

## Strength and Keeping Quality of Abomasum Rennet and its Influence on Yield and Quality of Halloumi Cheese made from Cow Milk

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**Abstract:** Halloumi cheese was made from cow milk using commercial rennet, and bovine and ovine rennet. But in Ethiopia commercial rennet is expensive and unavailable for smallholder dairy farmers. To solve this problem an experiment was conducted to evaluate strength, quality and suitability of locally available bovine and ovine abomasum rennet for halloumi cheese making in comparison with commercial rennet. The treatments consisted of bovine rennet, ovine rennet and commercial rennet (control) arranged in a completely randomized design (CRD). Rennet was extracted from bovine and ovine abomasa and comparisons were made with commercial rennet. Shelf life and clotting activity of the extracted rennet were determined using Halloumi cheese making. The result of the study indicated that commercial rennet ( $0.189 \pm 0.02$ ) had a higher clotting activity and short clotting time than bovine ( $0.179 \pm 0.02$ ) and ovine ( $0.023 \pm 0.02$ ) rennet. Bovine rennet was active up to nine weeks of storage while ovine rennet was active only up to six weeks of storage after which both types of rennet start losing their clotting activity. A highly significant difference was observed between locally and commercially produced rennet in terms of rennet and curd pH. The use of bovine and ovine rennet in the Halloumi cheese making was acceptable to the sensory panelist and scored more than the average for overall acceptances (Score >3.5). In conclusion, bovine rennet showed better strength and quality compared to ovine rennet and could be used for halloumi cheese making where commercial rennet is not available.

**Keywords:** Bovine rennet; Clotting activity; Curd strength; Ovine rennet; Shelf life

### 1. Introduction

Milk-clotting enzyme is the first active agent in cheese-making and an essential ingredient for the production of various cheese types. Clotting of milk is usually an enzyme-driven step during which rennet enzymes bring about cleavage of Met105–Phe106 of  $\kappa$ -casein. Today, nearly all types of cheese are manufactured by clotting the milk with a milk clotting enzyme and then processing the curd in various ways (Moschopoulou, 2011). Considering the nutritional, economic and value addition importance of cheeses as well as the unaffordable price of commercially available rennet to most resource poor producers, it is essential to search for locally available and affordable rennet. Moreover, a significant number of livestock are slaughtered for domestic meat consumption as well as export (CSA, 2013). This means that, a substantial volume of liquid rennet can be prepared from adult livestock. However, there is very limited information available on production and efficiency of rennet making from locally available sources. Therefore, the objectives of this research were to compare the strength and shelf life of bovine and ovine rennet with commercial ones and to evaluate their suitability of liquid rennet extracted from mature bovine and ovine abomasum for Halloumi cheese making.

### 2. Materials and Methods

#### 2.1. Description of the Study Area

The experiment was conducted in the Dairy Laboratory of Holetta Agricultural Research Center (HARC) of the Ethiopian Institute of Agricultural Research (EIAR). It is located 34 km to the West of Addis Ababa in the central highlands of Ethiopia (2400 m above sea level, 38.5°E longitude and 9.8°N latitude). The average maximum and minimum daily temperatures are 21°C and 6°C (<http://www.eiar.gov.et/index.php/holetta-agricultural-research-center>).

#### 2.2. Experimental Materials and Preparation of Liquid Rennet

Fresh abomasums of adult cattle and sheep were collected from one abattoir and one slaughter house in the vicinity of Holetta town. Commercial pepsin powder was purchased from Addis Ababa market. Rennet was prepared according to the method described by O'Connor (1993) as shown in Figure 1. After removing the internal contents, abomasal tissues were cleaned with tap water, and veins and fat contents were removed. Then they were inflated with air and dried in a wooden-wire mesh cabinet placed in an open air. After drying, each abomasum was cut into very thin strips separately, weighed and soaked in 1000 ml solution of 150 g NaCl, 40 g calcium chloride and 10 ml

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acetic acid solution individually. The solution was stored at 24-25 °C. The extract was filtered with muslin cloth. After filtration a yellowish clarified solution (approximately 950 ml) was obtained.

