Asian Australas. J. Biosci. Biotechnol. 2016, 1 (3), 504-513

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Article

Isolation and identification of bacteria with determination of bacterial loads from different brands of butter and cheese

Marshia Parvin, Mir Rowshan Akter*, Md. Mostafizer Rahman and Md. Fakhruzzaman

Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

*Corresponding Author: Dr. Mir Rowshan Akter, Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh. E-mail: akter.rowshan@gmail.com

Received: 03 November 2016/Accepted: 11 December 2016/ Published: 28 December 2016

Abstract: The present study was conducted to isolate and identify the organisms in butter and cheese manufactured by different plants namely Milk Vita and Quality sold in retail stores at Dinajpur, Bangladesh were collected and transported aseptically to the laboratory for bacteriological analyses. The samples were analyzed to determine the hygienic status of butter and cheeses and also for the total viable count (TVC), presence of gram-positive and gram-negative bacteria. TVC was performed according to the American Public Health Association, using plate count agar medium for TVC and Eosine methylene blue (EMB) agar for total *E. coli* count and Staphylococcus agar no. 110 for total Staphylococcus count. The average TVC for Milk Vita butter 3.28 x 10^5 CFU/gm (log 5.5) was lower than local butter 5.45 x 10^6 CFU/gm (log 6.7) and average TVC for Quality cheese 4.5 x 10^5 CFU/gm (log 5.7) was lower than local cheese 5.14 x 10^6CFU/gm (log 6.7). The bacterial loads of butter and cheese samples were within the acceptable limit of public health safety, since the count was well below the acceptable limit. The highest extent of bacterial contamination and proliferation of viable bacteria occurred in local butter and local cheese. The numbers of *E. coli* in local butter and cheese were little bit higher indicating poor hygienic practices during manufacture, post process contamination and unsatisfactory transportation. Statistically the *E. coli* were more closely related to TVC than the staphylococcal counts in the samples of Milk Vita butter 2.68 x 10^3 CFU/gm (log 3.4), Local butter 3.15x 10^4 CFU/gm (log 4.5), Quality cheese 4.46 x 10^3 CFU/gm (log 3.6) and Local cheese 1.19 x 10^4 CFU/gm (log 4.0) respectively. The results demonstrated that Milk Vita butter and Quality cheese are of superior quality product in respect of sanitary condition.

Keywords: bacteria; butter; cheese

1. Introduction

Butter, cheese and other dairy products are important source of food borne pathogens and numerous epidemiological reports have implicated inadequate heat treated milk by products as the major factors responsible for illnesses caused by food borne pathogens. Cross contamination with pathogenic microorganisms can gain access to butter and cheese during packaging. Cheese manufacture and ripening are affected by the metabolic activity of different types of microorganisms. When milk of optimal hygienic quality is used, the dairy microbial consortia can be simple when starter cultures are employed, or a higher degree of complexity can occur in the case of natural fermentations. The environmental microbiota from the processing plant has been often addressed as a source of microbes that may play a role in the cheese making (Bokulich and Mills, 2013; Didienne *et al.*, 2012; Irlinger and Mounier, 2009; Ksontini *et al.*, 2013).

In cheese, wide range of microorganism is frequently reported. Their majority is destroyed during pasteurization but some like *Pseudomonas fluorescens* can produce exogenous proteolytic and lipolytic enzymes which are

thermostable and capable to alternate the pasteurized milk. Pathogens that have been involved in food borne outbreaks associated with the consumption of milk by products include *Listeria monocytogenes, Salmonella* spp., *E. coli and Staphylococcus aureus.* The presence of these pathogenic bacteria in butter and cheese emerged major public health concerns (Tassew, 2007). Although cows' milk is the most widely produced and processed milk, milk from goats, sheep and camels is also consumed mainly in the pastoral lowland areas of the country. The major dairy products produced and consumed in different parts of the country include fresh whole milk, sour milk, butter, Arera (defatted sour milk) and a traditional cheese (O'Mahony, 1988). Cheese of West Gojam Zone of the Amhara National Regional State is an example of traditional dairy products which has recently been reported by Tassew (2007). About 45-50 per cent of total milk produced is converted into variety of traditional milk products by heat and acid coagulation, heat desiccation and fermentation. Indian traditional products market is estimated to be more than Rs. 6,500 crores (Dolci, 2007).

