



Original Research

Physicochemical Analysis and Microbial Quality of Cow Butter Obtained from Menz District of Amhara Region, Ethiopia

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Abstract

Menz has long been known for its quality butter production but traditional milk products are reported to be of substandard quality. Therefore, this investigation was conducted to access physicochemical and microbial quality of butter from Menz district along the market value chain. The microbiological count data were transformed to log₁₀ values before statistical analysis. Overall values of 15.05:82.62:2.09%, 14.26:83.44:2.77%, 14.25:83.30:1.03%, 14.58:83.82: 3.58% and 12.52: 83.96: 2.82% moisture, fat and free fatty acid contents were observed in samples from farmers, traders, made by investigators, Tarmaber and Addis Ababa, respectively. Overall mean score of total aerobic mesophilic bacterial count ranging from 3.26×10^9 (sample made by invigilator) to 4.20×10^9 (sample from Addis Ababa) were recorded. Similarly, the total coliform count ranges from 1.61×10^6 to 3.03×10^6 and the yeast and mold counts vary from 1.31×10^6 to 1.83×10^6 from samples collected at different butter market value chains. There is unhygienic production and processing of butter in the study area which calls for improvement.

Key words: Bacterial Count, Fat Content, Moisture Content

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Introduction

Ethiopia has one of the largest livestock inventories in Africa with 52.13 million heads of cattle, 2.5 million camels, 22.6 million goats, 24.2 million sheep and 7.73 million of equines CSA (2012). Given the considerable potential for smallholder income and employment generation from high-value dairy products, development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country (Tsehay, 2001). Fresh milk is easily perishable if it is not consumed immediately so when milk is surplus, it should be processed into different products like butter, soured milk and cheese. Butter



has long shelf life as compared to fresh milk, especially when heated to higher temperature (100-120°C) for 30 minutes it can stay for several months without spoilage (Lejko *et al.*, 2009). Butter is one of the primarily fat sources and an important source of dietary energy.

The quality of butter is closely related to its physico-chemical and microbiological characteristics. Besides fats, butter contains small percentages of proteins, milk sugar and water which make it a suitable substrate for microorganisms (Mahendra *et al.*, 2016 ; Singh *et al.*, 2011). Although butter spoilage is most often due to the development of chemical rancidity, microbiological problems do also occur in the form of cheesy, rotten or fruity odors and the rancid flavor produced by hydrolysis (Rady and Badr, 2003). The primary spoilage factors in butter are moulds and the majority of the moulds growing in butter are composed of the species of *Thamnidium*, *Cladosporium* and *Aspergillus* (Bereda *et al.*, 2014). Moreover, some pathogenic microorganisms like *Listeria monocytogenes*, verocytotoxin-producing *Escherichia coli* and *Staphylococcus aureus* which are known to cause food borne illness in human beings were also detected in butter (Pal, 2014).

Livestock and livestock products like milk, butter and meat are the main income generators for the farmer living specially in the highland areas of the North Shoa. Among those areas Menz districts one of the districts known for dairy production and the livelihood of the farmers also depend on dairy products (milk and butter). Menz districts are well known by their butter production in addition to milk and this product is also more preferred by consumers than cow milk butter collected from other areas. Even if the areas have good potential in butter and milk production, there is no enough scientific information on the quality of milk and butter made from cow milk. Therefore, the present study was done to assess physicochemical property and microbiological quality of cow butter collected from different areas of the Menz districts.

Materials and Methods

Study Area

The study was conducted in three purposively selected districts of North Shoa Zone (Menz Gera, Menz Mama and Menz Keya) based on their butter production potential from June-September 2017. North Shoa Zone is one of the ten administrative zones of Amhara National Regional State. This zone covers 17.7 thousand km² land areas. Of the total land area 38.2% is arable, 42.1% grazing and browsing, 7.5% natural vegetation, 2.1% unproductive and 11.0% unutilized. Traditionally the zone is divided into highland (37.4%), midland (30.1%) and lowland (32.5%) agro-climatic zones (ANRS –BOFED, 2001).

