Microbial and Organoleptic Evaluation of Traditional Butter Preservation Techniques

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Abstract

The climate in most parts of Ethiopia 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 of the country and cooling systems are not feasible due to lack of infrastructure. This study was, therefore conducted to assess traditional butter preservation methods in Ethiopia and compare their efficiency using microbial and organoleptic properties. Fresh butter samples were allocated to each preservation method, including traditional ghee, untreated, salted, spiced, melted, frozen (-20°C) and refrigerated butter (4°C). Microbial and organoleptic qualities of the samples were analyzed at one-month interval for three consecutive months. Microbial qualities of the preserved butter samples were substandard; but traditional ghee and salting were more efficient. Optimization of the utilization of spices as preservatives and comprehensive evaluation including oxidative deterioration appears to be very vital.

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, buttermilk and whey as a byproduct. Rural 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 hairdressing 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, saves milk from spoilage, and diversifies its uses (FAO, 1990).

The climate in Ethiopia 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, 1 987) . 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 this could make butter of good storage stability (O'Mahony and Peters, 1987). However, traditional butter preservation techniques and their efficiency in these areas are not studied. Therefore, the present study was initiated to evaluate traditional butter preservation techniques and compare their efficiency using microbial and organoleptic properties.

Materials and Methods

Sample collection and preparation

Seven kilograms of butter was bought from open markets and kept in an icebox and transported to Holetta dairy laboratory within 4 hrs after collection. The samples were thoroughly mixed to form composite sample, which was divided into seven equal parts each, with one kilogram of butter. The samples were then randomly allocated to seven treatments, namely traditional ghee, spiced, salted, melted, untreated, frozen (-20°C) and butter stored at 4°C. The samples were tested for their microbial and organoleptic qualities after 0, 1, 2 and 3 months of preservation.

Preparation of treatments

Traditional ghee/'Nitir Kibe': One-kilogram butter was placed in a saucepan and melted over a slow heating stove. White cumin, fenugreek, korerima, ginger, garlic, turmeric, black cumin and other herbs of desirable aroma, such as rue *(Ruta graucolence),* basil (*Ocimum spp* .) and 'Kussayyee'(*Ocimum hardiense*) were added to the boiling butter fat following the commonly used local method in Ethiopia. The melting butterfat and spices were stirred while boiling until foaming had stopped. Finally, the saucepan was removed from the stove and left aside until it was settled. The butterfat was filtered through metal sieve into high-density polyethylene bucket and kept at room temperature.

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. Spiced butter sample was placed in high-density polyethylene bucket and kept at room temperature.

Salted butter*:* One-kilogram butter was thoroughly mixed with 30 g of NaCl and kept in high-density polyethylene bucket and was placed at room temperature.

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 heating stove, kept in cool place to settle down and left there for overnight until it was completely solidified and impurities that were settled at the bottom of the saucepan were decanted off by opening the solidified butter in the saucepan from one side. Then, the sample was placed in high-density polyethylene bucket and kept at room temperature.

Untreated butter: One kilogram of fresh butter sample was kept in high-density polyethylene bucket and was placed at room temperature.

Frozen butter*:* One kilogram of butter sample was kept in high-density polyethylene bucket and placed in deep freeze at -20°C.

Refrigerated butter: One kilogram of composite butter sample was kept in high-density polyethylene bucket and placed in refrigerator at 4°C. Required amount of sample was taken from each treatment for evaluation of microbial load (aerobic mesophilic bacteria, total coliforms, total lactic acid bacteria, Enterobacteriaceae, yeast and mold counts), color, texture, odor and overall acceptability of the butter after 0, 1, 2 and 3 months of preservation. 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 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 hrs 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 by means of colony forming units of coliforms per milliliter. Plates were incubated at 32±1°C for 24±2 hrs. One milliliter of melted sample was serially diluted using peptone water and transferred into sterile Petri-dishes. Ten to fifteen 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 hrs. 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 $N=\Sigma c/(n1+n2)d$, where Σ c= Sum of all colonies on all plates counted 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 hrs, 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 x 90 ml Petri dishes and mixed with 20 ml of PDA containing an antibiotic; chloramphenicol solution. Then, after incubation at 25°C for 5 days, yeast and mould were counted for 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 hrs 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 parameters were evaluated by 10 semi-trained sensory panelists using 5 point hedonic rating scale, where 1 = dislike very much, 2 = dislike, 3 = neither like nor dislike, 4 = like and 5 = like very much. The sensory attributes used to evaluate butter samples were odor, texture, color and overall acceptability.