As the traditional dairy product preparation is labor intensive and the quality of finished products is highly variable in terms of physical, chemical, microbiological and sensoric properties, there is an urgent need to produce uniform and high quality products through modernization (Dolci, 2007). Food preservation by lactic acid bacteria (LAB) can be attributed to the reduction of pH that extends the shelf life of fermented food through the destruction of putrefactive, pathogenic and toxigenic bacteria thereby improving the microbial quality of food. In addition, LAB improves the sensory attributes and commercial value of products like cheese (Everard, 2007*).* Two groups of LAB occur in bacterial ripened cheeses. These are the starter culture LAB and the non-starter LAB. The latter group is composed of species of Lactobacilli, Pediococci and Micrococci that work in synergy to affect the typical cheese flavor in hard and semi-hard cheeses (Frank 2004). Butter is one of the most highly concentrated forms of fluid milk. Commercial butter is 80–82 percent milk fat, 16–17 percent water, and 1–2 percent milk solids other than fat (sometimes referred to as curd). It may contain salt, added directly to the butter in concentrations of 1 to 2 percent. Unsalted butter is often referred to as "sweet" butter. This should not be confused with "sweet cream" butter, which may or may not be salted. Reduced-fat, or "light," butter usually contains about 40 percent milk fat. Butter also contains protein, calcium and phosphorous (about 1.2%) and fat-soluble Vitamins A, D and E (Pinho *et al.*, 2004). Although there are over 120 different compounds that contribute to butter's unique flavor, the five primary factors responsible for butter's flavor including fatty acids, lactones, methyl ketones, diacetyl and dimethyl sulfide.

Although the butter is not a highly perishable food, it does undergo spoilage by bacteria and molds. The main source of microorganisms of butter is cream, whether sweet or sour, raw or pasteurized (Jay, 1996). Yeast and molds are important spoilage microorganisms of butter and can result in surface discoloration and off-flavor. Psychrotrophic Gram negative bacteria may develop and result proteolytic and lipolytic changes (ICMSF, 2005).

To develop public health nutrition policies based on scientific evidence, it is important to fight against misinformation of the general population about nutritional aspects of their daily food intake. Frequently, the benefits and risks are put down to dairy products on the basis of physiopathological review papers covering dairy products in both health promotion and disease prevention. Focus is placed on the critical analysis of the scientific literature, pointing out future needs in the research for evidence between dairy products and health. Therefore, the objectives of the present study were to isolate and identify the bacteria with determination of loads of bacteria from different brands of butter and cheese samples.

2. Materials and Methods

2.1. Laboratory and duration of the experiment

The present research work was performed in the Bacteriology Laboratory of the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh and the duration of the experiment was 6 months (July to December/2013).

2.2. Collection of sample

A total of 12 commercially produced butter and cheese samples belonging to 3 different brands were collected aseptically from different retail stores located at Dinajpur town with necessary precautions and the samples were transported in an ice box to the laboratory for subsequent bacteriological tests and other laboratory analyses.

2.3. Enumeration of total viable count (TVC)

For the determination of total bacterial count, 1 ml of each ten-fold dilution was transferred and spread on duplicate plate count agar using a fresh pipette for each dilution. The diluted samples were spread as quickly as

possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 30℃ for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organism or colony forming units per gram (CFU/gm) of butter and cheese sample.

2.4. Enumeration of total *E. coli* count (TEC)

For the determination of total *E. coli* count 1ml of each tenfold dilution was transferred to EMB agar. For each dilution five test plates containing EMB agar were used. All the agar plates were incubated at 30℃ temperature for 48 hours. The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organism or colony forming units per gram (CFU/gm) of butter and cheese sample.

2.5. Enumeration of total *Staphylococcal* count (TSC)

For the determination of total Staphylococcal count 1 ml of each tenfold dilution was transferred to staphylococcus agar no.110 agar. For each dilution five test plate containing Staphylococcus agar no.110 agar were used. All the agar plates were incubated at 37℃ temperature for 48 hours. The total staphylococcal count was calculated according to ISO (1995). The results of the total Staphylococcal count were expressed as the number of organism or colony forming units per gram (CFU/gm) of ice cream sample. A total of 3 samples of each brand were bacteriologically examined. The results were recorded and the organoleptic and microbiological quality was determined.