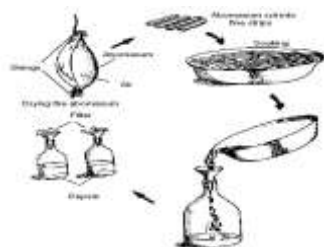


Figure 1. Schematic diagram of rennet making (O'Connor, 1993).

### 2.3. Treatments and Experimental Design

The treatments consisted of bovine rennet, ovine rennet, and commercial rennet. The experiment was designed as completely randomized design (CRD).

#### 2.4. Experimental Procedures

##### 2.4.1. Halloumi Cheese Making

Halloumi cheese was produced by slightly modifying the process indicated by O'Connor (1993) from cow milk. 120 L of cow milk was collected (at different time) from the dairy farm of Holetta Agricultural Research Center. Milk was pasteurized at 72°C for 20 seconds and cooled immediately with cold water to 32°C. Then, the crude abomasal rennet was added to the cheese milk. Curd was expected in 40-45 minutes. Then the curd was cut in to a 3 – 4 cm cube with a sharp cutting knife. Stirring of curd and whey removal was then done by heating at 38-42°C. The curd was left to settle to decant off the remaining whey. Once the curd was separated from the whey, the content was scooped into a mould lined with cheese cloth and pressed for about four hours. After the whey was removed, it was heat treated and the curd removed from the press cut into the desired size and placed in heated whey. After 20 minutes, the curd pieces were transferred into the draining table and allowed to cool. Finally, the cold curd was placed in clean, dried, and closed cup for quality and microbiology examination.

##### 2.4.2. Minimum Rennet Concentration for Curd Formation

Crude rennet extract had unknown pepsin content and it was, therefore, necessary to establish the strength of the rennet before applying it to milk coagulation. This is important to calculate the appropriate dosage required for optimal milk curdling and to produce a desired quality cheese. The correct amount of rennet to be added into the cheese milk was determined based on the standard developed by O'Connor (1993). Different rennet concentrations were set to identify the minimum curd forming concentration. Based on this procedure, the minimum curd forming bovine rennet

concentration was 2 ml/lit of milk and that of ovine rennet concentration was 30 ml/lit of milk. These rennet concentrations were selected and used for cheese making.

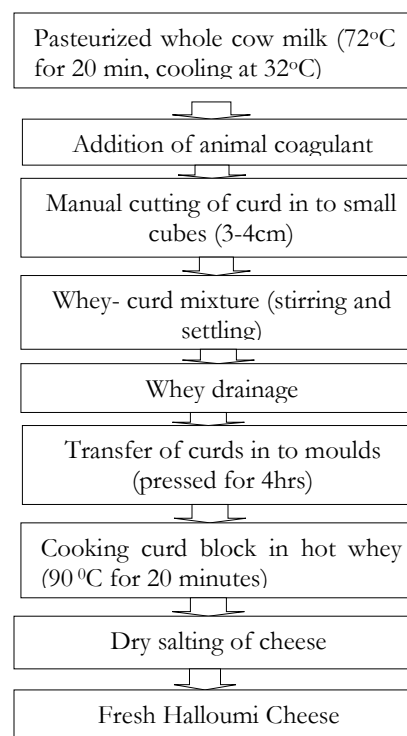


Figure 2. Halloumi cheese making flow chart Source: O'Connor, 1993.

#### 2.5. Observations

##### 2.5.1. Rennet Strength

Rennet strength is conventionally measured by determining the time required to coagulate milk under a standardized condition. The clotting activity (strength) of rennet types was measured and reported in clotting strength according to Berridge (1952). In addition, the time length (days) rennet maintains its strength was determined by testing the extracted rennet samples for strength every week. Rennet pH was measured in each analysis.