Menz Gera (also called *Mehal Meda*) with administrative center of Mehal Meda and a total population of 120,469, of whom 58,827 are men and 61,642 women; 11,055 or 9.18% are urban inhabitants, Menz Mam (also known as *Molale*) with administrative center of Molale and a total population of 85,129, of whom 42,102 are men and 43,027 women; 6,513 or 7.65% are urban inhabitants and Menz Keya (also called

Zemero) with administrative center of Zemero and a total population of 46,219, of whom 22,965 are men and 23,254 women; 2,623 or 5.68% are urban inhabitants.



Fig . Study area (left: map of Amhara Region and right: North Shoa with the study areas signaled by+ symbol)

Data Collection

Laboratory Analysis

Traditional butter was manipulated in the laboratory as described by Idoui *et al.* (2010). Butter samples were collected from each value chain actors (starting from the producer to the traders at different locations of Menz districts to Addis Ababa) and placed in sterile bottles and delivered to Holeta Dairy Laboratory under ice box. In each of the three districts a total of five samples were collected (three samples from the farmers, one sample from trader and fresh milk sample). In addition, two butter samples were collected from the butter market value chains one from Tarmaber and other from Addis Ababa for each of the districts. Totally 21 samples were collected from the three districts and their respective value chains. The fresh milk samples were processed to butter by investigators following the traditional way of butter making process. After the milk is left to turn in to yoghurt it was churned by hand churner till whipped cream became coarser and semi-solid butter granules were formed that rapidly increased in size and separated sharply from the liquid buttermilk. Butter was washed with cold water several times and the excess water was removed. Butter was filled in sterile disposable polyethylene bags and stored at 4°C till analysis.

Physiochemical Analysis

Moisture Content

First 5g of butter sample was placed in crucible and immersed into hot water at 39°C and shaken well until creamy consistency is obtained. Then it was dried in atmospheric oven (EDSC, 96H203: England) at

100±5°C for 24h until constant weight was obtained. After drying weigh the butter sample and the crucible. The IUPAC (1979) method was used to determine the moisture content of butter.

$$\text{Moisture content of butter} = (W1-W2/W1) \times 100$$

Where, W2 = weight of butter sample after drying (final weight); W1 = weight of butter sample before drying (original weight)

Fat Content

The fat content of butter samples was determined according to the Soxhlet (1879) method using petroleum ether and two grams of butter sample was extracted using Soxhlet Extractor (Bristol, BS19 1BW: Great Britain) for about 8 hours in triplicate. After the recovery of the fat, the flasks were transferred to an oven to complete evaporation of the solvent at 100°C until constant mass was obtained. Finally, the oil content was calculated as-

$$\text{Fat Content} = \frac{\text{Weight of flask containing oil} - \text{weight of empty flask}}{\text{Sample weight} \times \text{DM percentage}} \times 100$$

Analysis of Fatty Acids

The butter samples were esterified in a methanol solution of 2N KOH for 30 minutes at 50°C. The gas chromatographic analysis of fatty acid methyl esters was performed on a Perkin Elmer gas chromatograph, equipped with a flame ionization detector (Shimadzu QP2010): The column was a fused silica capillary SE 30 length 25 meters, diameter 0.25 µm. Helium is the carrier gas. The column temperature program was initially isotherm at 140°C for 10 min, an initial programmed rate of 1°C/min up to 160°C, then a second rate of 2°C/min up to 220°C and a final isotherm for 15 min. Samples were injected into the split mode. The apparatus itself carried out recording and integration.

Microbiological Analysis

For the microbiological analysis of butter samples total bacteria count (TBC), coliform count (CC) and yeast and mould counts (YMC) were considered. Butter samples used for microbial analysis were collected aseptically in sterile bottles after collecting. For these counts peptone, water was sterilized by autoclaving at 121°C for 15 minutes. Similarly, plate count agar (Oxoid, UK) used for determination of total viable organisms, potato dextrose agar (Oxoid, UK) used for determination of yeast and mould count, while violet red bile agar (Oxoid, V 37720: UK) used for determination of coliform count was sterilized by boiling (Richardson, 1985). For all tests, the media used was prepared according to the guidelines given by the manufacturers. Each analysis was made in duplicate. For total bacteria count(TBC) dilutions was selected

so that the total number of colonies grown on a plate is between 10 and 300, while for CC dilutions that resulted counts between 15 and 150 per plate was selected.