2.6. Culture in to bacteriological media

Each sample of butter and cheese earlier put into transport media was divided and inoculated separately in nutrient agar (NA) and plate count agar (PCA) to promote growth of bacteria. Each group of these media was incubated at 37^0C for overnight. The colonies on primary cultures were repeatedly sub-cultured by streak plate method (Cheesbrough, 1984) until the pure culture with homogenous colonies were obtained.

2.7. Morphological characterization of organisms by Gram's staining method

A loop full of sterile distilled water was placed in the center of a sterile, clean, dry, grease free glass slide. A Small colony was picked up with a bacteriological loop and was mixed with distilled water on the slide. The colony was made to thin smear on a slide. The smears were fixed by air drying. Then 0.5% crystal violet solution was applied on the smear for 1 minute. Gram's iodine solution was then added to act as mordant for 1 minute. Acetone alcohol was then added to decolorize for 1-2 seconds. Then the slide was washed with water. Safranine solution (2 %) was added as counter stain and allowed to stand for 1 minute. The slide was then washed with water. Then the slide was blotted with blot paper and was allowed to air dry. The slide was examined under Light Microscope with high power objective (100X) using immersion oil.

2.8. Isolation of bacteria in pure culture

For isolation of bacteria in pure culture, the mixed culture was inoculated into NA media by streak plate technique to obtain well isolated colonies. At first, an inoculum was picked up with a sterile inoculating loop and spread on an area of the medium in the petridish. The loop was sterilized by being heated as red hot in a flame. The inoculum was spread over the reminder of the plate by drawing the cooled parallel line. This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

2.9. Identification of *Escherichia coli*

2.9.1. Culture into different bacteriological media

Sterilized platinum loop was used for streaking the lactose broth culture on MacConkey and EMB agar to get isolates in pure culture. All inoculated media were kept at 37°C for overnight in an incubator. Materials from lactose fermentation tubes were inoculated into MacConkey agar plates and were incubated. Materials from lactose fermentation tubes were also inoculated into EMB agar plates which after incubation, showed metallic sheen if positive for *E. coli.*

2.9.2. Identification of *E. coli* by Gram's staining

Gram's staining was performed to determine the size, shape, and arrangement of bacteria. Gram staining reaction was performed according to the methods described by Merchant and Packer (1967).

2.9.3. Biochemical characterization *E. coli*

For Indole reaction, 2 ml of peptone water was inoculated with 5 ml of broth culture and incubated at 37°C for 48 hours. Then 0.5 ml of Kovac's reagent was added to the culture and mixed thoroughly. The tube was then allowed to stand for a while. For Triple sugar iron (TSI) agar slant, the test organisms were cultured into TSI agar slant by stab streak method. For Motility, Indole, Urease (MIU) test suspected colony was inoculated into the tube containing MIU medium. Then the medium was incubated at 37°C for overnight. The Methyl Red (MR) test was conducted by inoculating a pure colony of the test organism in 5 ml sterile glucose phosphate peptone broth. After 48 hours incubation at 37°C, 5 drops of methyl red indicator was added. A red coloration is positive and indicates an acid pH of 4.5 or less resulting from the fermentation of glucose (Cheesbrough, 1984). For Voges-Proskauer (VP) test 5 ml of sterile glucose phosphate peptone water were inoculated with a pure culture of test organisms, incubated at 37°C aerobically for 48 hours. Then 3 ml of 5 % α-naphthol were added and mixed well. 1 ml of 40% potassium hydroxide was added and mixed well to aerate. The bottle cap was removed and left for an hour at room temperature.

2.10. Identification of *Staphylococcus* spp.

2.10.1. Culture into different bacteriological media

A loop full of an aliquot was taken from the nutrient broth (NB) culture and streaked on to NA media to get pure culture. All inoculated media were incubated at 37^{o}C overnight. Materials from nutrient broth tubes were inoculated into nutrient agar plates. Materials from nutrient broth tubes were inoculated into Staphylococcus agar no. 110 containing plates.

2.10.2. Identification of *Staphylococcus* spp. by Gram's staining

Gram's staining was performed to determine the size, shape, and arrangement of bacteria. Gram's staining reaction was performed according to the methods described by Merchant and Packer (1967). Stained slides were examined under light microscope at 100X magnification with immersion oil.