Data analysis

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

Results and Discussion

Microbial properties of preserved butter samples

Aerobic mesophilic bacterial count

The mean total bacterial count (log cfu/g) for the treatments and preservation time is presented in Table 1. The aerobic mesophilic bacterial counts of butter preserved using traditional ghee, salt and spices for 0, 1, 2 and 3 months of preservation did not show significant differences ($P > 0.05$). While aerobic mesophilic bacterial counts at 0, 1, 2 and 3 months of preservation showed significant ($P<0.05$) differences for melted, untreated, frozen and butter stored in refrigerator at 4°C. Nevertheless, mean values of total aerobic mesophilic bacterial counts for traditional ghee, salting, spicing, melted, untreated, frozen and refrigerated butter (4^0C) showed significant difference ($P<0.05$). At initial time of preservation, greater counts of aerobic mesophilic bacterial were observed for salted (9.58 log cfu/g) and melted butter (9.65 log cfu/g), which were significantly different ($P<0.05$) from the other treatments. This might be due to the poor hygiene of the salt used in preserving the butter. On the other hand, the increase in the aerobic mesophilic bacterial count in melted butter might probably be attributed to post melting contamination. At initial preservation period, the mean aerobic mesophilic bacterial count in untreated butter wos 8.71 log cfu/g of butter sample. The current result was far beyond the maximum tolerable limit of 6 log cfu/g of aerobic mesophilic bacteria count set by Standards Authority of Ethiopia (QSAE, 2009), but it was similar to a report from Wolayita area in Southern Ethiopia, indicating total bacterial count of 8.10 log cfu/g of butter sample (Mekdes, 2008).

Aerobic mesophilic bacterial count of 9.37 log cfu/g was observed after one month of preservation and this was significantly different ($P<0.05$) from the other treatments. This might be due to the antagonistic effects of active ingredients of spices, heat treatments and low temperatures that inhibited bacterial growths. Similar study 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 kill the microorganisms or control 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 the treatments with duration are presented in Table 2. There were significant differences ($P < 0.05$) between traditional ghee, salted, spiced and melted butter at 0, 1, 2 and 3 months of preservation for total lactic acid bacterial counts. While the total lactic acid bacterial counts of butter preserved using untreated, frozen (-20°C) and refrigerated (4°C) butter showed no significant difference among the means of 0, 1, 2 and 3 months of preservation. Total lactic acid bacterial counts at 0 and 1 month of preservation showed no significant 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 ($P<0.05$) difference among traditional ghee, salted, spiced, melted and

refrigerated butter (4°C) , except for untreated and frozen butter (-20°C). Similarly, at the end of the third month of preservation, the mean total lactic acid bacterial counts for traditional ghee, salted, spiced, melted and refrigerated butter samples did not show significant difference, except for untreated and frozen butter, which did not significantly ($P > 0.05$) differ from each other.

The mean total lactic acid bacterial counts for 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 samples as local butter is usually made of spontaneously fermented whole milk. At initial time of preservation, the mean lactic acid bacterial count for traditional ghee was 6.06-log cfu/g and significantly differed from other treatments; except for salted butter, which was 6.09-log cfu/g of butter sample. These results were 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 area in Southern region. Mean lactic acid bacterial counts for spiced, melted butter and traditional ghee were relatively lower and significantly differed ($P<0.05$) from other treatments for the second to third months of preservation time. This might be due to heat treatment and antimicrobial effects of spices used in melted butter, traditional ghee and spiced butter, respectively.

