Hygienic Handling Practices and Quality of Ethiopian Traditional Butter (*Kibe*) along the Value Chain in Selected Areas of the Central Highlands

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Abstract

This study was conducted to understand production and market conditions and determine the microbial quality and safety of traditional butter in eight selected dairy potential sites in the Ethiopian central highlands. A total of 320 smallholder farmers, 8 primary dairy cooperatives, 80 open market butter traders and 40 dairy product shops were interviewed using a semi structured questionnaire. A total of 160 butter samples (from 80 smallholder farmers, 40 shops and 40 butter traders) were collected from a subset of previously interviewed respondents for microbial analysis. All respondent from smallholder producers, shops, and dairy cooperatives reported that they wash their hands prior to butter handling with about 74 - 100% reporting to wash their hands with cold water and detergent. However, none of the respondents from open market butter traders washed their hands and butter storage containers. About 75 - 88% of respondents from dairy producers, shops and cooperatives used plastic containers for storing butter, while the remaining used stainless steel. The majority of the sample smallholder dairy farmers, open market traders, shops, and cooperatives had no access to refrigerator instead store containers holding butter in cold water (5 - 30%) or at ambient temperature (67.7 - 92%). The average aerobic mesophilic bacterial, coliform, yeast and mould and lipolytic bacterial counts observed in the present study were 6.23, 2.5, 4.6 and 3.98 log cfuml^{-1}, respectively. Out of the 160 butter samples, on average, 3.88% were positive for Listeria monocytogenes, while no sample was positive for Salmonella test. Generally, the microbial quality of butter obtained in this study failed to comply with the minimum standard values set for quality butter. Therefore, care should be taken in the raw material used to producing butter, the equipment used for processing and storage, personnel hygiene of personnel handling the product in all the way from production to marketing.

Key words: *Butter, hygienic practices, microbial quality*

Introduction

A wide variety of dairy products are manufactured by processing milk, among which, butter is one of the primary source of fat and dietary energy. Milk fat being its major component, butter contains small percentages of proteins, lactose and water, which make it a suitable substrate for many bacteria, and yeast and mould that have been implicated in spoilage and lipolysis of butter even at low temperature (Rady

and Badr, 2003). Traditionally, butter has been viewed as a microbiologically safe or low risk product. However, internationally, a number of public health problems have been reported in connection with pathogens such as *Listeria monocytogenes* in Finland, USA and England (Chun *et al.*, 1990; ACMSF, 2003).

There is limited information on the microbial quality and safety of Ethiopian traditional butter (*Kibe*). Earlier reports revealed that the microbial quality of butter is substandard (Almaz *et al.*, 2001; Wondu2007, Zelalem *et al.*, 2007; Mekdes, 2008; Zelalem, 2010; Debela, 2015) and samples contained bacteria of public health concern. For instance, studies conducted by Zelalem *et al.* (2007) and Liyuwork*et al.* (2013) who identified *Enterobacter sakazakii, Klebsiella pneumoniae, Klebsiella oxytoca* and *Salmonella* from butter samples collected from the central highlands of Ethiopia. The microbiology of butter usually reflects the microflora present in the raw material, milk, the sanitary conditions of processing equipment, manufacturing environment and conditions under which the product is stored. Hence, controlling microbial contamination of butter and their subsequent growth can be achieved through properly keeping the hygienic quality of milk at farm level as well as control during processing, storage and packaging (Pandey and Voskuil, 2011).

Butter is one of the food items used in most traditional dishes on a regular basis thus is one of the most marketable dairy commodities in Ethiopia. Likewise, the most important selling attributes of butter is its unique and pleasant flavor, which is the main reason for its higher selling price than other fat sources (Rady and Badr, 2003). However, the flavor of butter considered to be of good quality is very delicate as even small amounts of bacterial growth can change its pleasant flavor and aroma. Information on the microbial quality and safety of traditional butter (*Kibe*) produced and marketed in the study areas is essential to understand the overall quality of the butter being consumed. Therefore, the objective of this study was to assess hygienic handling practices and evaluate the microbial quality and safety of Ethiopian traditional butter along its value chain.