2.10.3. Biochemical characterization of *Staphylococcus* spp.

The organisms were cultured into TSI agar slant by stab streak method and incubated at 37^{0} C. For Indole reaction, 2 ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. Kovac's reagent (0.5 ml) was added and mixed thoroughly. The tube was then allowed to stand for a while. MR test was conducted by inoculating a pure colony of the test organism in 5 ml sterile glucose phosphate peptone broth. After 48 hours incubation at 37°C, 5 drops of methyl red reagent was added, mixed and examined. For VP test 5 ml of sterile glucose phosphate peptone water were inoculated with a pure culture of test organisms, incubated at 37°C aerobically for 48 hours. Then 3 ml of 5 % α-naphthol were added and mixed well. 1 ml of 40% potassium hydroxide was added and mixed well to aerate. The bottle cap was removed and left for an hour at room temperature.

3. Results

3.1. Quality and total viable counts of bacteria

The sensory and microbiological attributes were analyzed and studied comparatively. Sensory characteristics were judged by a panel of experts and judgements for consumer's acceptance were scored. A presentation of the data showing the scores of characteristics of different butter and cheese samples are shown in Table 1. Sensory characteristics of hedonic scales obtaining scores 19-20 indicate excellent quality and 16-18 indicate good quality, 12-15 means fair; 9-11 scores recommend marginal acceptable; 7-8 considered unacceptable; 0–6 stands for bad . Five organoleptic quality characteristics were taken for the panel score and judgement for consumer's acceptance. For each of the characteristic, the maximum score given was 20 points. The grand total scores for the five characteristic parameters together become 100 points. Out of total score points 100, three different brand of butter and cheese samples were found to have obtained for Milk Vita butter 64 (64%) followed by Local butter 57 (57%) and Quality cheese 68 (68%), Local cheese 58 (58%) scores.

The result presented in Table 2 showed the total viable bacterial load of nine different butter and cheese samples of different brands. The bacterial loads were not uniform and varied quite considerably. The average counts/gm

of Milk Vita butter, Local butter , Quality cheese and Local cheese were 3.28 x 10^5CFU/gm (log 5.5), 5.45 x 10^6 CFU/gm (log 6.7), 4.5 x 10^5 CFU/gm (log 5.7) and 5.14 x 10^6CFU/gm (log 6.7) respectively .The maximum and minimum range of total bacterial load per gm of butter and cheese samples belonging to Milk Vita butter, Local butter ,Quality cheese, and Local cheese varied from log 5.6 to log 5.4, log 6.8 to log 6.6, log 5.9 to log 5.6, log 6.9 to log 6.5 respectively (Table 3). All brands of butter and cheese samples were within acceptable limit of public health safety because the samples did not exceed the total viable count 1.8x10^9 CFU /gm and 1.3x10^9- 7.8x10^{10} CFU /gm permitted under regulation.
The grading of samples was calculated according to the ANOVA table.

3.2. Identification of *E. coli* by different bacteriological methods

3.2.1. Cultural characterization

The isolated *E. coli* produced bright pink colored colonies on MacConkey (MC) agar. EMB agar plates streaked separately with the organism revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically. The growth was indicated by smooth, circular, black color colonies with metallic sheen on the agar plate.

3.2.2. Morphological characterization of by Gram's staining

The microscopic examination of Gram's stained smears from MC and EMB agar revealed Gram-negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain.

3.2.3. Results of biochemical test

TSI agar slant test revealed yellow slant, yellow butt, presence of gas bubbles and absence of black precipitate. MIU test demonstrated that the isolated *E. coli* produced turbidity and urease production with indole positive. In other biochemical tests it was demonstrated that all the isolates were indole test positive, MR test positive and VP test negative. Results of these tests are presented below Table 9.

3.3. Identification of *Staphylococcus* spp. by different bacteriological methods

3.3.1. Cultural and morphological characterization

The isolated organisms were observed as golden yellowish colonies on Staphylococcus agar no. 110. The organisms were observed as Gram-positive spherical shaped arranged in grape like clusters.

3.3.2. Biochemical characterization

In TSI agar slant test, yellow slant, yellow butt, absence of gas bubbles and absence of black precipitate. In Other biochemical tests, all the isolates were found to be MR test positive, VP test negative, MIU and indole test negative.

Table 1. Sensory characteristics of butter and cheese samples and evaluation were done by a group containing 4 people. Texture was evaluated by looking to the hardness and smooth structure of samples.

