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Comparison of traditional butter preservation techniques using microbial and organoleptic properties, West Shewa, Ethiopia

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The study was conducted in Dire Inchini and Ambo districts of West Shewa, Oromia, Ethiopia to assess traditional butter preservation methods and compare their efficiency. Semi-structured questionnaire was prepared, pretested and used to interview 120 women respondents having experience in butter making. Butter preservation methods identified were ghee making (100%), spicing (98.33%), melted butter (29.17%) and salting (11.67%). Commonly used spices were *Trachyspermum ammi*, *Nigella sativa*, *Aframomum angusti-folium,* Trigonella foenum, *Zingiber officinale* and *Allium sativum*. Based on the survey, 7 kg of fresh butter samples were purchased from open market in the two districts and taken to Holeta Dairy laboratory and randomly allocated to each treatment namely traditional ghee, untreated, salted, spiced, melted, frozen (-20°C) and refrigerated butter (4°C). Microbial and organoleptic qualities of butter were analyzed at one month interval for 3 consecutive months. Microbial qualities of the samples were substandard; but, traditional ghee and salting were more efficient. Optimization of utilization of spices and comprehensive evaluation including oxidative deterioration is vital.

Key words: Traditional, butter, preservation, microbial, organoleptic quality.

INTRODUCTION

Like most sub Saharan Africa countries, Ethiopia is unable to meet the increasing demand for dairy products for its increasing population (Azage et al., 2000; Tsehay, 2001). Smallholder farmers and pastoralists produce and supply 98% of the total annual milk production (Yonad, 2009). The majority of milk produced in rural areas of Ethiopia is processed into milk products at household level using traditional technologies (Muriuki and Thorpe, 2001). In rural areas, 40% of milk produced is spontaneously fermented for three to four days without addition of specific starter culture and is churned to make butter, butter milk and whey as a byproduct. Rural

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producers are forced to produce butter due to limited market outlet, shorter shelf life of milk, lower price for whole milk, ease of handling of butter and for product diversification (Ayantu, 2006; Kassahun, 2008). Traditional butter ferments slowly at ambient temperature, offering rural consumers a relatively shelf stable dairy product (LMP, 2007). According to Getachew (2003), of the total butter production, 80% is used as food ingredient and the remaining is used for hair dressing and other purposes. The same study revealed that 70% of butter produced in the country is used in rural areas; while 30% is channeled to the Addis Ababa market. Therefore, butter contributes much to the dietary requirements of the society and saves milk from spoilage and diversifies its use (FAO, 1990). Workneh and Ulfina (2011) observed the significant importance of butter in Ambo area of West Shoa Zone and indicated that 75% of dairy producers in Ambo area process milk into butter. However, in Ethiopia, the climate is hot and humid; leading dairy products to spoil easily during storage unless cooled or treated with preservatives. Moreover, commercial preservatives are not readily available in rural areas. Cooling systems are also not feasible because of lack of infrastructure (O'Mahoney and Peters, 1987). In rural areas of Ethiopia, producers use different traditional preservation methods to increase shelf life of butter (Lemma, 2004; Mekdes, 2008). The local preservatives are used as a principle of acidification and moisture reduction and can make butter of good storage stability (O'Mahony and Peters, 1987). However, traditional butter preservation techniques and their efficiency in these districts were not studied. Therefore, the study was initiated to assess traditional butter preservation techniques and to compare their efficiency through evaluation of microbial and organoleptic properties.

MATERIALS AND METHODS

Description of the study areas

The study was conducted in Ambo and Dire Inchini districts of West Shoa Zone of Oromia, Ethiopia which is located at 105 km to the west of Addis Ababa, Ethiopia.

Ambo district

It is located between 80 47'N -90 21'N and 370 32'E and located at 115 km to the West of Addis Ababa (ADADO unpublished data, 2014). It has a mean annual temperature and rain fall of 23 to 25°C and 1300 to 1700 mm, respectively. The altitude ranges between 500 and 3,200 m above sea level (ADADO Unpublished Data, 2014).

Dire Inchini district

It is located at 162 km to the West of Addis Ababa and 50 km from Ambo (Tamrat, 2007). The average annual rainfall ranges between 1000 and 1400 mm. Average minimum and maximum temperatures are 8.8 and 21.6°C, respectively (Tamrat, 2007).

Sampling techniques

The survey was conducted in three major ecologies of the districts as follows: Ya'i cabo (high altitude), Gosu qora (mid altitude), Sankele and Farisi '(low land) and, Waldo hindhe (high land), Nano Jidu (mid altitude) and Toke Abuye (low land) from Ambo and Dire Inchini district, respectively.

Data collection

Survey

One hundred twenty respondents who own at least one lactating cow and having experience in traditional butter preservation techniques were purposively selected. Semi structured questionnaire was prepared, pretested and used for individual interview by trained enumerators. The questionnaire focused on butter preservatives and preservation methods. The collected data was entered into SPSS computer program.