Table 1. Total aerobic mesophilic bacterial counts (log cfu/g) for butter samples preserved in different methods.

Preservation method	Preservation duration in months			
	0	1	2	3
Traditional ghee	9.48±0.08^{aB}	9.62±0.03^{aA}	9.64±0.04^{aA}	9.73±0.06^{aA}
Salted butter	9.39±0.09^{aB}	9.37±0.06^{aB}	9.66±0.06^{aA}	9.78±0.03^{aA}
Spiced butter	9.58±0.05^{aB}	9.62±0.04^{aB}	9.84±0.04^{aA}	9.89±0.06^{aA}
Melted butter	9.65±.02^{aB}	9.72±0.05^{aB}	9.23±0.06^{bC}	9.93±0.03^{aA}
Untreated butter	8.71 ±.03^{bC}	9.61±0.03^{ab}	9.68±0.04^{aB}	9.98±0.04^{aA}
Frozen butter	8.71 ±.02^{bC}	9.59±0.09^{aB}	9.61 ±.04^{aB}	9.24±.04^{bC}
Refrigerated butter	8.71 ±0.02^{bC}	9.59±0.09^{aB}	9.63±0.05^{aB}	9.97±0.03^{aA}

Means followed by similar lower case letters in a raw are not significantly different ($P > 0.05$) Means followed by similar upper case letters in a column are not significantly different ($P<0.05$).

Table 2. Total lactic acid bacteria counts (log cfu/g) of preserved butter samples

Preservation method	Preservation time in months			
	0	1	2	3
Traditional ghee	6.65±0.09^{aA}	6.06±0.06^{bC}	5.56±0.06^{cC}	5.09±0.11^{cD}
Salted butter	6.55±0.05^{aA}	6.31 ±0.06^{aBC}	5.1 9±0.09^{cC}	5.01±0.17^{cD}
Spiced butter	6.72±0.06^{aA}	6.09±0.13^{bD}	5.72±0.05^{cC}	5.53±0.04^{bD}
Melted butter	6.80±0.28^{aA}	6.40±0.05^{bC}	5.26±0.07^{cC}	5.01±0.14^{cD}
Untreated butter	6.77±0.03^{aA}	6.65±0.09^{aB}	6.60±0.07^{aB}	6.32±0.07^{aC}
Frozen butter	6.77±0.08^{aA}	6.23±0.04^{aB}	6.28±0.08^{aB}	6.04±0.10^{aC}
Refrigerated butter	6.77±0.07^{aA}	6.59±0.04^{aB}	6.12±0.1 0^{aC}	6.06±0.10^{aD}

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

Yeast and mold counts

The result of mean yeast and mold counts (log cfu/g) for treatments and preservation time is presented in Table 3. Mean yeast and mold counts at 0, 1, 2 and 3 months of preservation did not significantly ($P>0.05$) differ for traditional ghee, spiced and butter refrigerated at 4°C, the values for salted, spiced, melted, untreated and frozen butter samples were significantly different ($P<0.05$). However, the overall mean yeast and mold counts for traditional ghee, salted, spiced, melted, untreated, frozen and refrigerated butter samples did not show significant differences. 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 the values recorded for 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. Besides, suboptimal temperature in frozen butter might have hindered the growth of yeasts and molds, as the temperature range for yeast and mold growth is said to be 0 to 47°C, out of which, the growth of both organisms can be hampered.

