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MICROBIAL QUALITY OF TRADITIONALLY PRODUCED BUTTER AND CHEESE (*DATSHI*)

KINLEY CHOKI*, SONAM ZANGMO AND PHUNTSHO T NORBU

National Dairy Research and Development Centre, Department of Livestock, Ministry of Agriculture & Forests, Yusipang, Thimphu

*Author for correspondence: kchoki@moaf.gov.bt

ABSTRACT: This study was aimed to determine the microbiological quality of traditionally produced butter and cheese (*datshi*). Traditional dairy products are produced from unpasteurized milk and cream. A total of 100 random samples each of butter and *datshi* were collected and analyzed for their microbiological quality from different districts of Bhutan. The microbiological evaluation showed presence of yeast, mold, *E. coli* and *Staphylococcus aureus* in all the butter and *datshi* samples. Of the total samples examined, 22%, 94% and 28% of butter samples and 17%, 100% and 29% of *datshi* samples were found unsatisfactory for mold, yeast and *E. coli*, respectively. The study recorded presence of *Salmonella* in 3% of *datshi* samples, and none for the butter samples. The presence of these spoilage and pathogenic microorganisms in butter and *datshi* samples indicates poor hygienic and animal husbandry management practices resulting to poor quality products, short shelf life and it raises public health safety. This study emphasizes the need for strict implementation of clean milk production practices and development and adoption of standard operating procedures for the processing of traditional dairy products to improve microbial quality along the dairy value chain. Initially, pasteurization of raw milk to improve quality of dairy products may be emphasized and promoted for adoption.

Keywords: Butter; *datshi*; microbial quality; raw milk; unpasteurized.

1. INTRODUCTION

Traditional dairy products such as butter and cheese (*datshi)* form an integral part of Bhutanese cuisine. Annual domestic production of butter and cheese recorded in 2019 was 2,126.11MT and 4,090.56MT, respectively (Department of Livestock [DoL] 2019). Traditionally butter is obtained from churning of naturally fermented unpasteurized sour milk maintained at ambient temperature for a duration of two to three days in traditional wooden vessel. The washing and shaping of butter are achieved in cold water manually. *Datshi* is a soft unripen cheese, manufactured through addition of hot water or heat treatment resulting in coagulation of sour milk. The curd obtained is pressed and shaped into *datshi* balls manually. The current operational modalities of dairy farmers groups include marketing of fresh milk or processing milk into local butter and cheese at the milk processing units (MPU). In the MPU, the products are generally produced following the traditional method from unpasteurized milk or cream with the use of modern processing equipment such as cream separator and butter churner.

Milk is an ideal medium for growth of microorganisms due to its high nutritional value (Ashenafi & Beyene 1994; Claeys et al. 2013). Traditionally produced cheese belongs to a class of fresh soft cheese due to its high moisture content, high pH and low concentrations of salt and it serves as an ideal medium for bacterial growth (Giammanco et al. 2011). The incidences of pathogenic microorganisms in unpasteurized milk poses risk to human health (Claeys et al. 2013) and are associated with several epidemiological outbreaks (Sarkar 2015). The consumption of unpasteurized, inadequately heat-treated milk and post production contamination is associated with several food borne outbreaks (Baylis 2009; Oliver et al. 2005). Global outbreaks of food borne diseases are reported to be associated with consumption of raw milk cheeses (Choi et al. 2016; Gould et al. 2014) and raw milk products (De Buyser et al. 2001; Verraes et al. 2015). Pasteurization is a heat treatment process with a specified time and temperature combination (Holsinger et al. 1997) critical for elimination of spoilage and pathogenic microorganisms required to minimize food borne illness through consumption of milk (Griffiths 2010) and to improve milk hygiene (Yoon et al. 2016). Cheese manufactured from unpasteurized milk is stated to require a minimum of 60 day aging at ≥ 2°C to ensure a microbiologically safe cheese. However, the 60 day aging period to ensure microbial safety remained debatable as there are conflicting studies wherein some reported aging period is effective in inhibiting pathogenic microorganisms (Brooks et al. 2012) while others (D'AMICO et al. 2008 Schlesser et al. 2006) reported presence of pathogenic microorganisms even after 60 day of aging period.