##### 2.5.2. Measurement of Rennet Clotting Time

The method of Berridge (1952) was used to measure clotting time as follows. The standard substrate was prepared from cow milk heated at 32 °C at 10% (w/v) solution of CaCl<sub>2</sub> (0.01M) solution. The prepared animal rennet of 1ml/10ml of standard substrate was added and mixed manually and incubated in a water bath at 32°C. After thoroughly mixing three times, the “zero” clotting time started. The milk clotting activity of each extract was measured, with the assumption that all the soluble proteins from the extract were enzymes which coagulate milk at 32°C. The clotting activity equation as reported by Berridge (1952) in rennet units (RU) was used.

$$RU = 10 \times V / T_c \times Q \quad (1)$$

Where: RU = rennet unit; V = volume of standard substrate (milliliter); Q = volume of animal rennet (milliliter); T<sub>c</sub> = time of clotting (second)

### 2.5.3. Clotting Strength

The clotting activity of rennet types is reported in clotting strength of Soxhlet (F) based on the equation of Bourdier and Luquet (1981).

$$F = RU / 0.0045 \quad (2)$$

In the experiment, the major chemical composition of Halloumi cheese was evaluated for determination of fat, protein, moisture, total solids, ash, pH and acidity.

**Fat Content:** the fat content of halloumi cheese was determined by Gerber method following the procedures outlined by O'Connor (1994). Three gram of cheese sample was weighed in a piece of greaseproof paper. Then 10 ml of sulfuric acid was dispensed in to the butyrometer followed by careful addition of water so that it rests on the acid. The cheese sample was then wrapped from cylinder that fits in to the butyrometer. Then additional 4-5 ml water was added. One ml amyl alcohol was then added into the cheese samples. Then the butyrometer was securely closed with a stopper and shaken to dissolve the cheese. The butyrometer was placed in the heated water bath and removed periodically for mixing until the cheese was fully dissolved. Finally the butyrometer was centrifuged and reading was recorded as for milk and cream.

**pH:** The pH of each cheese sample was measured following the procedures outlined by O'Connor (1994) using electronic digital pH meter. The electrode was immersed directly into the cheese sample (curd) until the pH sensitive bulb was covered. The temperature of the sample was measured and the pH meter was activated and pH reading was recorded.

**Acidity:** Acidity was measured following the procedures outlined by O'Connor (1994) by titration. A ten gram of cheese sample was prepared, into which 105 ml of water was added at 40° C and the content was shaken vigorously. 25 ml portion (2.5 g) of the filtrate was titrated with standard 0.1 N NaOH solution (until definite pink color persisted) using a phenolphthalein indicator. The result was expressed as percent lactic acid.

$$\text{Lactic acid (\%)} = \left[ \frac{\text{ml } \frac{N}{10} \text{ NaOH} \times 0.009}{\text{ml of sample used}} \right] \times 100 \quad (3)$$

**Total Solid:** Five grams of Halloumi cheese samples were weighed on a crucible in a duplicate and kept in a constant temperature oven set at 105°C for 12 hrs until a constant weight was achieved. The loss in weight was calculated as percent moisture and the total solids was

calculated subtracting percent moisture from 100 (Kirk and Sawyer, 1991).

$$\text{Total solid} = \frac{\text{crucible weight} + \text{wet dry sample weight} - \text{crucible weight}}{\text{sample weight}} \times 100 \quad (4)$$

**Ash:** A fresh Halloumi cheese sample was ignited at 600°C for 3 hours and weighed after cooling under room temperature. Loss in weight was calculated as percent organic matter and the ash content was determined by subtracting the organic matter percentage from 100 (Kirk and Sawyer, 1991).

### 2.5.4. Cheese Quality

Cheese quality was determined through a sensory evaluation based on appearance, odor, and texture and consistency of the cheese samples. A panel of fifteen experienced volunteering cheese tasters was selected. They included mainly researchers and graduate students in the Dairy Science Department of Holetta Agricultural Research Center. Clean water held at room temperature was served for cleaning mouth during testing the samples. The panel evaluated the appearance, odor, texture, and consistency of Halloumi cheese. Five scale hedonic scales was used to evaluate the halloumi cheese in which, 5 refers to excellent, 4 to good, 3 to fair, 2 to poor and 1 for unacceptable quality of Halloumi cheese (Lawless and Heymann, 1999).