The results of the present study were also in agreement with the findings of Seriler (2003) who revealed the possibility of reducing mold growth on the surface of butter by salting. The mean yeast and mold count for untreated butter sample at initial time of preservation was 6.70-log cfu/g, which is beyond the maximum tolerable limit of 1 log cfu/g of yeast and mold count for butter samples recommended by the Ethiopian Standards Authority (QSAE, 2009). The present result was also higher than the mean yeast and mould count of 5.58 log cfu/g reported for butter samples from Wolayita area in Southern Ethiopia (Mekdes, 2008). Increasing trends of yeast and mold counts were observed with prolonged period of preservation for untreated, refrigerated (at 4°C), spiced and melted butter samples. In the case of spiced butter, poor hygiene of spices purchased from open market might have contributed to the high rate of contamination, while water activity might have been high and have created favorable environment for the growth of yeast and molds in untreated and refrigerated butter samples.

Table 3. Mean yeast and mold counts (log cfu/g) of butter samples preserved in different methods.

Preservation method	Preservation duration in month			
	0	1	2	3
Traditional ghee	5.70±0.08^{aB}	5.70±0.04^{aB}	6.1 6±0.08^{aA}	6.1 9±0.08^{aA}
Salted butter	6.54±0.03^{bC}	6.67±.03^{aB}	6.69±.04^{aB}	6.78±0.05^{aA}
Spiced butter	5.74±0.05^{aC}	6.36±0.05^{aA}	6.28±0.08^{aB}	6.37±0.06^{aA}
Melted butter	5.56±0.03^{dD}	5.78±0.09^{cC}	6.28±.07^{bB}	6.71±.07^{aA}
Untreated butter	6.70±.05^{bD}	6.74±0.03^{cC}	6.78±.03^{aB}	6.84±.05^{aA}
Frozen butter	6.70±0.04^{aA}	6.46±0.05^{bB}	6.14±0.10^{bC}	6.42±0.1 4^{bC}
Refrigerated butter	6.70±0.05^{aB}	6.72±0.06^{aB}	6.76±0.05^{aA}	6.79±0.04^{aA}

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

Total coliform counts

Mean total coliforms count (log cfu/g) for the treatments and time of preservation is presented in Table 4. There were no significant differences (P>0.05) between butter samples preserved using traditional ghee, salted, frozen and refrigerated for 0, 1, 2 and 3 months of preservation, while spiced, melted and untreated butter showed significant (P<0.05) differences at 0, 1, 2 and 3 months of preservation. Mean total coliform counts at initial time of preservation for traditional ghee, spiced, melted, untreated, frozen and refrigerated butter samples did not differ significantly, except for salted butter. Similarly, 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, except for salted and untreated butter, which did not significantly differ from each other.

At the end of second month of preservation, mean total coliforms counts for traditional ghee, spiced, melted, frozen and refrigerated butter samples did not significantly differ from each other, except for salted and untreated butter. On the other hand, there was no significant difference between the treatments (traditional ghee, spiced, melted, frozen, refrigerated, salted and untreated butter samples) at the end of the third month of preservation. Mean total coliform count of untreated butter at initial preservation time was 5.62-log cfu/g of butter sample. This value was far beyond the mean total coliform count (2-log cfu/g of butter sample) for samples collected from Wolayita zone (Mekdes, 2008). After two months of preservation period and onwards, mean total coliform count showed relatively decreasing trends for traditional ghee, frozen and salted butter compared to the other treatments. This might be associated with the inhibitory effects of heat treatment, low storage temperature (-20°C) and salt, respectively.

Table 4. Total coliform counts (log cfu/g) of butter samples preserved in different methods.

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

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

Enterobacteriaceae count

Mean Entrobacteriaceae count (log cfu/g) for the treatments and preservation time is presented in Table 5. There were no significant differences (P<0.05) between traditional ghee, salted, spiced, melted, frozen and refrigerated butter, except for untreated butter, at 0, 1, 2 and 3 months of preservation. Mean Entrobacteriaceae counts did not significantly (P<0.05) differ for samples of traditional ghee, spiced, melted and frozen

butter at 0 month of preservation, while the values for salted, untreated and refrigerated butter samples showed significant difference ($P<0.05$). A similar trend was also observed at the end of one month. However, the mean counts for untreated and refrigerated butter did not significantly differ from each other. On thr other hand, the mean Entrobacteriaceae counts at the end of second months of preservation did not significantly ($P>0.05$) differ for traditional ghee, spiced, untreated and refrigerated butter, while the values showed significant difference for salted, melted and frozen butter samples. However, at the end of third month of preservation, the mean Entrobacteriaceae counts did not significantly differ for traditional ghee, spiced, untreated, melted, refrigerated and frozen samples, except for salted butter.