Materials and Methods

Description of the study areas

The study was conducted in eight selected sites in the Ethiopian central highlands namely Debre Berhan, Sheno, Sendafa, Chancho, Fiche, Degem, Debre Zeit and Asella. These areas are located within a radius of about 175 km from Addis Ababa, capital city of the country with elevations ranging from 1600 to 3000 meters above sea level. According to each of the respective district level Bureaus of Livestock and

Fisheries, the mean annual temperature ranges from 2.4 to 28^{o}C while the rainfall ranges from 860 to 1200 mm.

Study methodology and approach

Two methods/approaches were used to collect the required information;

Survey using a questionnaire

Three hundred twenty dairy herd owners, 40 from dairy product shops and 80 from open market butter traders as well as 8 primary dairy cooperatives were interviewed using a semi structured questionnaire. The assessment mainly focused on hygienic practices during butter handling, source of water used for cleaning (containers and hands), storage conditions and materials of butter along the butter value chain.

Microbial analysis

A total of 160 butter samples; 80 from smallholder farmers (10 per each of the 8 study sites), 40 from dairy product shops (5 per each of the 8 study sites) and 40 from open market butter traders (5 per each of the 8 study sites) were collected from previously surveyed respondents. Approximately 250g butter sample was aseptically sampled from containers of each individual selected sample respondent into a sterile bottle. The butter samples were labeled, securely capped and kept in ice-cooler box immediately following collection and transported to Dairy Microbiology Laboratory of the Holetta Agricultural Research Center for analysis. Samples were refrigerated and analyzed within 24hrs of sampling. Butter samples were heated in a water bath at 37°C for ease of handling (HPA, 2003).

Serial dilutions of the samples (10^{-1} - 10^{-7}) were made and about 0.1ml (surface plating) and 1 ml (pour plating) dilutions were mixed with culture medium in pre-labeled sterile plates. For each sample analysis was made in duplicate plates. For all tests, the media were prepared according to the guidelines given by manufacturers. Except VRBA which was sterilized by boiling, peptone water and other media prepared for each test were autoclaved for 15 min at 121°C (Richardson, 1985). All samples were analyzed for Aerobic Mesophilic Bacterial Count (AMBC), Coliform Count (CC), Yeast and Mould Count (YMC) and Lipolytic Bacterial Count (LPBC). Samples were also tested for the prevalence of selected bacterial pathogens namely *Listeria monocytogenes* and *Salmonella* spp. The microbial loads, except for *Listeria monocytogenes* and *Salmonella* spp were counted and expressed as Colony Forming Units (CFU) per gram of butter samples.

Aerobic Mesophilic Bacterial Count (AMBC): With a sterile pipet, 1ml of test portion of sample dilutions were added into pre-labeled plates and 15 - 20 mlPlate Count Agar (PCA) (cooled to 45°C) was immediately added and then mixed thoroughly. Plated samples were then allowed to solidify and counts were made after incubating plates at 30ºC for 48hrs in an inverted position.

Coliform Count (CC): Appropriate dilutions of butter samples (1 ml) were placed in sterile plates and 15 - 20 ML Violet Red Bile Agar (VRBA) was added and mixed. The dried plates were incubated in an inverted position for 24hrs at 32^{o}C (Richardson 1985). Typical dark red colonies normally measuring at least 0.5 mm in diameter were considered as coliform colonies.

Yeast and Mould Count (YMC): One ml test portion of sample dilutions was added into pre-labeled sterile plates, and 15 - 20 mltempered Potato Dextrose Agar (PDA) was added with streptomycin and chloramphenicol. Sample dilutions and agar medium were then thoroughly mixed by alternate back and forth rotation of plates on the flat leveled surface and allowed to set. The dried plates were then incubated at 25^{0}C for 3 to 5 days (Yousef and Carlstrom 2003).

Lipolytic Bacteria Count (LPBC): After autoclaving, Tributyrin agar was cooled in a water bath to45^{0}C.About15 - 20 mlof the medium was added into a sterile plate and allowed to solidify. From an appropriate dilution 0.1 ml of inoculum was then spread over the surface of the solidified medium using a sterile bent glass rod. Finally, the plates were incubated at 32^{o}C for 48hrs in an inverted position (Richardson 1985).