### 2.5.5. Statistical Analysis

All treatments were replicated four times. A repeated measure analysis was applied to assess differences among the ovine rennet, bovine rennet, and the commercial enzymes by using SAS Software version 9.1 (SAS, 2005). Failure data analysis was run to fit the expiry date data to Weibull survival distribution to model the shelf-life data (rennet) as used by Schmidt and Bouma (1992).

Data for chemical composition of Halloumi cheese were subjected to analysis of variance (ANOVA) to test for significant differences at P < 0.05. For each treatment, mean comparisons were done using the least significant difference (LSD) test for variables of which F-values showed significant differences at 5 % significance level.

### Models

Observation Model:

$$Y_{ijkl} = \mu + R_i + S_j + \delta_{ij} + \epsilon_{ij} \quad (5)$$

Where: Y = processing parameters, quality parameters or shelf-life;  $\mu$  = Overall mean; R<sub>i</sub> = effect of coagulant rennet source (bovine, ovine, commercial rennet); S<sub>j</sub> = effect of storage periods (weeks);  $\delta_{ij}$  = Interaction of rennet source and storage period;  $\epsilon_{ij}$  = random error

**Weibull Model:**

The model used for failure analysis was Weibull distribution with the general function;

$$f(x; k, \lambda) = \frac{k}{\lambda} \left(\frac{x}{\lambda}\right)^{k-1} e^{-(x/\lambda)^k} \quad (6)$$

Where:  $x > 0$ . ( $x$  = failure day),  $k > 0$  is the *shape parameter* and  $\lambda > 0$  is the scale parameter of the distribution.  $K < 1$  indicates that the failure rate decreases over time. If the failure rate is constant over time, then  $k = 1$ .  $K > 1$  indicates the failure rate increases over time

**3. Results and Discussion****3.1. Clotting Activity**

Clotting time, activity and strength of Soxhelt (F) for the ovine, bovine and commercial rennet is presented in Table 1. At individual observation level, the clotting activity of the milk coagulants used ranged from the lowest 0.0074 RU for ovine rennet produced locally to the highest 0.196 RU for commercial rennet with the overall average value being 0.13 RU. On average, the highest clotting activity (0.189 RU) was observed for the commercial rennet, while the lowest value (0.023 RU) was recorded for ovine rennet. No apparent differences ( $P > 0.05$ ) were observed between commercial and bovine rennet in their clotting activity, while ovine rennet showed significantly lower ( $P < 0.05$ ) clotting activity compared with the other two types of rennet. It was observed that the average

clotting activity increased with increasing Soxhelt strength and decreasing clotting time. Accordingly, the highest clotting activity recorded for the commercial rennet corresponded with the highest Soxhelt strength and shortest clotting time.

Variation in milk clotting activity (MCA) and strength might be related to differences in the nature of the clotting ability of raw materials, the proteolytic efficiency and/or the concentration of the rennet types on the milk substrate used. For instance, as indicated by Guinee and Wilkinson (1992), the MCA of rennet relies on its ability to degrade casein micelles, the action being dependent on the chymosin and pepsin content of the rennet complex. As a critical parameter in the progress of curd formation, clotting time can be usefully related to curd firmness and curd yield. The weak clotting ability of ovine rennet observed in the present study might be attributed to the weak property of pepsin present in the rennet solution. This observation is in agreement with that of Vairo Cavalli *et al.* (2005), who indicated that the breakdown of cow milk by ovine caseinate took place much more slowly than that of bovine counterpart. The weak clotting power of ovine pepsin on cow milk might be related to species specificity. Consistent with this suggestion, Calvo and Fontecha (2004) also indicated that the clotting ability of each rennet proteolyses is stronger for their species specific casein compared with that of other species.

Table 1. Mean ( $\pm$  SD) clotting time, change in clotting activity (rennet unit: RU) and strength of Soxhelt (F) for bovine, ovine and commercial rennet.