Parameters (Sensory Characteristic)		Sources of samples			
		Milk Vita butter	Local butter	Quality cheese	Local cheese
Appearance	20 Marks	12	13	12	14
Color	20 Marks	11	12	15	12
Aroma	20 Marks	10	11	10	11
Texture	20 Marks	11	10	11	10
Salt Content	20 Marks	20	12	20	11
Grand Total Score		64	57	68	58
LSD		6.697	5.463	5.773	5.105
CV %		28.23	24.87	22.54	23.37

Legends: Maximum grade is 20. Data are the average values of 4 people.
LSD = Least significant difference
CV = Coefficient of variation

Table 2. Density of average total viable bacteria and other selected microbial groups per gram of butter and cheese samples of different brands.

Sources of samples	No. of representative samples analyzed	Total viable count/gm		*E. coli* count/gm		*Staphylococcal* count/gm	
		CFU	Log	CFU	Log	CFU	Log
Milk Vita butter	2	3.28×10^5	5.5	8.26×10^3	3.9	2.68×10^3	3.4
Local butter	2	5.45×10^6	6.7	6.25×10^5	5.7	3.15×10^4	4.5
Quality cheese	2	4.5×10^5	5.7	5.65×10^4	4.7	4.46×10^3	3.6
Local cheese	2	5.14×10^6	6.7	5.5×10^5	5.7	1.19×10^4	4.0
LSD	-	11000. 0.9134		7868.	1.225	817.7	1.102
CV %	-	1.43	7.44	1.27	12.26	3.24	14.23

LSD = Least significant difference
CV = Coefficient of variation
CFU = Colony forming unit

Table 3. Range of total viable bacterial concentration in commercial butter and cheese samples.

Brand specification	No. of representative samples analyzed	Range of TVC/gm		Average TVC/gm (mean value)	Standard Deviation
		Maximum	Minimum		
Milk Vita butter	2	5.6 b	5.4 b	5.5 b	0.1
Local butter	2	6.8 a	6.6 a	6.7 a	0.1
Quality cheese	2	5.9 b	5.6 b	5.8 b	0.2
Local cheese	2	6.9 a	6.5 a	6.7 a	0.2
LSD	-	0.4143	0.7555	0.5015	-
CV %	-	3.27	6.27	4.08	-

All counts are expressed in logarithms
* a to d rank order (highest - lowest)
TVC = Total viable count
LSD = Least significant difference
CV = Coefficient of variation

Table 4. Range of *E. coli* counts in commercial butter and cheese samples.

Brand specification	No. of representative samples analyzed	Range of *E. coli* count/gm		Average *E. coli* count/gm (mean value)	Standard Deviation
		Maximum	Minimum		
Milk Vita butter	2	3.9 c	3.7 c	3.8 c	0.1
Local butter	2	5.9 a	5.5 a	5.7 a	0.2
Quality cheese	2	4.8 b	4.6 b	4.7 b	0.1
Local cheese	2	5.9 a	5.6 a	5.8 a	0.2
LSD	-	0.3895	0.5615	0.5721	-
CV %	-	3.78	5.80	5.74	-

All counts are expressed are logarithms
* a to d rank order (highest - lowest)
LSD = Least significant difference
CV = Coefficient of variation

Table 5. Range of Staphylococcal counts in commercial butter and cheese samples.

Brand specification	No. of representative samples analyzed	Range of Staphylococcal count/gm		Average Staphylococcal count/gm (mean value)	Standard deviation
		Maximum	Minimum		
Milk Vita butter	2	3.6 d	3.3 b	3.5 b	0.2
Local butter	2	4.8 a	4.4 a	4.6 a	0.2
Quality cheese	2	3.7 bc	3.4 b	3.6 b	0.2
Local cheese	2	4.1 b	3.8 b	4.0 ab	0.2
LSD	-	0.4643	0.5508	0.7231	-
CV %	-	5.75	7.39	9.22	-

LSD = Least significant difference
CV = Coefficient of variation
All counts are expressed are logarithms.
* a to d rank order (highest - lowest)

Table 6. Statistical analysis of butter and cheese brand samples in relation to total viable *E. coli* and Staphylococcal counts.