Listeria monocytogenes**:** well mixed testing butter samples (25ml) were homogenized in 225 mlof Listeria Enrichment Broth A and B and incubated for 24hrs at 37^{o}C. After 24hrs, the enrichment culture broth was transferred using a loop and streaked over the surface of PolymyxinAcriflavin-Lithium Chloride Ceftazidime AesculinMannitol (PALCAM) agar plate and incubated for 48hrs at 37^{o}C. Colonies that are gray-green with black precipitate were considered as *Listeria monocytogenes*. Suspected *Listeria monocytogenes* colonies were then further characterized using gram staining and catalase test.

***Salmonella* spp**: twenty-five mlof butter samples was pre-enriched in 225 mlof Buffered Peptone Water (BPW) and incubated for 24hrs at 37^{o}C. A portion (0.1 ML) of the pre-enriched culture was transferred to 10 mlRappaport-Vassiliadis (RVs) broth and incubated at 42^{o}C for 24hrs. A loop full of a culture from the enrichment broth was streaked on the surface of Xylose Lysine Deoxycholate (XLD) agar plates and incubated at 37^{o}C for 24hrs (El-Shamy *et al.*,2008). Characteristic *Salmonella* colonies, having a slightly transparent zone of reddish color and black center, were sub-cultured on nutrient agar and confirmed biochemically using Triple Sugar Iron (TSI) and Simon citrate agar.

Data analysis

Percentage was used to compute different parameters related to hygienic practices involved in butter production using SPSS software version 16. Bacteria counts of each variable were first converted into logarithmic values ($\log_{10}$ cfu g^{-1}) and these transformed values were analyzed using the General Linear Model procedure of analysis of variance using SPSS version 16. Mean comparison was done using Duncan test for variables whose F value appeared to be statistically significant. Differences were considered as significant when P-value is <0.05.The model used for this study is $Y_{ij}=\mu+\beta_i+e_{ij}$, where, Y_{ij} = microbial count, μ = overall mean, β_i = butter sampling sources and e_{ij} = random error.

Results

Hygienic practices during traditional butter handling

The entire sample producers, dairy product shops and primary dairy cooperatives reported to wash their hands prior to butter handling (Table 1). Other related hygienic practices such as dressing gown and hair cover were usually common with primary dairy cooperatives and to some extent with dairy product shops and open market butter traders. However, none of the sample open market butter traders reported to wash hands prior to handling butter due to inaccessibility of water at market place. Sources of water used for cleaning (hands and utensils) varied along the butter value chain. For instance, only about 54% of the producers used tap water for the purpose; however, all of the sampled dairy cooperatives and dairy product shops reported that they had access to tap water for cleaning.

Up to 74% of the sample farm owners washed their hands with cold water and soap to remove the milk fat residuals whereas the proportion that used cold water and soap was higher in dairy product shops (95%) and primary dairy cooperatives (100%) (Table 1). The majority of the respondents use plastic materials to store butter along the butter value chain. However, 12 - 25% of the sample smallholder farmers, dairy shops and primary dairy cooperatives use stainless steel (Table 1). Concerning cleaning practices (hands and utensils), about 79 - 100% of the respondents used warm water and soap to clean butter storage containers. The rest (20.7%) of the sample dairy farmers and dairy product shops (11%) reported to use cold water and soap (Table 1).

Table 1: Butter hygienic practices followed along the butter value chain (%) (N=448)

Parameters	Producers (n=320)	Traders (n=80)	Dairy Shops (n=40)	Dairy cooperatives (n=8)
Sanitary practices				
Hand washing	100	0.0	100	100
Wearing gown	0.0	25.0	35.0	100
Cover hair	0.0	10.0	30.0	100
Water sources				
Tap	54.47	0.0	100	100
River	20.15	0.0	0.0	0.0
Spring	12.87	0.0	0.0	0.0
Bore well	12.50	0.0	0.0	0.0
Methods of hand washing				
Cold water	25.1	-	4.8	0.0
Cold water with soap	74.4	-	95.2	100
Storage equipment				
Plastic	87.8	100	83.8	75.0
Stainless steel	12.2	0	16.2	25.0
Methods of equipment cleaning				
Cold water and soap	20.7	-	11.2	0.0
Warm water and soap	79.3	-	88.8	100

Table 2: Butter storage conditions (%) (N = 448)