The current traditional practices of producing *datshi* and butter from unpasteurized milk and cream can facilitate growth of pathogenic and spoilage microorganisms. The presence of these microorganism might result to product spoilage and possibly pose a risk to public health safety. However, until now such information on quality and safety related to food borne pathogens are limited in Bhutan. Therefore, this study is aimed to determine the microbial quality of major traditional dairy products – butter and datshi.

2. MATERIALS AND METHODS

2.1 Sample collection

A total of 100 random samples each of butter and *datshi* were collected from local retailers, dairy sales outlet, road side vendors, milk processing units (MPU), and weekend markets of Chukha, Tsirang, Dagana, Trongsa and Wangduephodrang Districts. The samples were stored in sterile cool box with ice packs to maintain the temperature of product during sampling and transportation to the National Food Testing Laboratory (NFTL) at Yusipang, Thimphu for the analysis.

2.2 Microbial Count

The butter and *datshi* samples were tested for yeast and mold count (IS 5403:1999). The samples were tested for the presence and absence of *E.Coli* (IS 5887 (Part I) 1976, Reaf. 2005, *Salmonella* (IS 5887-III, 1999, Reaf. 2005), and *S.aureus* (IS 5887-II, 1976, Reaf. 2005) to evaluate the overall microbial quality. The samples were not tested for Total Plate Count (TPC) as both the traditional *datshi* and butter making process encourages natural fermentation which would show high TPC by default.

2.3 Statistical Analysis

The data were statistically analyzed using one way ANOVA in SPSS version 21 (Landau and Everitt 2004).

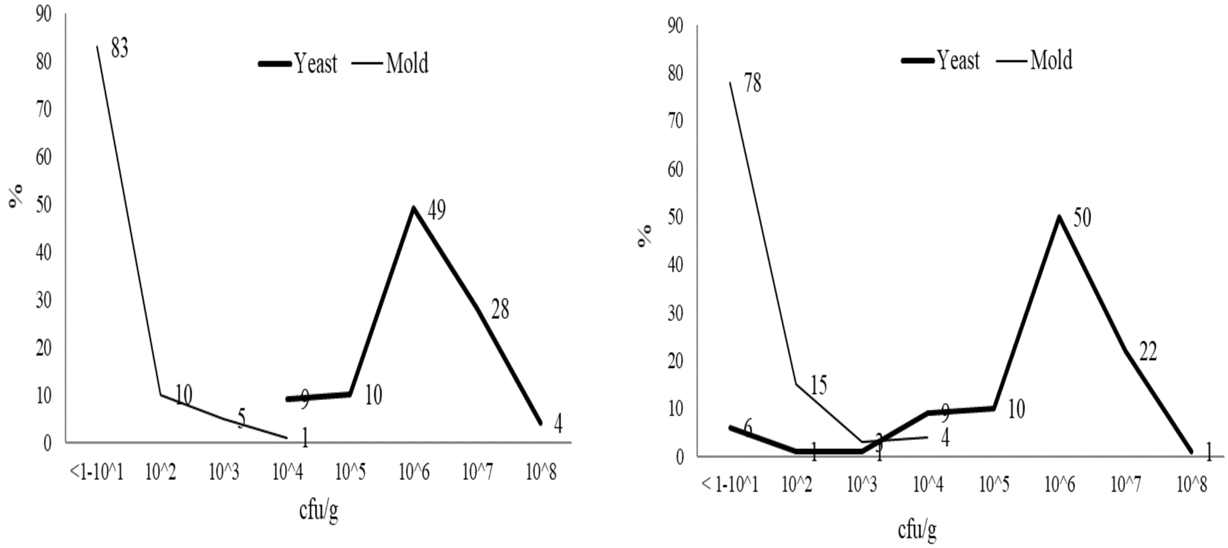
3. RESULTS AND DISCUSSIONS

Table 1 presents the count of yeast, mold and E. coli in butter and cheese. The butter samples collected for this study had recorded an overall mean of 12.0 x 10^6 cfu/g, 18.19 x 10^2 cfu/g and 118.5 MPN/g for yeast, mold and *E. coli* respectively.