Laboratory analysis

Collection of butter samples

Seven kilograms of butter was bought from open markets of the two districts to assess the microbial and organoleptic qualities. The purchased butter samples were kept in an icebox and transported to Holeta dairy laboratory within 4 h of collection. The samples were thoroughly mixed to form composite sample which was divided into seven equal weights each treatment receiving one kilogram of butter. The samples were randomly allocated to seven treatments namely traditional ghee, spiced, salted, melted, untreated, frozen (-20°C) and butter stored at 4°C. All analysis was done using duplicate samples.

Preparation of treatments

The samples were tested for their microbial and organoleptic qualities at 0, 1, 2 and 3 months of preservation. The treatments were prepared as follows.

Traditional ghee/'Nitir Kibe'

One kilogram butter was placed in a sauce pan and melted over a slow heating stove. White cumin, fenugreek, korerima, ginger, garlic, turmeric, black cumin and other herbs such as basil and other herbs of desirable aroma such as rue (*Ruta graucolence)*, basil (*Ocimum spp.) and '*Kussayyee'*(Ocimum hardiense)* aroma were added to the boiling butter fat based on the respondents' experience. The melting butter fat and spices were stirred while boiling until foaming had stopped. Finally, the sauce pan was removed from the stove and left aside until it was settled. The butter fat was filtered through metal sieve into high density poly ethylene bucket and kept at room temperature for a period of 3 months.

Spiced butter

One kilogram of butter was removed from composite butter sample and thoroughly mixed with about 45 g of fenugreek and black cumin powders, respectively. Based on the recommendation of the respondents, spiced butter sample was placed in high density poly ethylene bucket and kept at room temperature for 3 months.

Salted butter

One kilogram butter was thoroughly mixed with 30 g of NaCl and kept in high density poly ethylene bucket and was placed at room temperature for 3 months.

Melted butter/'Nigur kibe'

To make melted butter one kilogram of butter was placed in sauce pan and kept on a slow heating stove and stirred until the butter was completely melted. The melted butter was removed from the heat source and kept in cool place to settle down and left there for overnight until it has completely solidified. Then, impurities that were settled at the bottom of the sauce pan were decanted off by opening the solidified butter in sauce pan from one side. Then the sample was placed in high density poly ethylene bucket and kept at room temperature for 3 months.

Untreated butter

One kilogram of fresh butter sample was kept in high density poly ethylene bucket and was placed at room temperature for 3 months.

Frozen butter

One kilogram of butter sample was kept in high density poly ethylene bucket and placed in deep freeze at -20°C for 3 months.

Refrigerated butter

One kilogram of composite butter sample was kept in high density poly ethylene bucket and placed in refrigerator at 4°C for 3 months.

Each time at 0, 1, 2 and 3 months of preservation, required amount of sample was removed for analysis of aerobic mesophilic bacteria, total coliforms, total lactic acid bacteria, Enterobacteriaceae and yeast and mold counts and color, texture, odor and overall acceptability. The analysis of each treatment was performed in duplicates.

Microbial analysis

Aerobic mesophilic bacterial count: The butter samples were homogenized and aseptically transferred to stomacher bag and held for melting on a water bath adjusted at 46°C. Then, the samples were immediately serially diluted by adding 1 ml of butter into 9 ml of peptone water. One milliliter of the sample from a chosen dilution was placed on the Petri dish using pour plating technique. Then, plate count agar media of 15 to 20 ml was poured onto the petri-dish and thoroughly mixed with the sample and allowed to solidify for 15 min and incubated for 48±2 h at 35°C. Finally, the colonies were manually counted. The plate counts were calculated by multiplying the count on the dish by 10^n, in which n stands for the number of consecutive dilutions of the original sample (FAO, 1997; Michael and Joseph, 2004; FSSAI, 2012).

Total coli forms count: Samples were decimally diluted and plated with violet red bile agar media /VRBA into Petri dishes for enumeration of total coliforms bacteria as coliforms colony forming units per milliliter. Plates were incubated at t 32±1°C for 24±2 h. One milliliter of melted sample was serially diluted using peptone water and transferred into sterile Petri-dishes. 10 to 15 ml of violet red bile agar media tempered to a temperature of 44 to 46°C was added to the milk sample and thoroughly mixed and allowed to solidify for 5 to 10 min. The mixture was then overlaid with the same plating agar media of 3 to 4 ml to inhibit surface colony formation. The medium were allowed to solidify. The plates were inverted and incubated at 32 ±1°C for 24±2 h. Counts were made manually. Finally, the plate counts were calculated as N, the number of colony forming units of coliforms per milliliter of milk sample using the formula $N=\sum c/(n_1+n_2)d$ where $\sum c$= Sum of all colonies on all plates counted were conducted according to the standard procedures of FAO (1997), Michael and Jospeh (2004) and FSSAI (2012).

Lactic acid bacteria counts: 0.1 ml of appropriate decimal dilutions of butter sample was poured on Petri dishes in duplicates and mixed with MRS agar media. Then, after incubating the plates anaerobically at 30°C for 48 h, lactic acid bacteria were counted (FAO, 1997; Michael and Joseph, 2004; FSSAI, 2012).