	Producers (n=320)	Traders (n=80)	Shops (n=40)	Cooperatives (n=8)
Storage conditions				
Refrigerators	2.3	0.0	21.0	25.0
Cold water	30.0	7.6	5.2	0.0
Ambient temperature	67.7	92.4	74.8	75.0
Methods of extension shelf life				
Mixing with spice	100	5.0	11.0	0.0
Convert to ghee	100	0.0	0.0	0.0
Mixing salt	0.0	0.0	9.0	0.0
Butter storage time before sale				
One week	45	30.3	28.3	98.2
Two weeks	54	58.4	51.1	1.8
Three weeks	0.0	11.3	9.0	0.0
One month - two months	0.0	0.0	9.0	0.0
Longer than two months	0.0	0.0	2.6	0.0

The study revealed that about 67.7 - 92% of the interviewed respondents usually kept butter at room temperature until marketing, while about 21 and 25% of the sample dairy product shops and dairy cooperatives kept butter refrigerated, respectively (Table 2). The majority (54%) of the sample farmers supply butter to market within two weeks after processing, and about 58% and 51% of the butter traders

and dairy product shops, respectively kept the product up to two weeks. In the case of dairy cooperatives butter is usually sold within a week.

A few sample producers reported to store butter intended for their own family consumption as long as over a year. The respondent from smallholder farmers reported that they traditionally preserve butter using a combination of different plant spices such as *Tikur azmud (Nigella sativa),Besobla (Ocimum sanctum), Zengible (Zingiber officinale), Kororima (Aframomum angusti-folium), Garlic (Allium sativum), Rue/Tenadam (Ruta chalepensis), Abish (Trigonella foenum), Nech azmud (Trachyperimum copticulm), Kosert (Ocimum hardiense)* and *Tossign (Thymus vulgaris).* As noted from the farm households, converting butter into traditional ghee was one of the best options to extend the shelf life of butter using aforementioned traditional spices.

Microbial quality and safety of butter

Lipolyitc and aerobic mesophilic bacteria counts of butter samples significantly varied (*P<0.05*) among sampling sources. Butter sampled from producers had the lowest LPBC and AMBC, while highest counts were recorded for samples collected from open market butter traders (Table 3). Butter samples collected from producers had 2.39 and 4.49 log cfu g^{-1} of CC and YMC, respectively, which were not significantly different (P>0.05) from butter sampled from open markets and dairy product shops (Table 3). The highest prevalence of *Listeria monocytogenes* was observed in butter samples taken from open markets (6.2%) followed by shops (3.2%) and producers (2.26%). However, none of the butter samples were positive for *Salmonella* (Table 3).

Table 3: Microbial quality and safety butter collected from different sources - mean (SE)

Variables	Sample sources (log cfu g^{-1})			Overall mean (N=160)
	Producers (n=80)	Open market butter traders (n=40)	Dairy product shops (n=40)	
AMBC(log cfu/ml)	5.84(0.09)^{a}	6.54 (0.07)^{b}	6.22(0.09)^{c}	6.23 (0.05)
CC(log cfu/ml)	2.39(0.14)	2.61(0.21)	2.61(0.21)	2.50 (0.11)
YMC(log cfu/ml)	4.49(0.11)	4.67(0.08)	4.57(0.12)	4.60(0.61)
LPBC(log cfu/ml)	3.64(0.06)^{a}	4.07(0.04)^{b}	4.12(0.06)^{bc}	3.98(0.04)
L.monocytogenes(%)	2.26	6.20	3.20	3.88
Salmonella (%)	0.00	0.00	0.00	0.00

AMBC - Aerobic Mesophilic Bacterial Count, CC - Coliform Count, YMC - Yeast and Mould Count, LPBC - Lipolytic Bacterial Count. Mean with different letters within the same row show significant difference (P<0.05)

Discussion

Hygienic practices during traditional butter handling

Washing hands with clean water and detergent followed by drying with a clean towel before handling butter helps to protect the product from microbial and dirt contamination (Richard, 2002). All of the sample respondents reported that they wash their hands before handling the butter with the exception of the interviewed butter traders. Covering hair and dressing clean gown during handling of milk and milk products are also important practices that handlers need to practice. However, none of surveyed households followed this practice. In contrary, all sample dairy cooperatives, and about 25 and 35% butter traders and dairy product shops, respectively reported to wear gown and hair cover.