Types of sample	Total viable count	*E. coli* count	Staphylococcal count
Milk Vita butter	328000 d	8260 d	2680 d
Local butter	5450000 a	625000 a	31500 a
Quality cheese	450000 c	56500 c	4460 c
Local cheese	5140000 b	550000 b	11900 b
LSD	7579.0	7733.0	935.3
CV %	0.42	1.25	3.71

LSD = Least significant difference
CV = Coefficient of variation
* a to d rank order (highest - lowest)

Table 7. Ratio relationship between total viable bacterial count and *E. coli* density/gm butter and cheese samples.

Brand	No. of representative sample analyzed	Average total viable count/gm	Average *E. coli* count/gm	Ratio of *E. coli* count : TVC
Milk Vita butter	2	328000	8260	1: 39.7094
Local butter	2	5450000	625000	1: 8.72
Quality cheese	2	450000	56500	1: 7.9646
Local cheese	2	5140000	550000	1: 9.3454
LSD	-	14280.0	12520.0	-
CV %	-	0.25	2.02	-

LSD = Least significant difference
CV = Coefficient of variation
TVC = Total viable count

Table 8. Ratio relationship between total viable bacterial count and staphylococcal density/gm of butter and cheese samples.

Brand	No. of representative sample analyzed	Average total viable count/ gm	Average staphylococcal count/gm	Ratio of staphylococcal count : TVC
Milk Vita butter	2	328000	2680	1: 122.3880
Local butter	2	5450000	31500	1: 173.0158
Quality cheese	2	450000	4460	1: 100.8968
Local cheese	2	5140000	11900	1: 431.3327
LSD	-	8060.0	689.7	-
CV %	-	2.02	2.73	-

LSD = Least significant difference
CV = Coefficient of variation
TVC = Total viable count

Table 9. Result of biochemical test for E*. coli.*

Different biochemical tests	Result
Fermentation reaction with dulcitol	+
Indole	+
MR	+
VP	-

Table 10. Result of biochemical test for *Staphylococcus*.

Different biochemical tests	Result
Fermentation reaction with dulcitol	–
Indole	-
MR	+
VP	-

4. Discussion

The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used to manufacture the butter and the environmental and sanitary conditions during packaging and handling of such product (Richter *et al.*,1992). In the present study, it was demonstrated that *E. coli* counts were highest in local butter and local cheese, lowest in milk Vita butter and quality cheese (Table 2). The average count/gm were Milk Vita butter 8.26 x 10^3 (log 3.8), in local butter 6.25 x 10^5 (log 5.7), in Quality cheese 5.65 x 10^4 (log 4.7), in Local cheese 5.5 x 10^5 (log 5.8) respectively (Table 4).

The *E. coli* standards for butter and cheese should not be over 60/gm. The present investigation showed significantly high *E. coli* counts in samples belonging to Milk Vita butter, Local butter, Quality cheese and Local cheese. However, this study demonstrated that the samples of Milk Vita butter and Quality cheese met the recommended criteria of United States Public Health Service (USPHS). Hence, it could be taken into consideration as superior quality butter and cheese. It is known that *E. coli* play role in determining the hygienic quality index of food. In this study, *E. coli* counts positively and significantly correlated with total viable count of different brands of commercial butter and cheese. The average Staphylococcal counts/ml in different brand of butter and cheese samples were Milk Vita butter 2.68 x 10^3 CFU/gm (log 3.4), Local butter 3.15x 10^4 CFU/gm (log 4.5), Quality cheese 4.46 x 10^3 CFU/gm (log 3.6) and local cheese 1.19 x 10^4 CFU/gm (log 4.0) (Table 2). The maximum and minimum ranges varied from log 3.6 to log 3.3 in Milk Vita, log 4.8 to log 4.4 in Local butter, log 3.7 to log 3.4 in Quality cheese, log 4.1 to log 3.8 Local cheese respectively (Table 5). The average Staphylococcal counts of all four brands of butter and cheese was 8.65 x 10^2 CFU/gm (log 1.13) (Table 2). However, the highest count was observed in local butter and cheese sample and did not obtain in Milk Vita butter and Quality cheese sample. In certain situation *Staphylococci* organisms particularly *S. aureus* may be a pathogen, a source of enterotoxin and indicator of unsanitary practice. In man, the main reservoir of *S. aureus* is the nasal cavity and skin. From these sources *Staphylococci* find their way into air and dust, into clothing and in other place from which foods get contaminated. Since Staphylococcal food poisoning is an intoxication and depends on the ability of food concerned to support the growth of the *Staphylococci* which produce the toxin. It is therefore important to consider that the processing and handling of the food products should be so designed to minimize contamination and to make unfavorable medium for the growth of these organisms. When susceptible foods are produced with low numbers of *Staphylococci*, they will remain free enterotoxin if kept either below 4℃ until consumed. The factors that contribute mostly to Staphylococcal food-borne outbreaks may be due to inadequate refrigeration, preparing food far in advance of planned service, infected persons practicing poor personal hygiene, inadequate heat processing and holding food in warming devices at bacterial growth temperature. *Staphylococci* may come into milk and milk product from food handlers who may have acute infections or from healthy carriers who harbor the organisms in their nose or throats and also it is due to improperly stored and refrigerated milk and milk product that make excellent culture media for growth of these organisms.