Yeast and mold counts: Potato dextrose agar (PDA) media was autoclaved for 15 min at 121°C and tempered in water bath adjusted at 45°C. Appropriate decimal dilutions of butter sample (0.1 ml) were poured into a 15 × 90 ml Petri dishes and mixed with 20 ml of PDA containing antibiotic; chloramphenicol solution. Then, after incubation at 25°C for 5 days, yeast and mould were counted with plates containing 10 to 150 colonies (FAO, 1997; Michael, 2004; FSSAI, 2012).

Enterobacteriaceae count: One milliliter of homogenized melted butter sample was added into 9 ml peptone water to yield a dilution of 1:10 cfu/ml. Violet red bile glucose agar (VRBGA) medium was used to enumerate Entrobacteriaceae. The mixture was then overlaid with the same plating agar media of 3 to 4 ml. Plates were aerobically incubated for 24 h at 37°C and inspected for purple-red colonies surrounded by a purplish circle of light or halo color. The plate counts were calculated by multiplying the count on the dishes by 10^n, where n stands for the number of consecutive dilutions of the original sample (ILSI, 2011, FSSAI, 2012).

Organoleptic quality of butter: Organoleptic quality were evaluated by 10 semi-trained sensory panelists using 5 point hedonic rating scale where 1=dislike extremely, 2= dislike slightly, 3= neither like nor dislike, 4= like moderately and 5 = like extremely. The samples were coded for identification purpose.

Data analysis

Survey and organoleptic quality data were analyzed using descriptive statistics using SPSS (2011). Microbiological counts were transformed to log10 cfu/g and analyzed using the General Linear Model of SAS version 9.1 (SAS, 2009). Least significant difference was used to test the differences between treatment means and time of preservation.

RESULTS AND DISCUSSION

Traditional butter preservation techniques in the study areas

Traditional methods of butter preservation in Dire Inchini and Ambo districts were presented in Table 1. In the study areas, 100, 98.33, 29.17 and 11.67% of interviewed smallholder producers commonly preserve butter in the form of traditional ghee, spiced, melted and salted butter, respectively. This finding is in agreement with previous reports in Arsi Negele, Oromia, Walayita, Southern Ethiopia, North Western Ethiopia and East Wollega, Ethiopia by Lemma et al. (2004), Mekdes (2008), Eyassu (2014) and Alganesh and Yetenayet (2016), respectively.

Table 1. Traditional methods of butter preservation in the study areas.

Preservation method	Dire Inchini (N=60)		Ambo (N=60)		Overall mean (N=120)	
Traditional ghee	N	%	N	%	N	%
Mixing with spices	60	100	60	100	120	100
Melting	58	96.67	60	100	118	98.33
Salting	20	33.33	15	25	35	29.17

N = Number of respondents.

Table 2. Spices used for butter preservation techniques in the study areas.

Local name	Common name	Scientific name	N (%)
Habasuuda adii	White cumin	*Trachyspermum ammi*	120 (100)
Ogiyoo	Kororima	*Aframomum angusti-folium*	98(81.67)
Abishii	Fenugreek	*Trigonella foenum*	85(70.83)
Jinjibila	Ginger	*Zingiber officinale*	71(59.17)
Qullubbii adii	Garlic	*Allium sativum*	61(50.83)
Irdii	Turmeric	*Curcuma domestica*	3(2.5)
Qarafaa		*Cinnamomum verum*	3(2.5)
Dimbilaala	Basil	*Cordiandrum sativum*	1(0.83)
Qullubbii diimaa	Onion	*Allium cepa*	3(2.5)
Qundeberbere	Black peper	*Piper nigrum*	2(1.67)
Habasuuda gurraacha	Black cumin	*Nigella sativa*	104(86.67)

N= number of respondents.

In the study areas in wet season and during Ethiopian Orthodox fasting time, demand for butter is low and during such occasions, surplus butter is preserved using traditional preservation techniques. In the present study, spiced, melted and salted butter are used as raw materials for traditional ghee making and they are optional butter preservation techniques.

Spices used for ghee making and spicing butter (spiced butter)

The result for the spices used for spiced butter and traditional ghee making in the study areas were indicated in Table 2. For traditional ghee making smallholder producers in the study areas mainly use *Trachyspermum ammi*, *Aframomum angusti-folium, Nigella sativa, Trigonella foenum, Zingiber officinale, Allium sativum* and *Curcuma domestica* is used for coloring purpose. While for making spiced butter, they use *Trigonella foenum and* Nigella *sativa*. Similar study by Joe et al. (2009) stated that spices are used to enhance aroma, flavor and for preservation of food substances.