Milk Coagulants	No. of observations	Clotting time (min)	Clotting activity (RU)	Strength of Soxhelt (F)
Bovine rennet	4	9.50 <sup>a</sup>	0.179 <sup>a</sup> $\pm$ 0.02	39.9 <sup>a</sup> $\pm$ 2.42
Ovine rennet	4	22 <sup>b</sup>	0.023 <sup>b</sup> $\pm$ 0.02	16.9 <sup>b</sup> $\pm$ 2.42
Commercial rennet (C)	4	8.80 <sup>a</sup>	0.189 <sup>a</sup> $\pm$ 0.02	42.2 <sup>a</sup> $\pm$ 2.42
Overall Mean	12	13.4	0.130 $\pm$ 0.14	33.3 $\pm$ 1.57

Note: <sup>abc</sup> Means in the same column without common letter are significantly different at  $P < 0.05$

**3.2. Stability of Rennet from Bovine, Ovine and Commercial Source**

The cumulative failure days (risk condition) of different rennet sources is shown in Figure 3. The stability of all rennet sources was not affected during the 6 weeks of storage i.e. the failure probability (hazard) of losing stability was zero. Due to the short period of the experiment, the highest shelf life (stability) was observed for commercial rennet which shows no failure date during the ten weeks of storage while ovine rennet started to lose its stability after six weeks of storage. On the other hand, bovine rennet was active up to the 9<sup>th</sup> weeks of storage. The changes that take place during storage may depend on manufacturing process, temperature of storage and extent of exposure of the rennet to light. Arvanitoyannis (2009) indicated that rennet can resist even very high temperatures when it is dry. However, liquid rennet solutions are less stable and the substance decomposes in various speeds (Pometto

*et al.*, 2006). In the current study, rennet was stored in a dark closed container that may help the enzyme to retain its stability. However, temperature of storage (room temperature) may affect their stability by creating variations in heat. The efficiency of liquid rennet reduces at room temperature, the intensity of which is related to the strength of the solution, and the rennet strength decreases when the substance is handled and stored improperly, with light, heat and shaking exerting clear detrimental effects on the rennet (Arvanitoyannis, 2009).

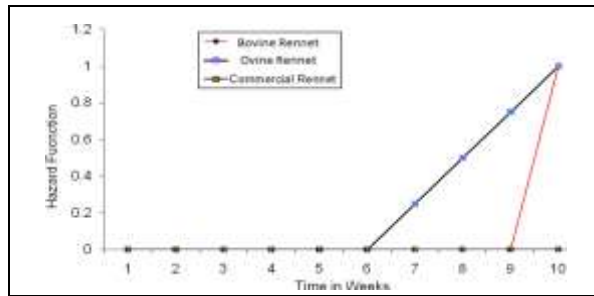


Figure 3. Cumulative failure plots of rennet extracted from bovine and ovine abomasums and commercial rennet.

The pH value of stored rennet ranged from 3.45 to 4.21. The repeated measure analysis revealed that a significant difference ( $P < 0.05$ ) was observed between pH values of locally produced and commercial rennet. Commercial rennet showed no variation in terms of rennet pH. pH plays a significant role in the stability of coagulant enzyme. During the storage period, the pH value of locally produced liquid rennet decreased (Figure 4). The decline in pH values of bovine and ovine rennet might be because of lack of purification methods and storage temperature used in the processing. As reported by De Caro *et al.* (1995) high recorded loss of rennet may be related to conventional ways of manufacture and lack of purification processes. In the current study, bovine and ovine rennet were crude extracts and stored at room temperature. Hooydonk and Van Den Berg (1988) further proposed that the strength of natural rennet could not be maintained at a constant level. When rennet is kept at high temperatures, pepsin activity increases in whey proteins (Hooydonk and Van Den Berg, 1988). In addition, lactic acid bacteria may contribute to the development of acidity there by reducing stability and pH.