Considering the total viable count, *E. coli* count and Staphylococcal count, the butter and cheese samples ranked into milk Vita butter ‘d’ local butter ‘a’ and quality cheese ‘c’ local cheese ‘b’. The analysis from the bacteriological context demonstrated that the lower count of bacteria in any food product signified comparatively better quality product, of course it should have to meet with international standard. In this respect, butter and cheese sample of milk Vita butter and quality cheese were the best quality product and also it fulfilled the gradation ‘a’. Local butter and cheese sample was of poor quality and graded into ‘b’ categories. In

case of staphylococcal count, milk Vita butter and quality cheese samples ranked into 'd' and 'c' local butter and cheese samples 'a' and 'b'. It is also numerically arranged and in this context Milk Vita butter and Quality cheese samples were of the best quality product. The results demonstrated that Milk Vita butter and Quality cheese is the best quality product in respect of bacteriological context as well as hygienic point of view. In this study, a ratio relationship obtained between total viable count and *E. coli* count of three brands of butter and cheese, separately. These were in case of Milk Vita butter 1: 39.7094, Local butter, 1: 8.72 and Quality cheese 1: 7.9646, local cheese 1: 9.3454 (Table 7). From this result, a conclusion could be drawn that higher density of total viable count always contained less number of *E. coli*, since *E. coli* is the index of sanitary quality, the butter and cheese samples of milk Vita were best and also Quality cheese have been found to possess the property of hygienically produced butter and cheese. In this study, Staphylococcal counts were positively and significantly correlated with total viable count in different brands of commercial butter and cheese. These were in case of milk Vita butter 1: 122.3880, local butter, 1: 173.0175 and Quality 1: 100.8965, local cheese 1: 431.3327 (Table 8).

The frequency distribution of different species of bacterial isolates in different butter and cheese samples were found variable. Results of the present study indicate that all the two different types of bacteria were not present in the same butter and cheese sample collected from local market.

Buxton and Fraser (1977) stated that, in white cheeses produced in three different plants with traditional methods total aerobic count was found to be between $3.1x10^6$ -$2.1x10^7$CFU/g.

The results of our recent study indicated that the butter and cheese samples contained Gram positive non-motile and Gram negative motile organisms. Colony characteristics in special media and fermentation ability with five basic sugars were similar. An interesting finding of the colony characteristics of the isolates was observed. The butter and cheese isolates were able to produce yellowish colonies on Staphylococcus agar no. 110, characteristic metallic sheen colony on EMB agar and bright pink colored colony on MacConkey agar.

In Gram's staining, the isolated bacteria exhibited Gram-positive (violet color) cocci arranged in groups or grape like clusters; short coccobacilli or rods arranged in bundles and singly also and Gram-negative (pink color) small rod-shape, arranged singly or in pairs which were supported by several authors (Buxton and Fraser, 1977).

In the present study, biochemical tests used for characterization of bacterial pathogens revealed that among two isolates, *E. coli* produced acid and gas by fermenting various sugars and gave positive reaction to indole, Motility Indole Urease, Methyl red and Catalase test but negative reaction to Voges-Proskeur test which satisfy the statement of Buxton and Fraser (1977). *Staphylococcus* spp. produced acid without gas by fermenting various sugars and gave positive reaction to Catalase and Methyl red test but negative reaction to indole and VP test.