Microbial properties of butter preserved using traditional preservation methods

Aerobic mesophilic bacterial count

The mean total bacterial count (log cfu/g) for the treatments and preservation time is presented in Table 3. The aerobic mesophilic bacterial counts of butter preserved using traditional ghee, salted and spiced butter at 0, ends of 1, 2 and 3 months of preservation did not show significant difference at $P<0.05$. While aerobic mesophilic bacterial counts of melted, untreated, frozen and butter stored in refrigerator at 4°C showed significant difference among treatments at 0, ends of 1, 2 and 3 months at $P<0.05$ level of significance. The total aerobic mesophilic bacterial counts for traditional ghee, salting, spicing, melted, untreated, frozen and refrigerated butter (4^0c) showed significant difference among the treatment means at $P<0.05$ level of significance. At initial preservation time, greater counts of aerobic mesophilic bacterial counts was observed in salted (9.58 log cfu/g) and melted butter (9.65 log cfu/g), which were significantly different ($P<0.05$) from other treatments. This might be due to the poor hygiene of the salt used in preserving the butter. In the case of melted butter, the increase in the aerobic mesophilic bacterial count might probably be as a result of post melting contamination. At initial preservation period, the mean aerobic mesophilic bacteria in untreated butter were 8.71 log cfu/g of butter samples. The current result is far beyond the maximum tolerable limit of 6 log cfu/g of aerobic mesophilic bacteria counts set by Standards Authority of Ethiopia (QSAE, 2009). The sensory attributes used to evaluate butter samples in this experiment were odor, texture, color and overall acceptability. So there was no risk on the health

Table 3. Mean total aerobic mesophilic bacterial counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	Maxlimit set by ESA
Aerobic mesophilic bacterial	0	9.48±0.08^{aB}	9.39±0.09^{aB}	9.58±0.05^{aB}	9.65±.02^{aB}	8.71±.03^{bC}	8.71±.02^{aCD}	8.71±0.02^{bD}	6
	1	9.62±0.03^{aA}	9.37±.06^{aB}	9.62±0.04^{aB}	9.72±0.05^{aB}	9.61±0.03^{aB}	9.59±0.09^{aC}	9.59±0.09^{aB}	
	2	9.64±0.04^{aA}	9.66±0.06^{aA}	9.84±0.04^{aA}	9.23±0.06^{bC}	9.68±0.04^{aB}	9.61±.04^{aB}	9.63±0.05^{aB}	
	3	9.73±0.06^{aA}	9.78±0.03^{aC}	9.89±0.06^{aA}	9.93±0.03^{aA}	9.98±0.04^{aA}	9.24±.04^{bD}	9.97±0.03^{aB}	

Means followed by similar lower case letters in a column are not significantly different (P<0.05), Means followed by similar upper case letters in a row are not significantly different (P<0.05). ESA: Ethiopian Standard Authority.

Table 4. Mean total lactic acid bacteria counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter
Total lactic acid bacteria	0	6.65±0.09^{aA}	6.55±0.05^{aA}	6.72±0.06^{aA}	6.80±0.28^{aA}	6.77±0.03^{aA}	6.77±0.08^{aA}	6.77±0.07^{aA}
	1	6.06±0.06^{bC}	6.31±0.06^{abC}	6.09±0.13^{bD}	6.40±0.05^{bC}	6.65±0.09^{aB}	6.23±0.04^{aB}	6.59±0.04^{aB}
	2	5.56±0.06^{cC}	5.19±0.09^{cC}	5.72±0.05^{cC}	5.26±0.07^{cC}	6.60±0.07^{aB}	6.28±0.08^{aB}	6.12±0.10^{aC}
	3	5.09±0.11^{cD}	5.01±0.17^{cD}	5.53±0.04^{bD}	5.01±0.14^{cD}	6.32±0.07^{aC}	6.04±0.10^{aC}	6.06±0.10^{aD}

Means followed by similar lower case letters in a column are not significantly different (P<0.05), Means followed by similar upper case letters in a row are not significantly different (P<0.05)

and safety of the panelists since they evaluated the butter by smelling to check for the odor, checked the texture using smoothness, solidity and appropriate degree of firmness by their hands and visual observation of the color of the butter samples. The current result is also similar to a report from Wolayita in Southern Ethiopia of 8.10 log cfu/g of butter samples of total bacterial count (Mekdes, 2008). After one month of preservation, aerobic mesophilic bacterial counts of 9.37 log cfu/g was observed and this was significantly different (P<0.05) from the other treatments. Relatively lower mean aerobic mesophilic bacterial counts were observed in frozen butter, traditional ghee and spiced butter. This might be due to the antagonistic effects of active ingredients of spices, heat treatment and low temperatures in spiced butter, traditional ghee, melted butter, butter kept at 4 and -20°C, respectively on bacterial growth and survival. Similar studied by Kilcast and Subramaniam (2000) confirmed that shelf life of products can be extended by the use of processing treatments such as heat and radiation which kills the microorganisms or control of microbial growth by chilling, freezing, reducing the water content and addition of preservatives.

Lactic acid bacterial count

The mean total lactic acid bacteria counts (log cfu/g) of treatments and preservation time are described in Table 4. There were significant differences (P<0.05) between total lactic acid bacterial counts in traditional ghee, salted, spiced and melted butter at 0, ends of 1, 2 and 3 months of preservation. While the total lactic acid bacterial counts of butter preserved using untreated, frozen (-20°C) and refrigerated (4°C) butter showed no significant difference at P<0.05 among the means at 0, 1, 2 and 3 months of preservation, respectively. Total lactic acid bacterial counts at 0 and 1 month of preservation showed no significant (P<0.05) difference for traditional ghee, salted, spiced, melted, untreated, frozen and refrigerated (4°C) butter samples. While, the total lactic acid bacterial counts at the end of the second month of preservation did not show significant (P<0.05)