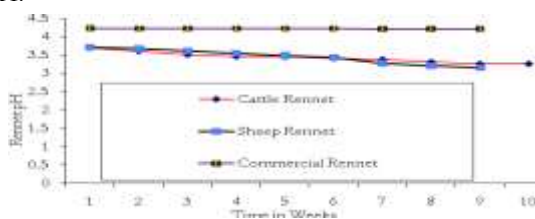


Figure 4. pH of stored rennet from bovine, ovine and commercial rennet sources.

Curd pH value of stored rennet ranged from 5.34 to 6.12. Mean curd pH value of repeated measure analysis revealed significant differences ( $P < 0.05$ ) among bovine, ovine, and commercial rennet. During the storage period, curd was formed even though the pH declined (Figure 5). The result showed a consistent decline in curd pH with storage period indicating its diminishing shelf life. Milci *et al.* (2005) revealed that average pH values of Halloumi cheeses manufactured

from bovine milk ranged from 5.37 - 6.45 which is in agreement with the current findings.

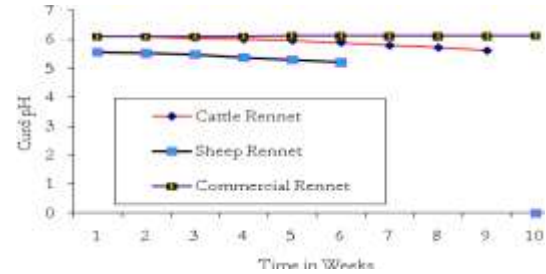


Figure 5. pH of curd obtained from bovine, ovine and commercial rennet sources.

### 3.3. Effect of Locally Produced Rennet on Halloumi Cheese Yield and Quality

#### 3.3.1. Chemical Composition of Milk

The fat, protein, and pH value of the milk used in the manufacture of Halloumi cheese were 4.28, 3.25 and 6.22, respectively. Fat and protein are the two primary milk components that are recovered in the cheese making process and are directly related to cheese yield (Fox *et al.*, 2000). These constituents influence the casein to fat ratio, total solids, lactose, mineral content, consequently moisture levels and extent of acid development in the finished cheese (Traordinary Dairy, 2001). The casein fraction of milk protein is the dominant factor affecting curd firmness, syneresis rate, moisture retention, and ultimately affecting cheese quality and yield (Lawrence, 1993).

#### 3.3.2. Chemical Composition of Halloumi Cheese

Chemical composition of Halloumi cheese manufactured using bovine, ovine and commercial rennet is presented in Table 2. The composition of cheese has a marked influence on all aspects of quality, including sensory properties, texture, and cooking properties (Guinee and Fox, 2004; Amenu and Deeth, 2007; Tunick *et al.*, 2007). The yield of Halloumi cheese sample ranged from 124 to 134 g per a liter of milk. The average yield was 129 g. All treatments had a significant ( $P < 0.05$ ) effect on the yield of Halloumi cheese. Halloumi cheese manufactured using fresh bovine rennet had higher yield compared to Halloumi cheese manufactured using ovine and commercial rennet (Table 2). Differences in the cheese yield among the different types of coagulants might be due to their proteolytic specificity, as highly specific coagulants provide higher cheese yields. This is substantiated in numerous cheese yield trials and quality studies comparing various rennet and coagulant types (Emmons *et al.*, 1990; Emmons and Binns, 1991; Banks, 1992; Guinee and Wilkinson, 1992; Quade and Rudiger, 1998) and reviewed by Emmons and Binns (1991) and Garg and Johri (1994). Bovine and commercial rennet had higher clotting and short coagulation time. Milk with favorable coagulation properties (short coagulation

and curd firming times and firm curd) is expected to give more cheese yield (Kubarsepp *et al.*, 2005). The weak clotting power and non-specificity of ovine rennet to bovine milk results in lower cheese yield.