Data Analysis

The study involved laboratory based investigation. Data on the microbial and physiochemical quality of butter were analyzed using general Linear Model (GLM) in SPSS version 20. Mean cut-off was carried out using the least significant differences between means. The microbiological count data were transformed to log₁₀ values. Mean values and frequencies were used to compare data.

Result and Discussion

Physicochemical Property

As shown in the Table 1 the mean value of moisture content in the three districts was 14.17, 15.25 and 15.05 in *Mehal Meda*, *Molale* and *Zemero* respectively with an overall mean value of 15.05. Highest values of moisture content were observed in *Zemero* (16.90% at kebele 01) and *Molale* (16.75% at kebele 06). All of these results are below the maximum legal compositional standards for butter moisture (16%).

Table 1: Moisture and fat content of samples from farmers

| Districts | % Moisture Content | % Fat |
|---------------------|--------------------|--------------|
| Mehal Meda | | |
| <i>Kebele 02</i> | 14.22 | 83.889 |
| <i>Kebele 03</i> | 14.83 | 82.431 |
| <i>Kebele 07</i> | 13.451 | 84.47 |
| Mean | 14.17 | 83.59 |
| Molale | | |
| <i>Kebele 01</i> | 12.561 | 85.94 |
| <i>Kebele 02</i> | 16.45 | 80.937 |
| <i>Kebele 06</i> | 16.75 | 80.688 |
| Mean | 15.25 | 82.52 |
| Zemero | | |
| <i>Kebele 01</i> | 16.9 | 79.33 |
| <i>Kebele 02</i> | 14.604 | 83.769 |
| <i>Kebele 08</i> | 15.674 | 82.127 |
| Mean | 15.72 | 81.74 |
| Overall mean | 15.05 | 82.62 |

Table 2 shows almost equal mean value of moisture content for samples from traders (14.36%) and butter made by investigators (14.25%). This is lower than the moisture content registered in samples from farmers. The moisture content of traditional Ethiopian butter ranges from 20 to 40% compared to international standard butter of 16 % (Tolosa Muleta, 2016).

Table 2: Moisture and fat content of samples from traders and other markets

| Type of Butter | Districts | % Moisture Content | % Fat |
|----------------|-------------|--------------------|--------------|
| Trader | Mehal meda | 15.42 | 83.44 |
| | Molale | 12.706 | 84.92 |
| | Zemero | 14.672 | 81.955 |
| Mean | | 14.26 | 83.44 |
| Churned | Mehal meda | 14.387 | 83.749 |
| | Molale | 16 | 81.127 |
| | Zemero | 12.38 | 85.05 |
| Mean | | 14.25 | 83.3 |
| | Tarmaber | 14.58 | 83.82 |
| | Addis Ababa | 12.52 | 83.96 |

The high level of moisture in traditional butter may have an influence on its microbiological and physicochemical quality since the presence of water in butter can activate lipases, stimulate the growth of microorganisms and cause the hydrolysis of triglycerides, spoilage when stored at room temperature (Ronholt *et al.*, 2013). Ashenafi (2006) has reported that traditional butter has 17.2% moisture, 1.3% protein, 81.2% fat, 0.1% carbohydrate, 0.2% ash, 0.024% calcium and 0.0015% iron. The average moisture content of butter collected from open markets of Delbo and Kucha, Ethiopia was 18.86%± 1.02 % /gram of butter samples Mekdes (2008). Traditionally and legally, however, butter must contain >81% of only milk fat (Gebremedhin *et al.*, 2014). The mean fat content of butter collected from Mehal Meda, Molale and Zemero farmers were 83.5%, 82.52% and 81.74% respectively with an overall mean of 82.68%. Highest values of 85.94% (at Molale, Kebele 01) and 84.47 % (at Mehal meda, kebele 07) fat% were observed. The value of fat percentage in samples from traders (83.44%) and sample from butter made by investigators (83.30%) were higher than the overall mean seen in samples from farmers. As shown in Fig.1 the moisture content of all samples was below the maximum standard (16%) expected in butter.