Table 1: Mean ± SE counts of Yeast, Mold and *E. coli* in butter and *datshi*

District (N)	Butter			*Datshi*		
	Yeast (10^6cfu/g)	Mold (10^2cfu/g)	*E. coli* (MPN/g)	Yeast (10^6cfu/g)	Mold (10^2cfu/g)	*E. coli* (MPN/g)
Chukha (20)	39.78±8.70 *	89.80±41.20*	3.00±0.00	2.80±0.90	84.00±59.30	30.30±8.70
Wangdue (20)	4.00±1.70	0.30±0.09	3.00±0.00	4.20±1.10	0.80±0.20	3.00±0.00
Trongsa (20)	4.50±1.60	0.64±0.10	3.00±0.00	43.00±14.80*	0.20±0.07	3.40±0.30
Tsirang (20)	4.50±0.40	0.10±0.40	566.80±113.30*	5.20±0.20	0.10±0.00	347.40±113.1*
Dagana (20)	7.20±1.20	0.10±0.00	16.80±7.50	29.70±6.50	0.10±0.00	3.00±0.00
Overall (100)	12.00±2.30	18.20±8.80	118.50 ± 35.70	17.00±3.60	17.00±12.10	77.40±26.00

*cfu/g= colony forming unit per gram, MPN= most probable number, N= total number of samples, * significant difference (p<0.05)*



(b) (a)

Figure 1: Distribution of yeast count and mold count in butter (b) and datshi (a)

Similarly, in *datshi* samples, the overall mean recorded was 17.0 x10^6 cfu/g, 17.0 x 10^2 cfu/g and 77.4 MPN/g for yeast, mold and *E. coli*, respectively. The yeast and mold count in butter samples of Chukha district were significantly higher (p<0.05) than other four districts. Accordingly, the yeast in *datshi* samples from Trongsa were also significantly higher than other districts (p<0.05). However, there was no significant difference (p>0.05) for mold in *datshi* samples among the districts. *E. coli* in both butter and *datshi* samples of Tsirang district were significantly higher (p<0.05) than other four districts.

The growth of yeast and mold in butter is not desirable as they grow at different temperature ranges and pH values causing color and flavor defects (Meshref 2010). Rady & Badr (2003) stated that butter is not highly perishable dairy product; however, it does undergo spoilage by bacteria and mold and the main source of these microorganisms are from cream (sweet or sour, unpasteurized). The yeast count in butter ranged from <1.0 x 10 cfu/g to 1.1x 10^8 cfu/g and mold count ranged from <1.0 x 10 cfu/g to 5.5 x 10^4 cfu/g (Figure 1(b)). Similarly, in *datshi* the yeast count ranged from 1.0 x 10^4 cfu/g to 2.3 x 10^8 cfu/g and mold count ranged from 1.0 x 10^5 cfu/g to 1.2 x 10^9 cfu/g (Figure 1(a). The mold count in 78% of butter samples and 83% of *datshi* samples were found to be within the acceptable limit of 10 x 10 cfu/g (Bhutan Standard Bureau [BSB] 2020). The yeast count in 94% of butter samples and 100% of *datshi* samples were found to be above the acceptable limit of <5.0 x 10 cfu/g (BSB 2020).