The moisture content of Halloumi cheese samples ranged from 46.8 to 51.6%. The average moisture content was 49.2% (Table 2). There were no significant ( $P > 0.05$ ) differences between cheeses manufactured using bovine and commercial rennet with respect to moisture attributes. These values are in agreement with the results of Pezeshki *et al.* (2011) where cheeses manufactured with *Withania coagulans* had no significant difference in moisture content compared to the moisture contents of cheeses produced using different rennet preparations. Raphaelides *et al.* (2006) reported moisture contents of 47.4% for Halloumi cheese

manufactured from bovine milk. In producing cheese using ovine rennet, a large amount of rennet (30ml/1Lt) was added to the cheese milk which significantly reduced the moisture content of the cheese. This could be ascribed to the large amount of enzyme extracting salts that increases syneresis due to acidity that facilitates squeezing out of the moisture from the cheese. This observation is in agreement with the findings of Guven *et al.* (2008) who reported that moisture content of cheese decrease as rennet concentration increases. The value of the moisture content of Halloumi cheese observed in this study suggests that it is an intermediate moisture cheese and is much lower than the corresponding value (79%) reported for the traditional Ethiopian cottage cheese (Mogessie, 1992).

Table 2. Proximate composition of Halloumi cheese made using different rennet sources.

Constituents	Treatment		
	Bovine rennet	Ovine rennet	Commercial rennet
Yield	134 ± 5.68 <sup>a</sup>	125 ± 5.68 <sup>b</sup>	127 ± 5.68 <sup>ab</sup>
Fat	23.8 ± 4.24 <sup>a</sup>	21.5 ± 4.24 <sup>a</sup>	22.5 ± 4.24 <sup>a</sup>
Protein	18.9 ± 1.12 <sup>a</sup>	19.7 ± 1.12 <sup>a</sup>	18.8 ± 1.12 <sup>a</sup>
Moisture	51.6 ± 1.71 <sup>a</sup>	47.6 ± 1.71 <sup>b</sup>	49.8 ± 1.71 <sup>a</sup>
Total solid	53.2 ± 1.71 <sup>a</sup>	53.4 ± 1.71 <sup>a</sup>	48.4 ± 1.71 <sup>b</sup>
pH	6.12 ± 0.07 <sup>a</sup>	5.72 ± 0.07 <sup>b</sup>	6.16 ± 0.07 <sup>a</sup>
Acidity	0.027 ± 0.01 <sup>b</sup>	0.049 ± 0.01 <sup>a</sup>	0.026 ± 0.01 <sup>b</sup>
Ash	5.13 ± 1.49 <sup>a</sup>	5.05 ± 1.49 <sup>a</sup>	5.01 ± 1.49 <sup>a</sup>

Note: <sup>abc</sup> Means in the same row without common letter are significantly different at  $P < 0.05$

The pH value of Halloumi cheese ranged from 5.60 to 6.22 with the average pH values of 6.0. No significant ( $P > 0.05$ ) difference was observed between Halloumi cheese manufactured using commercial and bovine pepsins in pH while Halloumi cheese manufactured using ovine rennet showed significantly lower pH. The acidic features of the rennet and the minimum requirements of ovine rennet to form curd (30 ml/l) could be a factor for this significant difference. Hynes *et al.* (2001) indicated that reduced pH causes the protein matrix in the curd to contract and squeeze out moisture and finally affect cheese yield. According to the findings of Milci *et al.* (2005) and Raphaelides *et al.* (2006), the average pH values of Halloumi cheeses manufactured from bovine milk ranged from 5.37- 6.45. The pH value of the cheese determines microbial stability, and susceptibility to mould and spoilage microorganisms.

The average titrable acidity of Halloumi cheese ranged from 0.0495 to 0.0261 and was significantly ( $P < 0.05$ ) affected by the treatments. The LSD test showed that the titrable acidity of Halloumi cheese made using bovine and ovine rennet was differed significantly which could be attributed to the pH and the concentration of rennet. In the current study, bovine and ovine rennet differed in their minimum curd forming concentration. Thus, the high concentration of ovine rennet significantly lowers the pH of the cheese.

The fat content of Halloumi cheese (Table 2) ranged from 21.3% to 23.8%. Proximate analysis revealed that the fat content of Halloumi cheese was not affected ( $P > 0.05$ ) by different rennet types. The fat content of Halloumi cheese obtained in the present study was lower than the values reported in earlier studies 44.5% (Fasakin and Unokiwedi, 1992) and 25.4% (Milci *et al.*, 2005).