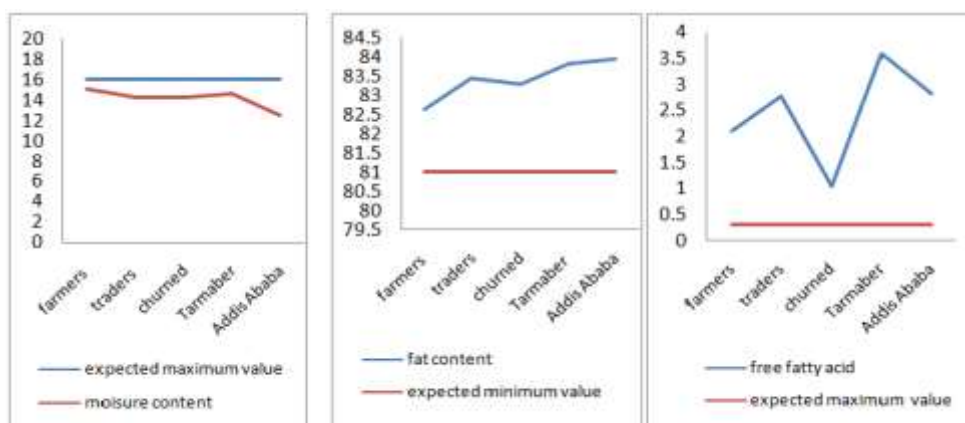


Fig 1 . Comparison of Moisture, fat and free fatty acid contents of butter with standard values

Fig.1: Comparison of moisture, fat and free fatty acid contents of butter with standard values. Compares the physiochemical property of butter from Menz District collected at each market value chain. The straight line shows the maximum or minimum acceptable value for each property (That is 16% maximum value of moisture, 81% minimum value of fat and less than 0.5% of free fatty acid content).

Lower moisture content were seen at Tarmaber and Addis Ababa which may be due to long time of storage and evaporation moisture of butter at the final markets away from the production area. The fat content of the samples is also above the minimum required value (81%). But the free fatty acid content indicates unhygienic processing and handling of butter in all samples with the lowest value attained from butter sample made by investigators.

Free Fatty Acid Composition

Hydrolysis of butter fat causes liberation of free fatty acids in the presence of a lipase enzyme. Spoilage bacteria provide a heat stable lipase when they exceed normal levels. Fat that has been lipolysed tastes rancid and smells rancid. The reduction in quality was caused by rancidity and bitterness that are related to high levels of free fatty acids and break down of proteins (Dieffenbacher *et al.*, 2000). The standard specifies butter to have 0.4% maximum free fatty acids, 80% fat and maximum of 16% water FAO and WHO (2011). The mean value of free fatty acid in Mehal Meda, Molale and Zemero are 2.06, 1.55 and 2.67 respectively with an overall mean of 2.09%. Mean value of free fatty acid in butter from traders and butter made by investigators were 2.77% and 1.03% percent respectively (Table 3). Highest values were seen in samples from Tarmaber (3.58%) and Addis Ababa (2.82%). The content of free fatty acids of butter sold in rural markets varied from 0.23 to 1.20%. Content of free fatty acids was high as 23% in the Addis Ababa market and between 0.07 and 3.32% in Debre zehit O'Mahony and Ephraim (1985).