Concerning sources of water used for cleaning (hands and containers), noticeable differences were observed along the butter value chain. Only about 54% of smallholder farmers had access to tap water, while all of the interviewed dairy cooperatives and dairy product shops used tap water for the same purpose. Previous study in the central highlands of Ethiopia also reported the use of tap water by 53% of the farmers (Zelalem and Faye, 2006). The quality of water sources other than tap water used for cleaning may not fulfill the required standard thus can contribute to the poor quality of milk and milk products (Zelalem, 2010). Therefore, it is important that producers should at least filter and heat treat it before use since the quality of water determines the number of bacterial counts (Biruk *et al.,* 2012).

The major sources of microbial contaminations of butter are related to unclean surfaces of the churn, storage utensils and wash water (O'Connor, 1995). With regard to storage containers, the majority (75 - 100%) of the respondents used plastic containers; this is in agreement with earlier reports in the central highlands of Ethiopia (Zelalem, 2010; Hiwot, 2013). Contrary to the current study, in East Shewa zone of Oromia, clay pot is commonly used for butter storage (Lemma, 2004). A few (12%) farmers, dairy product shops (16%) and primary dairy cooperatives (25%) used stainless steel for butter storage.

Plastic materials used for butter storage and transportation are not generally easy to clean, and are scratched by repeated cleaning, which provides a hiding place for microbial multiplication. Such conditions can represent a potential source of microbial contamination of butter, despite washing with hot water (Pandey and Voskuil, 2011). Therefore, replacements of plastic containers with stainless steel or aluminum containers can improve the flavor and hygienic conditions of the product considerably (Budhkar *et al.,* 2014).

The use of detergent and good quality water for cleaning the equipment could remove butter residues together with potentially existing spoilage as well as pathogenic microorganisms. As understood from the

current study, most (79 - 100%) of the respondents used warm water and soap to clean butter containers, however, all the surveyed butter traders did not practice any butter storage container cleaning.

Butter holding materials such as cups and leaves can also be important sources of contamination (O'Connor, 1995). Butter producers use cups, plastic containers and other local containers to transport butter by wrapping/topping with plastic sheets or leaves of *Enset (Ensete ventricosum)*, castor bean and eucalyptus (*nech bahirzaf)*. These leaves can usually harbor several kinds of worms such as snails infested with dirt and microbes, thus may affect the quality of product as well as consumers' health. Asrat (2009) reported that *Enset* leaf used for cheese packaging is usually wilted using fire, thus can have the advantage of destroying potentially existing worm and/or insect larva.

Storage temperature of the product should get the highest priority to ensure safe foods with low risk of contamination. In this study, smallholder farmers used different mechanisms to maintain the keeping quality of butter before being supplied to the market. Out of the interviewed dairy cattle producers, about 30% stored butter in containers with cold water, while the remaining 67.7% of the respondents kept the product at room temperature. The high percentage of value chain actors storing butter at ambient temperature observed in the current study is similar to that practiced by smallholder farmers (68.6%) reported in the central highlands of Ethiopia (Biruk *et al.*, 2012). However, 21 - 25% of the sample dairy product shops and primary dairy cooperatives kept butter in refrigerator until sale.

The majority (92.4%) of open market butter traders reported to keep butter at ambient temperature. Moreover, the marketing of butter had been taking place where a lot of dirt arises due to motor traffic, garbage dumps and open sewage. Such practice can result in butter contaminated with various microorganisms. Across the study areas, butter produced by smallholder farmers was either sold or preserved by mixing with different traditional spices to extend its shelf life and eventually improve its flavor. Farmers fetch butter to market at varying frequencies. For instance, about 45% of the sample households sold butter once a week, while the remaining (54%) sold butter fortnightly. The majority of open market butter traders and shops reportedly complete selling the butter within two weeks following collection.

As reported by sample smallholder farmers, the minimum and maximum shelf life of butter treated with traditional spices and stored under ambient temperature in this study was one month and 18 months, respectively. Other findings showed that, the keeping quality of butter can be increased by adding salt (O'Connor, 1995). However, salting butter was not observed to be a common practice among sample respondents in the current study, which is in line with that reported by Lemma (2004). Moreover, higher cooking temperatures when combined with different traditional spices are also reported to enhance the keeping quality of butter. As is the case in many other parts of the country, a variety of plant species such

as *Nigella sativa, Ocimum sanctum, Zingiber officinale, Aframomum angusti-folium, Allium sativum, Ruta chalepensis, Trigonella foenum, Trachyperimum copticulm, Ocimum hardiense* and *Thymus vulgaris* are the most commonly reported butter preservatives in the study areas.

