its composition.

Statistical analysis specified that the overall acceptability of various cheese samples prepared from different blends of milk showed significance ($p<0.0001$) effect by the blending proportion. The control cheese sample containing 100% cow milk had scored the highest overall acceptability (4.67) by panelists among the other cheese samples. This study was in agreement with Hanna who developed soft cheese from cow milk scored the highest in overall acceptability [16]. Pre-ripened provolone cheese prepared from (60% cow, 10% doe, 10% ewe and 20% camel) milk (T_{10}) sample had lower score for overall acceptability. Overall acceptability of provolone cheese appeared to be slightly higher than that reported by Pinto et al, who found a mean score of 2.23 to 2.99 [60]. On the other hand, overall acceptability scores were not in agreement with the findings of Drake et al, as they observed significantly lower scores [66]. Hence, concluded that source of milk and its composition have significantly influenced the overall acceptability of the current provolone cheese.

CONCLUSION

In this study, proximate analysis result pre-ripened provolone cheese, which was prepared from (60% cow, 10% doe and 30% ewe) milk (T_{12}) was best quality in terms of protein, total solid, fat content and moisture content. Cheese sample contained 20% camel milk (T_{10}) was best based on ash content. The finding of this study also showed that pure cow milk was best by lactose content from the other cheese samples. The mineral content and bioactive contents (vitamin C and total polyphenols) of pre-ripened provolone cheeses were significantly affected ($p<0.0001$) by blending proportions. In this finding the pre-ripened provolone cheese, prepared from (60% cow, 10% doe, 10% ewe and 20% camel) milk (T_{10}) had better mineral content. Beside this, it was best by vitamin C and total polyphenols content from the other cheese samples. In this finding, cheese samples which have high proportion of camel milk from the other treatments was best hygienic quality in terms of total coliforms. This finding showed that the color, taste flavor, appearance and overall acceptability of control (100% cow) milk cheese were better liked. Finally, it can be concluded that the development of pre-ripened provolone cheese making technology from blends of cow, doe, ewe and camel milk is functional not only for the investors but also for the development of the country.

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