Table 3: Free fatty acid composition of samples from farmers

| % Free Fatty Acid | Districts | | | | | | | | |
|-------------------|-------------|-----------|-----------|-------------|-----------|-----------|-------------|-----------|-----------|
| | Mehal meda | | | Molale | | | Zemero | | |
| | Kebele 02 | Kebele 03 | Kebele 07 | Kebele 01 | Kebele 02 | Kebele 06 | Kebele 01 | Kebele 02 | Kebele 08 |
| | 2.87 | 1.49 | 1.81 | 2 | 1.1 | 1.56 | 3.01 | 3.77 | 1.24 |
| Mean | 2.06 | | | 1.55 | | | 2.67 | | |

A study conducted in Algeria by Idoui *et al.* (2010) revealed that traditional cows' butter with palmitic acid (24.33 % - 36.95%), myristic acid (18.49% - 27.35 %) and stearic acid (7.68%-14.05%). In other study palmitic acid was reported to be the major saturated fatty acid (22.81%) followed by stearic acid (10.21 %) (Rady and Badr, 2003).

Table 4: Free fatty acid composition of Samples from traders and other markets.

| | Districts | Free Fatty Acid |
|----------------|-------------|-----------------|
| Trader | Mehal meda | 1.89 |
| | Molale | 3.02 |
| | Zemero | 3.4 |
| Mean | | 2.77 |
| Churned | Mehal meda | 1.2 |
| | Molale | 0.92 |
| | Zemero | 0.96 |
| Mean | | 1.03 |
| | Tarmaber | 3.58 |
| | Addis Ababa | 2.82 |

Microbial Properties

Microbial criteria requires that specific microorganisms or toxins must not be present at all or be present at less than a specified number or amount in a given quantity of a food ingredient (Michael and Joseph, 2004). Average value of Total Aerobic Mesophilic Bacterial Count (TAMBC) from farmers sample was 3.94×10^9 with the maximum value of 4.11×10^9 at Zemero district Kebele 01. A mean value of 3.89×10^9 , 3.99×10^9 and 3.94×10^9 TAMBC was recorded from Mehal Meda, Molale and Zemero respectively (Table 5).

Table 5: Microbial quality of samples from farmers

| Microbial Quality | | | |
|---------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Districts | TAMBC(CFU/g) | T. Coiform (CFU/g) | Yeast & Moulds (CFU/g) |
| Mehal Meda | | | |
| <i>Kebele 02</i> | 4.07×10^9 | 3.16×10^6 | 1.90×10^6 |
| <i>Kebele 03</i> | 3.43×10^9 | 2.89×10^6 | 1.65×10^6 |
| <i>Kebele 07</i> | 4.17×10^9 | 2.45×10^6 | 1.82×10^6 |
| Mean | 3.89×10^9 | 2.83×10^6 | 1.79×10^6 |
| Molale | | | |
| <i>Kebele 01</i> | 4.08×10^9 | 2.58×10^6 | 2.32×10^6 |
| <i>Kebele 02</i> | 3.85×10^9 | 2.31×10^6 | 1.68×10^6 |
| <i>Kebele 06</i> | 4.06×10^9 | 2.48×10^6 | 1.75×10^6 |
| Mean | 3.99×10^9 | 2.46×10^6 | 1.92×10^6 |
| Zemero | | | |
| <i>Kebele 01</i> | 4.11×10^9 | 2.63×10^6 | 1.37×10^6 |
| <i>Kebele 02</i> | 3.57×10^9 | 2.12×10^6 | 1.75×10^6 |
| <i>Kebele 08</i> | 4.15×10^9 | 3.32×10^6 | 2.22×10^6 |
| Mean | 3.94×10^9 | 2.69×10^6 | 1.78×10^6 |
| Overall mean | 3.94×10^9 | 2.66×10^6 | 1.83×10^6 |

In samples taken from butter made by the investigators (*churned*) lowest values of 3.26×10^9 , 3.21×10^9 and 3.32×10^9 were recorded as shown in Table 6. But much higher results were seen at Tarmaber (4.19×10^9) and at Addis Ababa (4.20×10^9).