Microbial properties of butter

Aerobic Mesophilic Bacteria Count (AMBC): The number of bacteria present in any given food product indicates the conditions under which it was produced and handled, and it also determines the food's keeping quality. The average values of AMBC (6.23 log cfu g^{-1}) found in the current study was lower than that of earlier findings (6.67 - 8.71 log cfu g^{-1}) reported in different parts of the country (Wondu, 2007; Mekdes, 2008; Zelalem, 2010; Debela, 2015).

The discrepancy in counts of aerobic mesophilic bacteria in butter sampled from different sources in this study might be attributed to differences in cleanliness of storage equipment and conditions, wash water used, handling materials and market place hygienic conditions. However, the overall mean AMBC observed in the present study failed to comply with the standard value (4.6 log cfu g^{-1}) given for good quality butter (COMESA, 2003).

The high counts of aerobic mesophilic bacteria observed in the present study could probably be related to the high initial microbial load in the raw material milk, absence of pasteurization, poor sanitary conditions of equipment used to manufacture the product, storage and transportation conditions, inadequate market facilities and the poor sanitary conditions practiced during butter handling. It is therefore advisable to adopt strict hygienic measures during milk handling to prevent contamination and improve the quality of its derivative products, in addition to proper heat treatment of milk and subsequent product handling (Meshref, 2010).

Coliform Count (CC): The average CC of the butter samples considered in this study was estimated at 2.5 log cfu g^{-1}. Higher CC (4.0 - 5.62 log cfu g^{-1}) was, on the contrary, reported for butter samples in the central highlands of Ethiopia (Zelalem, 2010; Debela, 2015). Aleme *et al.* (2013) also reported higher CC of (3.07 log cfu g^{-1}) butter samples made from the blend of goat and camel milk. However, lower coliform counts (0.90 to 1.66 log cfu g^{-1}) were reported for traditional butter made from camel milk in four regions of Algerian Sahara (Kacem and Karam, 2006).

The average CC observed in the present study is much higher than the acceptable limit of 1log cfu g^{-1} (Mostert and Jooste, 2002).This high count could be considered as indicative of potential product contamination with enteric pathogens thus represent potential health risk to consumers. The occurrence of coliform in a high number in this study could show substandard sanitation measures taken during product

handling (milking, processing and packaging); fecal contamination or improper storage; and/or contamination while transportation following manufacturing. Moreover, wash water used from sources other than tap could also be a source of contamination by coliform bacteria.

Yeast and Mould Count (YMC): Yeast and mould are considered to be the major group of microorganisms causing butter quality deterioration during storage. Such quality deterioration could lead not only to a reduction of the nutritional value of the product but also render the product to produce undesirable flavor (Idoui *et al.*, 2013). The average YMC observed in this study was estimated to be 4.6 log cfu g^{-1}. This figure is much higher than the maximum (50 cfu g^{-1}) value set for acceptable quality butter in the COMESA countries (COMESA, 2003). Values of YMC (ranging from 5.58 to 8.32 log cfu g^{-1}) higher than that observed in the current study were, however, reported for butter samples collected from various regions of Ethiopia (Mekdes, 2008; Zelalem, 2010; Debela, 2015). On the contrary, lower average values (2.08 - 3.0 log cfu g^{-1}) of YMC were also reported in Algerian traditional butter manufactured from goat and camel milk (Kacem and Karam, 2006; Idoui *et al.*, 2013). The overall mean counts of yeast and mould observed in this study are in congruent with the value (4.80 log cfu g^{-1}) reported by Samet-Bali *et al.* (2009) in Turkish butter samples.

In a similar growth environment, yeast and mould can usually grow faster than bacteria and accordingly can cause rapid food spoilage with high acidity, low moisture and water activity (Walstra *et al.*, 2006). In addition to causing food spoilage, some yeast and mould species are capable of producing dangerous mycotoxins (Ramazan *et al.*, 2010), aflatoxin being an example worth mentioning of the reports in all the milk and dairy feeds samples in Addis Ababa milk shed (Dawit *et al.*, 2016). The presence of high counts of yeast and mould in butter could likely be related among others to lack of hygienic practices during butter making process and/or packaging. Yeast and mould are widely distributed in the environment and can enter into food products such as butter through inadequately sanitized equipment and/or as airborne contaminants. Warm weather and improper storage conditions can also be considered to be among the principal causes for butter contamination (Moreira *et al.*, 2001).