Table 5. Mean yeast and mold counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	Max. limit set by ESA
Yeast and molds	0	5.70±0.08^{aB}	6.54±0.03^{bC}	5.74±0.05^{aC}	5.56±0.03^{dD}	6.70±.05^{bD}	6.70±0.04^{aA}	6.70±0.05^{aB}	1
	1	5.70±0.04^{aB}	6.67±.03^{aB}	6.36±0.05^{aA}	5.78±0.09^{cC}	6.74±0.03^{cC}	6.46±0.05^{bB}	6.72±0.06^{aB}	
	2	6.16±0.08^{aA}	6.69±.04^{aB}	6.28±0.08^{aB}	6.28±.07^{bB}	6.78±.03^{aB}	6.14±0.10^{bC}	6.76±0.05^{aA}	
	3	6.19±0.08^{aA}	6.78±0.05^{aA}	6.37±0.06^{aA}	6.71±.07^{aA}	6.84±.05^{aA}	6.42±0.14^{bC}	6.79±0.04^{aA}	

Means followed by similar lower case letters in a column are not significantly different from each other (P<0.05), Means followed by similar upper case letters in a row are not significantly different from each other (P<0.05). ESA: Ethiopian Standard Authority

difference among traditional ghee, salted, spiced, melted and refrigerated butter (4°C) except for untreated and frozen butter (-20°C). Whereas, at the end of the third month of preservation, the mean total lactic acid bacterial counts in traditional ghee, salted , spiced, melted and refrigerated butter samples did not show significant (P<0.05) difference among their mean counts except for untreated and frozen butter which did not significantly (P<0.05) differ from each other. The mean total lactic acid bacterial counts of the treatments at initial time of preservation ranged from 6.55 to 6.80 log cfu/g. This could be due to prior fermentation of composite sample as local butter is usually made of spontaneously fermented whole milk. At initial time of preservation, the mean lactic acid bacterial count in traditional ghee was 6.06 log cfu/g and it significantly differed at P<0.05 from other treatments; except for salted butter which was 6.09 log cfu/g of butter samples. The current result is also similar with the finding of Mekdes (2008) who reported mean lactic acid bacterial count of 7.51 log cfu/g for butter sample collected from Wolayita in Southern region. From the second to third months of preservation time, mean lactic acid bacterial count of spiced, melted butter and traditional ghee were relatively lower and significantly differed (P<0.05) from other treatments. This might be due to heat treatment and antimicrobial effects of spices used in melted butter, traditional ghee and spiced butter, respectively.

Yeast and mold counts

The result for the mean yeast and mold counts (log cfu/g) of treatments and preservation time is indicated in Table 5. The mean yeast and mold counts during initial 0, 1, 2 and 3 months of preservation for traditional ghee, spiced and butter refrigerated at 4°C did not significantly (P<0.05) differ from each other. The mean yeast and mold counts (log cfu/g) of salted, spiced, melted, untreated and frozen butter samples significantly differed (P<0.05) from each other. The mean yeast and mold counts (log cfu/g) of traditional ghee, salted, spiced, melted, untreated, frozen and refrigerated butter samples did not show significant difference (P<0.05) between the treatment means. Mean yeast and mold counts of butter at initial preservation time for traditional ghee (5.70 log cfu/g), salted butter (5.74 log cfu/g) and melted butter (5.56 log cfu/g) significantly differed (P<0.05) from spiced, untreated, frozen (-20°C) and refrigerated (4°C) butter. This might be attributable to the effect of heat treatment; antimicrobial properties of spices used to treat butter samples and reduced water activity in salted butter. The temperature range for yeast and mold growth is 0 to 47°C, out of which, the growth of yeast and mold can be hampered. This is in agreement with Seriler (2003) who revealed the possibility of reducing mold growth on the surface of butter by salting. The mean yeast and mold count of untreated butter sample at initial time of preservation was 6.70 log cfu/g. The current finding is beyond the maximum tolerable limit of 1 log cfu/g of yeast and mold count of butter sample recommended by the Ethiopian Standards Authority (QSAE, 2009). The present result is also higher than the mean yeast and mould count of 5.58 log cfu/g of butter samples reported in Wolayita Southern Ethiopia (Mekdes, 2008). Increasing trends of yeast and mold counts have been observed from one month of preservation period to the end in untreated, refrigerated (at 4°C), spiced and melted butter and these results were significantly different (P<0.05) from other treatments. In the case of spiced butter poor hygiene of spices purchased from open market might have contributed to the high rate of contamination. In untreated and refrigerated butter samples water activity might have been high and

Table 6. Mean total coliform counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	ESA
Total coliform	0	5.70±0.08^{aA}	6.54±0.03^{aB}	5.74±0.05^{aC}	5.56±0.03^{aD}	6.70±0.05^{aB}	6.70±0.04^{aA}	6.70±0.05^{aA}	Absent
	1	5.70±0.04^{aA}	6.67±0.03^{aB}	6.36±0.05^{aB}	5.78±0.09^{aC}	6.74±0.03^{bC}	6.46±0.05^{aA}	6.72±0.06^{aA}	
	2	6.16±0.08^{aA}	6.69±0.04^{aB}	6.28±0.08^{aB}	6.28±0.07^{aB}	6.78±0.03^{aB}	6.14±0.10^{aA}	6.76±0.05^{aA}	
	3	6.37±0.08^{aA}	6.96±0.11^{aA}	6.54±0.05^{aC}	6.86±0.09^{aB}	6.98±0.09^{aA}	6.34±0.07^{aA}	6.62±.05^{aA}	

Means followed by similar letters in a column are not significantly different from each other (P<0.05); means followed by similar bold letters in a row are not significantly different from each other (P<0.05). ESA: Ethiopian standard Authority.

have created favorable environment for the growth of yeast and molds.