Table 6: Microbial quality of samples from traders and churned by investigators

| Microbial Quality of Butter Collected from Traders and Churned | | | |
|--|----------------------|----------------------|------------------------|
| Districts | TAMBC(CFU/g) | T. Coiform (CFU/g) | Yeast & Moulds (CFU/g) |
| Mehal Meda | | | |
| Traders | 3.64x10 ⁹ | 3.00x10 ⁶ | 1.92x10 ⁶ |
| Churned | 3.26x10 ⁹ | 1.38x10 ⁶ | 1.99x10 ⁶ |
| Molale | | | |
| Traders | 3.23x10 ⁹ | 3.16x10 ⁶ | 1.37x10 ⁶ |
| Churned | 3.21x10 ⁹ | 2.10x10 ⁶ | 1.79x10 ⁶ |
| Zemero | | | |
| Traders | 3.45x10 ⁹ | 2.92x10 ⁶ | 6.27x10 ⁵ |
| Churned | 3.32x10 ⁹ | 1.36x10 ⁶ | 1.52x10 ⁶ |

In general, an overall mean of 3.94 x10⁹ TAMBC from farmers sample, 3.44 x10⁹ from traders, 3.26 x10⁹ from churned samples, 4.19x10⁹ from Tarmaber and 4.20x10⁹ from Addis Ababa were recorded. These values are higher than the acceptable limit of 5×10⁴ cfu/g Mostert and Jooste (2002). Highly contaminated butter was found at Addis Ababa and Tarmaber followed by butter collected from farmers. Necessary measures were taken to avoid collection of old butter which may rule out age as a source of variation. And there was no seasonal variation since all samples were collected and tested on month of August which is supposed to be rainy season. Accordingly, the source of contamination seems to be at secondary/ final market places and at farmers (Table 7). Mamo (2007) reported total microbial load of 3.15 × 10⁷ were higher counts recorded in samples collected from open markets/rural producers compared to samples from dairy farms/urban producers. Average total bacterial counts of 7.25 cfu/g in Selale area Zelalem (2010) and 8.19 ± 0.12 log cfu/g in Southern Ethiopia Mekedes (2008) was reported. The mean TAMBC in fresh butter samples collected from Ambo and Dire Inchini districts of West Shewa revealed 8.71log cfu/g Alganesh (2017).

Table 7: Microbial quality of samples from Tarmaber and Addis Ababa

| Microbial Quality | | | |
|-------------------|----------------------|----------------------|------------------------|
| Places | TAMBC(CFU/g) | T. Coiform (CFU/g) | Yeast & Moulds (CFU/g) |
| Tarmaber | 4.19x10 ⁹ | 2.69x10 ⁶ | 1.56x10 ⁶ |
| Addis Ababa | 4.20x10 ⁹ | 2.10x10 ⁶ | 1.45x10 ⁶ |

A study conducted in other parts of the world recorded higher values of TAMBC than the present study. In Sudan log₁₀ 7.24 cfu/g, log₁₀ 7.68 cfu/g and log₁₀ 7.89 cfu/g values were seen respectively for butter traditionally made by farmers, butter manufactured in dairy plant and butter made by investigators (Ahmed *et al.*, 2016). Samet-Bali *et al.* (2009) also reported total microbial count of log 4.70±0.05 in traditional Tunisian butter. Additionally, a mean total bacteria count of log 5.18 – 6.08 cfu/g in traditional butter from Turkey was reported Gökce *et al.* (2010). High total bacteria count in butter may be attributed to high

microbial load initially present in the milk, absence of pasteurization and salt, and the effect of both separation and churning processes on the breaking up of bacterial clumps which increases their number (Idoui *et al.*, 2010).

Butter was classified according to its total aerobic mesophilic bacteria as very good quality ($< 1.0 \times 10^6$ cfu/g), good quality ($1.0 \times 10^6 - 2.0 \times 10^6$ cfu/g) and low quality ($> 2.0 \times 10^6$ cfu/g) (Gökce *et al.*, 2010). In this respect quality of butter collected from present study belongs to low quality because the lowest value observed in the study area is 3.21×10^9 from butter sample made by investigators at Molale district. The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used in the manufacture of butter and the environmental and sanitary conditions during packaging and handling so it is advisable to adopt strict hygienic measures (Meshref, 2010). Total coliforms as hygiene indicator can be used as important criteria for determination of microbiological quality of butter (Zelalem, 2010). Overall average mean value of 2.66×10^6 total coliform counts was observed from farmer's sample. Looking in to the three districts separately mean value of 2.83×10^6 , 2.46×10^6 and 2.69×10^6 were gathered from Mehal meda, Molale and Zemero respectively. Unlike in the case of TAMBC the values for total coliform counts were higher in samples collected from traders in Mehal Meda (3.00×10^6), Molale (3.16×10^6) and Zemero (2.92×10^6) (overall average being 3.03×10^6). Lowest values were obtained from butter samples manufactured by investigators (1.38×10^6 , 2.10×10^6 and 1.36×10^6 in Mehal Meda, Molale and Zemero districts respectively with overall value of 1.61×10^6). Total coliform counts of 2.69×10^6 and 2.10×10^6 were seen for samples collected from Tarmaber and Addis Ababa (Table 6). These high deviations from the acceptable value of 10cfu/g (Mostert and Jooste, 2002) indicate substandard handling conditions at all stages in the milk chain.