Lipolytic BacteriaCount (LPBC): Being psychotropic and natural inhabitants of soil, water and animals, lipolytic bacteria count are ubiquitous and able to grow at refrigeration temperature. Lipolytic bacteria are the most frequently occurring spoilage microorganisms in butter therefore are used to predict the keeping quality of butter (Lejko *et al.*, 2009). Lipolytic bacteria have lipolytic activity often responsible for rancidity or loss of flavors (Idoui *et al.*, 2013). Minimizing lipolytic microorganisms therefore means increasing fat retention and high butter yield with desirable flavor.

The average LPBC of butter samples collected from producers is significantly lower than that obtained from dairy product shops and open markets. Under acceptable condition, counts of lipolytic bacteria should not exceed 1.69 log cfu g^{-1} if the butter is to be considered as good quality (Richard, 2002). However, in the current study the observed counts are much higher in lipolytic bacteria (3.98 log cfu g^{-1}) than the acceptable limit. Studies carried out by Kacem and Karam (2006) and Rady and Badr (2003) reported lower average LPBC of 2.33 and 3.07 log cfu g^{-1}, respectively. However, no lipolytic bacteria were detected in butter manufactured from cow's milk in East Algeria (Adams and Moss 2008). The cleanliness of the milkers, the udder of the cow, the washing water, the milking environment, and processing and storage equipment represent major sources of contamination of the raw material milk as well as the final processed product.

Listeria monocytogenes: A food unfit for human consumption may not necessarily be spoiled and contain pathogens. *Listeria monocytogenes* is considered as one of the most important pathogens responsible for food-borne infection (Ryser and Marth, 2007). The prevalence of *Listeria monocytogenes* is higher (6.2%) in butter samples obtained from open market butter traders compared with those sampled from dairy product shops (3.2%) and smallholder farmers (2.26%). Similar to the present study, several positive *Listeria monocytogenes* results were reported in different countries such as Finland, Turkey and Iran (Lyytikainen *et al.*, 2000; Aygun and Pehlivanlar, 2006; Rahimi *et al.*, 2012). Detection of *Listeria monocytogenes* in product samples can reflect fecal and environmental contamination during milking, storage and transport as well as infected cows in dairy farms and poor silage quality (Husu, 2010). The presence of *Listeria monocytogenes* in butter may also indicate a significant failure of hygiene standards during the preparation and/or storage of such products.

***Salmonella* spp:** Studies made on pathogens isolation and food borne illness associated with the consumption of butter contaminated with *Salmonella* and *Listeria monocytogenes* had not been fully documented in Ethiopia. *Salmonella* species are of public health concern given the ability to produce infection ranging from a mild self-limiting form of gastroenteritis to septicemia and life-threatening typhoid fever (Fook *et al.*, 2004). In this study, *Salmonella* was not detected in 160 tested butter samples collected from different sources, which contrasts with the 1% prevalence of *Salmonella* in butter sampled from Addis Ababa (Liyuwork *et al.*, 2013). Foods of animal origin, particularly milk and milk products, meat and egg, are considered to be primary sources of human *Salmonellosis* (Acha and Szyfers, 2001). Most of these food products become contaminated during milking, slaughter, processing in contaminated environment and because of faulty during transportation, handling and storage facilities.

Conclusion

Based on results of the current study, butter produced and marketed in the study areas can generally be considered to be of poor quality, which is evidenced by the observed microbial quality that did not fulfill the required acceptable limits set for quality butter. The microbial contamination of butter could be attributed to factors such as the raw material (unpasteurized whole milk) coupled with traditional methods of processing, handling, storage and marketing. Therefore, it is essential to consider all butter quality affecting factors and accordingly take corrective measures to avoid microbial contamination of butter. Such quality measures should be taken all the way starting from the production of raw milk till the butter is consumed. This is important in terms of ensuring food security, public health and better financial return for all market actors involved in the butter value chain in the current study areas.

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