The growth of yeast and mold in butter and *datshi* indicates contamination or unhygienic processing conditions during packing and handling arising from traditional methods. Contamination could be attributed to the lack of pasteurization process and unhygienic storage conditions along the supply chain. Yeast and mold in cheese gains entry from air in the packing room and dusty and improperly stored packaging materials (Robinson 2005). Contaminated water supply traced back to equipment, churns, vats, pumps and pipelines was identified as main source of contamination in the development of defects in butter (Sorensen 1940).

In addition, the working of butter manually in traditional practices may result in uneven distribution of water droplets in butter favoring growth of bacteria. As per Long & Hammer (1938), bacterial growth in butter is mostly restricted to contaminated water droplets in water in oil phase of butter, the thorough working of butter results in finer dispersion of water droplets

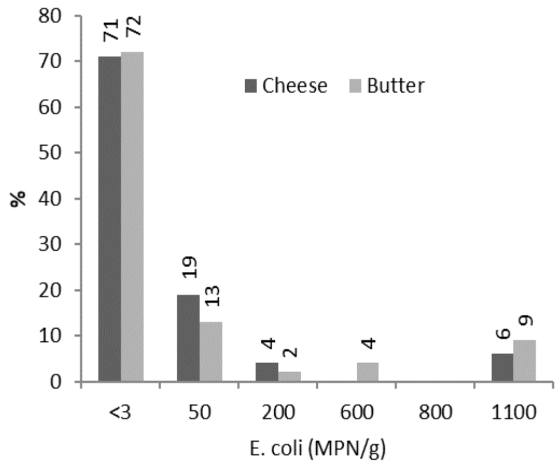


Figure 2: Distribution of E. coli in butter and *datshi*

resulting in decreased number of contaminated droplets and nutrients available for the bacterial growth.

E. coli, ranging from <3 MPN/g to 1100 MPN/g (Figure 2) were detected in all butter and *datshi* samples. 72% of butter samples were found to be within the acceptable limit of <3 MPN/g of *E. coli* in butter (BSB 2020); whereas, 71% of *datshi* samples were found to be within the acceptable limit of <10 MPN/g of *E. coli* (BSB 2020).

E. coli forms the normal intestinal micro flora of humans and other mammalian species and is moslty harmless (Kaper et al. 2004). However, the isolation of *E. coli* also indicates possible existence of enteropathogenic or toxigenic strains (Soomro et al. 2002) which causes intestinal and extraintestinal diseases through its virulence factors (Kaper et al. 2004). *E. coli* is also an indicator bacteria and are used as indicators of standard hygienic quality and post pasteurization contamination in food and dairy industries (Masiello et al. 2016). Trmcic et al. (2016), suggests that unpasteurized milk is a source of coliform in cheese and the probability of positive detection of coliforms in unpasteurized milk is 4.6 times. These microbes are able to persist in the final products as well as along the entire dairy supply chain. Thus, the presence of these indicator bacteria in the tested samples indicates unhygienic practices during processing and lack of heat treatment during production.

Moreover, the detection of *E. coli* indicates fecal contamination, which could arise from cow dung/manure at the farm or due to fecal contaminated water source at the farm or at the production areas. Ribeiro et al. (2019) identified cattle feces at the farm as the main source of *E. coli* contamination in water and unpasteurized milk. The teats and udders of cows can be contaminated with feces and can hence contaminate milk during unhygienic milking practices (Fremaux et al. 2006). All butter samples tested positive for presence of *S. aureus* (Table 2) while 3% of the samples from Tsirang tested positive for presence of *Salmonella*. All the *datshi* samples tested positive for presence for *S. aureus* and negative for the presence of *Salmonella*. The acceptable limit of *S. aureus* in butter is <1000 cfu/g and in *datshi* is <50 cfu/g while, *Salmonella* should be absent/25g for both *datshi* and butter (BSB 2020).