Higher coliform count was reported from Sudan Ahmed *et al* (2016) with values of $\log_{10} 2.51$ cfu/g, $\log_{10} 2.38$ cfu/g and $\log_{10} 2.41$ cfu/g for butter from farmers, dairy plant and made by investigators respectively. Coliforms are indicators of cleanliness of handling of milk and cream, premises and equipment (Idoui *et al.*, 2010). Coliform was found in fresh butter from rural and public butter markets in Addis Ababa which indicates poor hygienic practices ILCA (1992). Zelalem (2010) reported coliform counts ranging from 1.92 to 4.5 \log cfu/gram of butter. Similarly high values were reported by Gökce *et al.* (2010), Meshref (2010), Idoui *et al.* (2010) and Asresie *et al.* (2013). Kacem and Karam (2006) reported coliform bacteria count of 0.90-1.66 \log cfu/g at refrigerator temperature. Karaozlu and Eronul (2008) reported that coliform and total fecal coliform count of the samples were found between $<3->1400$ cfu/g. The existence of coliform bacteria in food material is of greatest importance because it indicates that the food product is exposed to an insufficient heat treatment or is re-contaminated afterwards (Gökce *et al.*, 2010).

Average yeast and mold counts of 1.83×10^6 was recorded from farmers butter with the highest record of 2.32×10^6 (Kebele 01, Molale) and lowest value of 1.37×10^6 (Kebele 01, Zemero). The mean yeast and mold count in the three districts was 1.79×10^6 , 1.92×10^6 and 1.78×10^6 in Mehal Meda, Molale and Zemero respectively. Lower values were observed from trader's sample than farmer's except in Mehal Meda (Table 6). Overall mean value from butter samples made by the investigators was 1.77×10^6 . Lowest values were observed from samples taken from Tarmaber (1.56×10^6) and Addis Ababa (1.45×10^6).

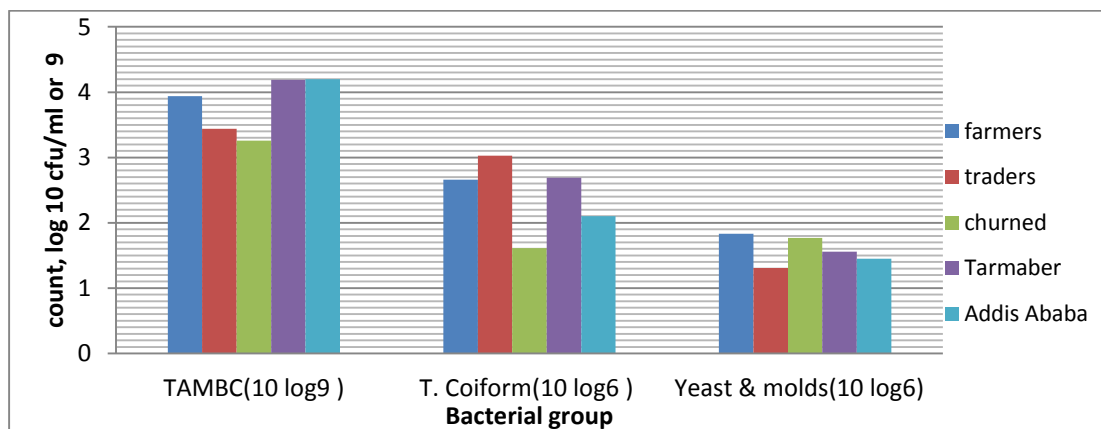


Fig. 2: Microbial counts of butter from Menz district. Elaborates the microbial quality of butter collected along the market value chain. These values are counted by Colony Forming Units (CFU) which measure only viable bacterial cells. The results are given as CFU/g. The calculation was done by using a formula $\text{cfu/g} = (\text{number of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$ then the value is converted in to Log value.