The Protein content of Halloumi cheese was not also affected ( $P > 0.05$ ) by the rennet type used (Table 2). The protein content of Halloumi cheese obtained in the present study ranged from 18.8 to 19.7%, which was slightly lower than the value reported by Milci *et al.* (2005), 22.8%. Similarly, the Halloumi cheese samples analyzed have higher crude protein content than the Ethiopian traditional cottage cheese *Ayib* which was reported to be 15 g/100 g protein (O'Mahony, 1988; Mogessie, 1992; Binyam, 2008).

Gross compositions of total solids and ash value in the present study were not affected ( $P > 0.05$ ) by different rennet types (Table 2). The total solid value of Halloumi cheese ranged from 48.4 to 53.4. An ash content of the Halloumi cheese ranged from 5.01 % to 5.1%. This is slightly lower than the value reported by Milci *et al.* (2005) who reported that the average ash content of the Halloumi cheese produced by using bovine, ovine milk was 6.52 %. The ash content of the

Halloumi samples analyzed in the present study was also higher than the ash content (1.16%) of Ethiopian cottage cheese, *Ayib*, reported by Binyam, (2008). The high ash content of the Halloumi cheese could serve as a good source of minerals.

### 3.4. Sensory Quality of Halloumi Cheese Made using Different Rennet Sources

Mean scores of sensory attributes of Halloumi cheese made using different rennet sources are illustrated in Table 3. All the sensory scores of Halloumi cheese made using bovine, ovine, and commercial rennet were found to be within the acceptable sensory score (score > 3.5) implying that treatments had no significant effect on the sensory quality of cheese. The average value of

odor and consistency was 3.96 and 4.05, respectively. This is in agreement with the findings of Gaborit *et al.* (2001) and Martinez-Cuesta *et al.* (2001) who reported that cheeses elaborated with animal rennet and powdered vegetable coagulants had no significant ( $P > 0.05$ ) differences in any of the odor and consistency characteristics studied. No significant ( $P > 0.05$ ) difference was observed between Halloumi cheese manufactured using commercial and bovine pepsins in their taste score, while Halloumi cheese manufactured using ovine pepsin showed a significantly ( $P < 0.05$ ) lower taste score compared to the other two. This could be related to the high amount of ovine rennet (30 ml/lt) added to the cheese milk which gives the cheese a characteristic salty taste.

Table 3. Mean Sensory scores of Halloumi cheeses treated with different rennet sources.

Treatments	Appearance	Odor	Taste	Consistency
Bovine rennet	4.1 ± 0.56 <sup>a</sup>	4.0 ± 0.31 <sup>a</sup>	4.2 ± 0.37 <sup>a</sup>	4.15 ± 0.53 <sup>a</sup>
Ovine rennet	4.35 ± 0.56 <sup>a</sup>	4.1 ± 0.31 <sup>a</sup>	3.65 ± 0.37 <sup>b</sup>	3.95 ± 0.53 <sup>a</sup>
Commercial rennet	4.2 ± 0.56 <sup>a</sup>	3.8 ± 0.31 <sup>a</sup>	3.95 ± 0.37 <sup>a</sup>	4.05 ± 0.53 <sup>a</sup>

Note: <sup>abc</sup> Means in the same column without common letter are significantly different at  $P < 0.05$

## 4. Conclusions

The results of this study have demonstrated that a better cheese yield and lower minimum coagulant cost were recorded for cheese made using bovine rennet than ovine rennet. However, considering rennet quality and strength, commercial rennet was stronger in making cheese with shorter rennet coagulation time. All the sensory scores of Halloumi cheese made using bovine, ovine and commercial rennet were found to be within the acceptable sensory score (score > 3.5). In general, in areas where commercial rennet is unavailable and expensive, bovine rennet could be the best alternative for Halloumi cheese making with regard to cost effectiveness, better clotting strength, shelf life, availability and better cheese yield. Production of bovine rennet in the country could also create employment opportunities.

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