Total coliform counts

Mean total coliforms count (log cfu/g) for the treatments and time of preservation is presented in Table 6. There were no significant difference (P<0.05) between the mean total coliforms bacterial counts of butter preserved using traditional ghee, salted, frozen and refrigerated butter at 0, 1, 2 and 3 months of preservation, while, the total coliforms bacterial counts of spiced, melted and untreated butter showed significant (P<0.05) difference among treatments at 0, 1, 2 and 3 months of preservation, respectively. Mean total coliform counts at initial time of preservation for traditional ghee, spiced, melted, untreated, frozen and refrigerated butter samples did not differ (P<0.05) significantly except for salted butter. The mean total coliforms counts at the end of one month of preservation for traditional ghee, spiced, melted, frozen and refrigerated butter samples were not significantly different at P<0.05 except for salted and untreated butter. While the mean total coliform counts at the end of one month of preservation for salted and untreated butter samples did not significantly (P<0.05) differ from each other. At the end of second month of preservation, the mean total coliforms counts of traditional ghee, spiced, melted, frozen and refrigerated butter samples did not significantly (P<0.05) differ from each other except for salted and untreated butter. At the end of the third month of preservation, the mean total coliforms counts of traditional ghee, spiced, melted, frozen, refrigerated, salted and untreated butter samples did not significantly (P<0.05) differ from each other. Mean total coliform count of untreated butter at initial preservation period was 5.62 log cfu/g of butter sample. The current result is by far beyond the mean total coliform count of 2 log cfu/g of butter samples collected from Wolayita zone (Mekdes, 2008). From two months of preservation period and onwards, relatively decreasing trends of total coliform count were observed in traditional ghee, frozen and salted butter compared to other treatments. This might be associated with the inhibitory effects of heat treatment, low storage temperature and addition of salt in ghee, butter kept at-20°C and in salted butter, respectively.

Enterobacteriaceae count

Mean Entrobacteriaceae count (log cfu/g) for the treatments and preservation time is presented in Table 7. There were no significant difference (P<0.05) between mean Entrobacteriaceae counts of traditional ghee, salted, spiced, melted, frozen and refrigerated butter except for untreated butter at 0, 1, 2 and 3 months of preservation, respectively. The mean Entrobacteriaceae counts of butter samples for traditional ghee, spiced, melted and frozen butter at 0 month of preservation did not significantly (P<0.05) differ from each other. While the mean Entrobacteriaceae counts of butter samples for salted, untreated and refrigerated butter at initial preservation time significantly (P<0.05) differed from other treatments but the mean counts for untreated and refrigerated butter did not significantly differ from each other. The mean Entrobacteriaceae counts of samples for traditional ghee, spiced, melted and frozen butter at the end of one month did not significantly (P<0.05) differ from each other. While, the mean Entrobacteriaceae counts of samples for salted, untreated and refrigerated butter at initial preservation time significantly (P<0.05) differed from other treatments. But the mean counts for untreated and refrigerated butter did not significantly differ from each other. The mean Entrobacteriaceae counts of butter samples at the end of second months of preservation for

Table 7. Mean Entrobacteriaceae counts (log cfu/g) of traditionally preserved butter.

Variable	Time (month)	Traditional ghee	Salted butter	Spiced butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter	EUS limit
Enterobacteriaceae	0	4.26±0.04^{aA}	6.70±0.07^{aB}	5.83±0.06^{aA}	6.27±0.03^{aA}	5.07±0.08^{cC}	5.07±0.33^{aA}	5.07±0.31^{aC}	3
	1	5.79±0.08^{aB}	6.84±0.03^{aA}	5.94±0.06^{aB}	6.42±0.03^{aB}	5.64±.04^{aC}	5.57±0.04^{aB}	5.67±0.05^{aC}	
	2	5.84±0.06^{aA}	6.52±0.03^{aB}	5.97±0.06^{aA}	6.57±0.07^{aB}	6.23±0.09^{aB}	5.69±0.04^{aB}	5.96±0.11^{aA}	
	3	6.12±0.17^{aA}	6.43±0.03^{aB}	6.23±0.10^{aA}	6.61±0.06^{aA}	6.71±0.06^{aA}	5.69±0.09^{aA}	6.36±0.08^{aA}	

Means followed by similar lowercase letters in a column are not significantly different from each other (P<0.05); Means followed by similar uppercase letters in a row are not significantly different from each other (P<0.05). EUS: European Standard.