The presence of mould contamination in butter indicates contamination by water or air after production. The mean yeast and mould count observed in the Ethiopian highlands was 8 cfu/g of butter (Zelalem, 2010). According to Mekdes (2008) yeast and mould counts ranged between 4.3 and 6.86 log cfu/g of butter sampled from Wollayta area. In Sudan higher values of log₁₀ 3.39 cfu/g, log₁₀ 3.03 cfu/g and log₁₀ 3.08 cfu/g were reported for butter samples from traders, butter samples from dairy plants and samples from butter made by investigators (Ahmed *et al.*, 2016). Yeasts and moulds count of log₁₀ 4.80±0.00 (Samet-Bali *et al.*, 2009) and < log₁₀ 1.00-6.62 cfu/g (Karagözlü and Ergonul, 2008) in Turkish butter and mean count of $6.3 \times 10^3 \pm 1.07 \times 10^3$ cfu/g in Egypt (Meshref, 2010) were reported. Moulds and yeasts grow faster than bacteria and cause spoilage in food with low water activity. Beside spoilage, mycotoxin risk also exists, and the high amount of moulds and yeasts is as an indicator of incorrect processing and packaging (Gökce *et al.*, 2010).

Conclusion and Recommendation

The fat content of butter samples collected from different actors in the market value chain are greater than 80% except in one sample collected from farmers in Zemero district (79.33%) ranging from 80.68%-

85.94% which is adequate amount since butter must contain $\geq 81\%$ of only milk fat. The moisture content of butter in most of the samples is also less than the maximum amount of moisture expected in butter (16%). Moisture content of butter samples vary from 12.38% to 16.9%. Values of free fatty acids which are indicators of quality deterioration are also variable in the samples ranging from 1.10-3.77%. Different values of Total Aerobic Mesophilic Bacterial Count (TAMBC) were observed in samples collected from different butter market actors. 3.94×10^9 cfu/g (mean value of farmers), 3.44×10^9 cfu/g (mean value of traders), 3.26×10^9 cfu/g (mean value of butter made by investigators), 4.19×10^9 cfu/g (Tarmaber) and 4.20×10^9 cfu/g (Addis Ababa) were recorded. Even if these records are generally lower than records in previous studies, the results reflect low quality butter compared to the acceptable limit of 5×10^4 cfu/g. Coliforms were found in all samples that indicate poor hygienic practices. The total coliform counts(cfu/g) were 2.66×10^6 , 3.03×10^6 , 1.61×10^6 , 2.69×10^6 cfu/g and 2.10×10^6 cfu/g in samples from farmers, traders, butter made by investigators, Tarmaber and Addis Ababa respectively. Higher value of yeast and mold counts were seen in samples collected from farmers (1.83×10^6 cfu/g) and in butter made by investigators (1.77×10^6 cfu/g).

According to the current study cooking butter is produced under unhygienic condition. Therefore, there is a necessity for developing the hygienic status of locally produced butter through provision of information to rural women on good hygiene practice (GHP). Maintenance of the proper hygienic conditions during the processing of milk can reduce the prevalence of bacteria, which spoil the milk product. There is a need for further research and authentication policy guidelines of dairy products focusing on spoilage and pathogenic microorganisms and adulteration practices. It would be a great interest if further investigations are to be carried out to identify and isolate different species of pathogenic microorganisms that might cause public health importance. There is also a need for assessment of Seasonal variation of physiochemical and microbial quality of butter.

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Conflict of Interests

The authors declared that there is no any conflict of interests.

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