At the initial preservation time, relatively smaller mean Enterobacteriaceae count (4.26-log cfu/g of butter) was observed for traditional ghee compared to the other treatments. This might be attributed to the heat treatment and, thus, moisture removal from butter during ghee making. In line with this, a report by Mattick *et al*. (2001) has shown 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) have also reported that ghee is preserved by a combination of heat, which destroys enzymes and contaminant microorganisms by removing moisture from the butter oil during storage. Similarly, Samaraweera *et al*. (2001) have confirmed that lowering moisture content substantially reduces the growth rate of some Enterobacteriaceae. On the other hand, relatively higher counts of Enterobacteriaceae (6.70 log cfu/g) were observed for spiced butter than for the other treatments during the initial preservation perio. This might be attributable to poor hygienic status of the spices purchased from local open markets.

In general, relatively smaller mean Enterobacteriaceae count was observed for frozen, refrigerated butter and traditional ghee compared to the other treatments throughout the preservation period. 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 making. In agreement with the results of the present study, Mattick *et al*. (2001) have reported that cooling of food to normal refrigeration temperatures of 0 to 8°C inhibits Enterobacteriaceae growth in storage facilitates. Rhea (2009) has also reported that deep freezing retards the growth of undesirable microorganisms and proper salting of butter also removes moisture droplets and negatively affects their growth.

Table 5. Entrobacteriaceae counts (log cfu/g) of butter samples preserved in different methods.

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

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

Organoleptic

texture of spiced butter were rated neither as like nor disliked, while the color and aroma of melted and untreated butter were slightly disliked, except for their texture.

End of three months of preservation

The hedonic rating scale values given to butter samples at the end of three months of preservation are presented in Table 6. Accordingly, color, texture and odor values for traditional ghee and salted butter showed extreme and moderate likeness, respectively, while frozen and refrigerated butter were moderately liked by the panelists. The odor and texture of spiced butter were neither rated as like nor disliked, while the color and aroma of melted and untreated butter were slightly disliked, except for their texture.

Overall acceptance of preserved butter at the end of three months

The hedonic rating scale values for overall acceptance of butter samples at the end of three months of preservation showed that, among the treatments, traditional ghee was extremely liked, followed by salted butter, which was rated between moderate and extreme likeness. On the other hand, refrigerated and frozen butter were moderately liked by the sensory panelists. The relative reduction in likeness in the overall acceptability of refrigerated and frozen butter might be attributable to the change in odor because of metabolic and enzymatic activities of psychrophilic bacteria that can multiply under low temperature.

Table 6. 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
	0	4.70±0.15a	3.50±0.40^{b}	4.70±0.1 5a	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}
Odor	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±0.14a	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}
	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
Texture	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}	3.1 4±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}
	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}
Color	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)

Conclusions and Recommendation

In the present study, it was observed that microbial quality of butter samples was substandard starting from initial preservation time. Moreover, microbial and organoleptic properties of the samples deteriorated with prolonged storage time, except for traditional ghee and salted butter. Hygiene of spices, herbs and salt used to preserve butter should be maintained to reduce contamination. Appropriate amount of spices and plant materials used to preserve butter need to be optimized. Moreover, though traditional ghee followed by salted butter was more liked, comprehensive evaluation, including oxidative deterioration of traditionally preserved butter appears to be very vital to come up with more conclusions that are comprehensive.

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