traditional ghee, spiced, untreated and refrigerated butter did not significantly (P<0.05) differ from each other. While, the mean Entrobacteriaceae counts of butter samples for salted, melted and frozen butter at the end of second months of preservation significantly (P<0.05) differed from other treatments. However, at the end of third months of preservation, the mean Entrobacteriaceae counts of traditional ghee, spiced, untreated, melted, refrigerated and frozen samples did not significantly differ (P<0.05) except for salted butter. During the initial preservation time, relatively smaller mean Enterobacteriaceae count of 4.26 log cfu/g of butter was observed in traditional ghee compared to other treatments. This might be ascribed due to the heat treatment and moisture removal from butter during ghee making. A report by Mattick et al. (2001) stated that some thermo tolerant Enterobacteriaceae comprising a sub-group of mesophiles are capable of growth at up to 44°C, with an optimum growth temperature of 37°C. Fellows (2008) also reported that ghee is preserved by a combination of heat which destroys enzymes and contaminant microorganisms by removing moisture from the butter oil to prevent microorganisms growing during storage. Samaraweera et al. (2001) confirmed that lowering moisture content substantially reduces the growth rate of some Enterobacteriaceae. During the initial preservation time, relatively higher counts of Entero-bacteriaceae of 6.70 log cfu/g was observed in spiced butter than in other treatments. This might be attributable to poor hygienic status of spices purchased from open market. Throughout the preservation period, relatively smaller mean Enterobacteriaceae count was observed in frozen, refrigerated butter and traditional ghee compared to the other treatments. This could be explained in terms of the inhibitory effects of low storage temperatures in refrigerated and frozen butter and heat treatment in traditional ghee. Mattick et al. (2001) confirmed that cooling of food to normal refrigeration temperatures of 0 to 8°C inhibits Enterobacteriaceae growth in storage facilitates. Rhea (2009) also reported that deep freeze retards the growth of undesirable microorganisms and proper salting of butter removes moisture droplets and negatively affects the growth of undesirable microorganisms.

Organoleptic quality of butter preserved using traditional preservation techniques

Organoleptic quality of butter at the beginning of preservation

The hedonic rating scale at the end of one month of preservation for color, texture and odor is presented in Table 8. The hedonic rating scale of color, texture and odor of butter samples preserved using traditional ghee; salted, spiced, melted, untreated, frozen and refrigerated butter is presented in Figure 1. At the initial time of preservation, color, texture and odor of traditional ghee, spiced, salted, melted, untreated and butter stored in deep freeze and in refrigerator at 4°C were in acceptable range except for spiced butter which was relatively lower compared to others. This might be attributable to darkening of the color of spiced butter due to mixing with spices such as black cumin. At initial time of preservation, traditional ghee and salted butter were extremely liked compared to untreated, butter stored in refrigerator at 4°C and in deep freezer which were liked moderately. The color and odor of traditional ghee and salted butter were extremely liked. Except in spiced butter the color of butter in the other treatments were extremely liked.

Organoleptic quality of butter at the end of one month of preservation

The hedonic rating scale at the end of one month of preservation for color, texture and odor is presented in Table 8. At the end of one month of preservation, the color, texture and odor of butter samples for traditional ghee, spiced, salted,

Table 8. Mean scores for descriptive sensory attribute of butter treated under different preservation methods.

Sensory attribute	Duration (months)	Preservation technique						
		Traditional ghee	Spiced butter	Salted butter	Melted butter	Untreated butter	Frozen butter	Refrigerated butter
Odor	0	4.70±0.15^{a}	3.50±0.40^{b}	4.70±0.15^{a}	4.00±0.39^{ab}	4.20±0.25^{ab}	4.20±0.25^{ab}	4.20±0.25^{ab}
	1	4.50±0.22^{a}	3.10±0.23^{b}	4.80+0.13^{a}	3.70±0.34^{b}	3.10±0.23^{b}	4.50±0.23^{a}	3.70 ±0.34^{b}
	2	4.43±0.20^{a}	2.71±0.36^{b}	3.71±0.36^{a}	2.14±..34^{bc}	1.57±0.30^{c}	4.14±0.26^{a}	3.71 ±0.18^{a}
	3	4.85±0.14^{a}	1.71±0.42^{c}	3.43±0.30^{b}	1.71±0.36^{c}	1.43±0.30^{c}	3.86±0.40^{b}	3.57 ±0.20^{b}
Texture	0	4.30±0.26^{a}	3.10±0.23^{b}	4.40±0.22^{a}	4.50±0.17^{a}	4.30±0.15^{a}	4.30±0.15^{a}	4.30 ± 0.15 ^{a}
	1	4.50±0.17^{a}	3.50±0.40^{b}	4.70±0.21^{a}	3.50±0.40^{b}	4.50±0.17^{a}	4.50±0.17^{a}	3.50 ± 0.40 ^{b}
	2	4.43±0.20^{a}	3.00±0.38^{bc}	3.71±0.42^{ab}	2.71±0.29^{bc}	2.29±0.42^{c}	3.14±0.26^{b}	3.00±0.31^{b}
	3	4.71±0.18^{a}	2.57±0.53^{b}	3.43±0.37^{b}	2.43±0.43^{b}	2.29±0.42^{b}	2.86±0.40^{b}	2.86 ±0.34^{b}
Color	0	4.70±0.21^{a}	2.40±0.27^{c}	4.70±0.21^{a}	3.70±0.34^{b}	4.50±0.22^{a}	4.50±0.22^{a}	4.50 ±0.22^{a}
	1	4.70±0.15^{a}	2.40±0.27^{c}	4.50±0.22^{a}	3.50±0.40^{b}	3.50±0.40^{b}	4.70±0.21^{a}	4.70 ±0.21^{a}
	2	4.86±0.14^{a}	1.71±0.36^{c}	4.14±0.14^{ab}	4.14±0.14^{ab}	1.86±0.34^{c}	1.71±0.29^{c}	3.57 ±0.20^{b}
	3	4.86±0.14^{a}	1.29±0.18^{c}	3.71±0.47^{b}	1.57±0.30^{c}	1.29±0.18^{c}	3.71±0.52^{b}	3.43 ±0.30^{b}