5. Conclusions

It may be concluded that microbial loads of raw butter and cheese are not satisfactory. Therefore, it could be assumed that the handler of local butter and cheese did not maintain good personal hygiene. All butter and cheese tested in this study are low quality based on BSTI standard. Since, butter and cheese is consumed mainly by children in Bangladesh, therefore, a Standard Sanitation Operating Procedure (SSOP) should be maintained, which is prerequisite program of Hazard Analysis and Critical Control Point (HACCP), in order to minimize the risk of contamination for safety purpose.

In the context of our recent study, it may also be concluded that, the presence of *E. coli* and *Staphylococcus* spp. in most of the samples are public health concern. Total viable count of organisms was successfully performed from different butter and cheese samples. High counts of bacteria in all local butter and cheese samples indicate the low quality from the sanitary point of view.

Acknowledgements

The authors are expressing their heartfelt thanks and gratitude to all the stuffs of the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh to complete the experimental work successfully and also for providing continuous support and sincerest cooperation.

Conflict of interest

None to declare.

References

Bokulich NA and DA Mills, 2013. Facility specific "house" microbiome drives landscapes of artisan cheese-making plants. Appl. Environ. Microbiol., 17: 5214–5223.

Buxton A and G Fraser, 1977. Animal Microbiology. Vol. 1. Blackwell Scientific Publications, Oxford, London, Edinburg, Melbourne : 177-183.

Cheesbrough M, 1984. Medical Laboratory Manual for Tropical Countries. 1st edn.Vol.02 Microbiology, English Language Book Society, London. 355: 40-57.

Didienne R, C Defargues, C Callon, T Meylheuc, S Hulin and MC Montel, 2012. Characteristics of microbial biofilm on wooden vats ('gerles') in PDO Salers cheese. Int J Food Microbiol., 156:91–101.

Dolci P, V Alessandria, K Rantsiou, L Rolle, G Zeppa and L Cocolin, 2007. Microbial dynamics of Castel magno PDO, a traditional Italian cheese, with a focus on lactic acid bacteria ecology. Int. J. Food Microbiol., 122: 302-311.

Everard, CD, CP O'Donnell, DJ O'Callaghan, EM Sheehan, CM Delahunty and BT O'Kennedy, 2007. A Three-poınt bendıng test for predıctıon of sensory texture ın processed cheese. J. Texture Stud., 38: 438–456.

Frank DC, CM Owen and J Patterson, 2004. Solid phase microextraction (SPME) combined with gas-chromatography and olfactometry-mass spectrometry for characterization of cheese aroma compounds. Lebensm.-Wiss. u.-Technol., 37: 139-154.

ICMSF, 2005. Microrganisms in Foods. 2nd Edition. Microbial Ecology of food commodities: Oil and Fat based foods. Kluwer Academic/ Plenum Publishers, New York. pp. 480–521.

Irlinger F and J Mounier, 2009. Microbial interactions in cheese: implication for cheese quality and safety. Curr Opin Biotechnol., 20:142–148.

ISO, 1995. Recommendation of the meeting of the subcommittee, International Organization for Standardization on meat and meat products. ISO/TC-36/Sc-6. The Netherlands. 10-18.

Jay J, 1996. Modern food microbiology. 5th edition. Chapman and Hall publisher, USA. Fermentation and fermented dairy products; pp. 131–148.

Ksontini H, F Kachouri and M Hamdi, 2013. Dairy biofilm: impact of microbial community on raw milk quality. J Food Qual., 36:282–290.

Merchant IA and Packer RA, 1967. Veterinary bacteriology and Virology. Seventh edi. The Iwoa University Press, Ames, Iwoa, USA, pp. 286-306.

O'Mahony F, 1988. Rural Dairy Technology: Experiences in Ethiopia. ILCA Manual No. 4. Addis Ababa, Ethiopia: International Livestock Center for Africa (ILCA).

Pinho O, E Mendes, MM Alves and IMPLVO Ferreira. (2004). Chemical, physical, and sensorial characteristics of "terrincho" ewe cheese: changes during ripening and ıntravarietal comparison. J. Dairy Sci., 87: 249–257.

Richter RL, RA Ledford and SC Murphy, 1992. Compendium of Methods for the Microbiological examination of foods. American Public Health Association, Washington DC. pp. 837–856.

Tassew A, 2007. Production, handling, traditional processing practices and quality of milk in Bahir Dar milkshed area, Ethiopia. Haramaya, Ethiopia: Haramaya University, MSc thesis.