The prevalence of *Salmonella* in butter samples could be related to the use of unpasteurized contaminated milk as it is generally

Table 2: Presence of *Salmonella* and *S. aureus* in butter and *datshi*

Districts	Microbiology	N	Butter			*Datshi*		
			Positive samples		Present/ Absent	Positive samples		Present/ Absent
			N	%		N	%	
Chukha	*Salmonella*	20	20	100	Absent/25g	20	100	Absent/25g
	S. aureus		20	100	Present/g	20	100	Present/g
Wangduephodrang	*Salmonella*	20	20	100	Absent/25g	20	100	Absent/25g
	S. aureus		20	100	Present/g	20	100	Present/g
Trongsa	*Salmonella*	20	20	100	Absent/25g	20	100	Absent/25g
	S. aureus		20	100	Present/g	20	100	Present/g
Tsirang	*Salmonella*	20	17	85	Absent/25g	20	100	Absent/25g
	S. aureus		20	100	Present/g	20	100	Present/g
Dagana	*Salmonella*	20	20	100	Absent/25g	20	100	Absent/25g
	S. aureus	20	20	100	Present/g	20	100	Present/g

killed during the pasteurization process. Outbreaks of salmonellosis has been associated with consumption of unpasteurized milk, improperly pasteurized milk or post pasteurization milk contamination (El-Gazzar & Marth 1992).

The presence of *S. aureus* in butter and *datshi* may be from the use of unpasteurized cream or unhygienic practices during production. The possible sources of *S. aureus* could be from cows suffering from mastitis because *S. aureus* is said to be ubiquitous gram-negative organism associated with mastitis in cattle (Cremonesi et al. 2007). Rabello et al. (2007) recovered *S. aureus* isolate in milk from mastitis cows in a Brazilian herd. The study of Kummel et al. (2016) reported that *S. aureus* can effectively gain entry in the dairy production chain via contaminated milk from cattle with subclinical mastitis. Possible sources apart from unpasteurized milk might be contamination during product processing from the dirty equipment and contaminated water, packaging materials and from food handlers themselves. Enterotoxin producing *S. aureus* are found in the hands and noses of food handlers which serve as a major source of contamination via indirect contact or respiratory secretions (Kluytmans & Wertheim 2005). The study of Fadel and Ismail (2009) demonstrated 60% and 70% prevalence of *S. aureus* from the swabs of dairy handlers' hands and nose respectively. Some strains of *S. aureus* can produce heat stable enterotoxin which causes food poisoning after 2-4 hours following ingestion with symptoms such as abdominal cramps, nausea, vomiting with or without diarrhea (Balaban & Rasooly 2000; Tranter 1990). After ingestion of contaminated milk products prepared from unpasteurized milk, the development of disease is dependent on numerous factors such as the quantity of ingested microorganisms, the pathogenicity of the strain, the physical state of the microorganism, the health condition of the consumer at the time of consumption. Although anyone can be infected including the healthy persons, the young, old, pregnant and the immunocompromised persons (YOPIs) are at greater risk of infection (Verraes et al. 2015).

4. CONCLUSION & RECOMMENDATION

Traditional dairy products mainly butter and cheese are made from unpasturized milk or cream produced under unhygenic conditions. The traditional products in general have higher microbial load resulting in reduced shelf life. Majority of the butter and cheese samples showed yeast and mold count within the acceptable limit as per the Bhutan Standards Bureau. Yet, the presence of pathogenic bacteria *E.coli*, *S. aureus* and *Salmonella* are of concern for the public health safety. The traditional products are mainly used for cooking purposes and not for direct consumption. The heating process during cooking may eliminate heat sensitive microbes but cannot destroy the heat stable toxin generated, thus there is likely chances of causing diseases depending on quantity of ingested microbes, age groups and the health status of the individual. Therefore, it is recommended for the development and adoption of Standard Operating Procedures for the processing of traditional dairy products and strict implementation of clean milk production practices at the farm and along the dairy value chain must be emphasized. In addition, to have deeper understanding on the different strains of pathogens in milk and milk products further study on isolation and identification of pathogenic bacteria is recommended.

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