Means followed by similar letters in a row are not significantly different from each other (P<0.05)

melted, untreated and butter stored in deep freeze and in refrigerator at 4°C were in acceptable range except for the color of spiced butter which ranged between dislike slightly to neither like nor dislike. At the end of one month of preservation, the sensory panelists extremely liked traditional ghee, salted, refrigerated and butter kept in deep freeze. While, spiced butter was neither liked nor disliked by the sensory panelists. Melted and untreated butter samples were moderately liked.

Organoleptic quality of butter at the end of two months of preservation

The hedonic rating scale result of color, texture and aroma of the treatments is presented in Table 8. At the end of two months of preservation, the sensory acceptability of spiced, melted and untreated butter highly deteriorated compared to others. Traditional ghee and salted butter were rated as extremely liked for their color, aroma and texture. This is in agreement with a report of Illingworth et al. (2009) that revealed that application of heat during preparation of ghee and removal of moisture and solid non-fat contribute to a product of unique color, flavor and texture.

Organoleptic quality of butter at the end of three months of preservation

The hedonic rating scale of the butter samples at the end of three months of preservation based on color, texture and odor is presented in Table 8. The color, texture and odor of traditional ghee and salted butter were extreme and moderate likeness, respectively. While frozen and refrigerated butter were moderately liked by the sensory panelists. Whereas, the odor and texture of spiced butter is rated as neither like nor disliked. The color and aroma of melted and untreated butter were slightly disliked except for their texture.

Overall acceptance of traditionally preserved butter at the end of three months

The hedonic rating scale on the overall acceptance of color, texture and odor of butter samples at the end of three months of preservation is presented in Figure 1. Among all the treatments traditional ghee was extremely liked followed by salted butter which was rated between moderate and extreme likeness. At the end of three months, refrigerated and frozen butter were moderately liked by the sensory panels. The relative reduction in likeness in the overall acceptability of refrigerated and frozen might be attributable to the change in odor as a result of the metabolic and enzymatic activities of psychrophilic bacteria that can multiply under low temperature.

CONCLUSION AND RECOMMENDATION

Major traditional butter preservation methods in the present study are traditional ghee making, spicing, melting and salting. Major spices used in traditional ghee making are *Trachyspermum ammi*, *Aframomum angusti-*

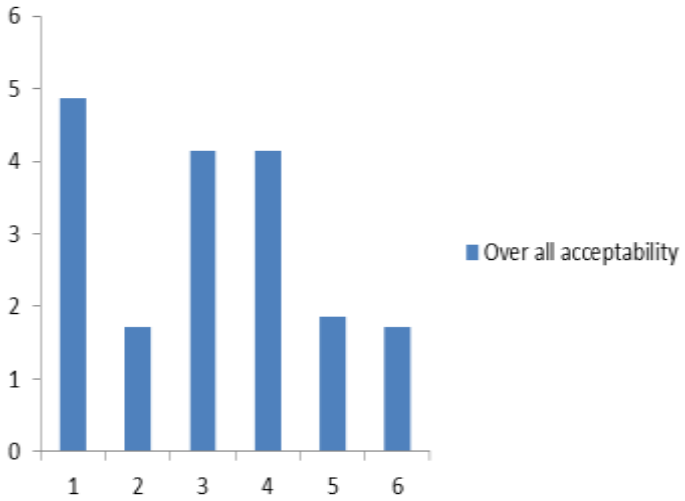

Figure 1. Overall acceptability of traditionally preserved butter at the end of three months of preservation.

folium, Nigella sativa, Trigonella foenum, Zingiber officinale, Allium sativum and *Curcuma domestica*. For spicing butter *rigonella foenum and Nigella sativa* are mainly used. Microbial quality of the butter samples were substandard starting from initial preservation time. Moreover microbial and organoleptic properties of the samples deteriorated as the storage time elapsed except for traditional ghee and salting. Hygiene of spices, herbs and salt used to preserve butter should be kept to reduce contamination. Appropriate application level of spices and plants materials used per unit weight of butter need to be optimized. Moreover, comprehensive evaluation including oxidative deterioration of traditionally preserved butter is vital.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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