PROCESS STANDARDIZATION OF TRADITIONALLY PRODUCED CHEESE (*DATSHI*) AND ITS EFFECT ON IMPROVING SENSORY ATTRIBUTES, MICROBIAL QUALITY AND SHELF LIFE

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ABSTRACT: This study aimed to determine the process standardization of traditionally produced *datshi* and to assess the microbial quality of raw milk, pasteurized skimmed milk and *datshi*. A total of 60 samples of raw milk, pasteurized skimmed milk and *datshi* were collected from Thimphu (n=24) and Paro (n=36) respectively. All the samples were analyzed for microbial load using pour plate method and microbial contamination. The overall mean total bacterial count for the raw milk, pasteurized skimmed milk and *datshi* were 6.20±0.61, 4.79±1.06 and 6.31±0.39 log10cfu/ml respectively. The overall bacterial count for raw milk in Paro (n=12) was higher than Thimphu (n=8) and was statistically significant($p<0.05$). However, there was no significant difference for pasteurized skimmed milk and *datshi* in the two Dzongkhags. High bacterial count in raw milk was observed in Tshaluna (6.74 log10cfu/ml) and Lamgong (6.78 log10cfu/ml) amongst five MPUs. Highest bacterial count in pasteurized skimmed milk was in Tshaluna (5.83 log10cfu/ml) and the lowest was in Laykha and Taba (4.15 log10cfu/ml). Similarly, the highest bacteria count for *datshi* was in Shari (6.80 log10cfu/ml) and the lowest in Lamgong (5.76 log10cfu/ml). The microbiological contamination showed the presence of *staphylococcus aureus* in the raw milk of Taba only. The yeast count in *datshi* ranged from $<1.0x10^1$ to $1.9x10^7$ cfu/gm and mold count from $<1.0\ X10^1$ to $1.0\ X10^1$ cfu/gm in all samples. The detection of microbes in raw milk and *datshi* indicates poor quality of milk and unhygienic management and processing practices in the study sites. Thus, it is suggested and warrants pasteurization process at the collection and processing units to improve the quality and shelf life of milk and dairy products and food borne illnesses.

Keywords: *Datshi*, raw milk, pasteurized skimmed milk, total bacteria count, process standardization, microbial contamination

1. INTRODUCTION

Milk and milk products in the form of local butter and local cheese *(datshi)* forms the core component of Bhutanese cuisine and diet. According to Integrated Agriculture and Livestock Census, the amount of milk produced stands at 42,254.94 MT of which 1508.12 MT and 2382.23 MT was processed into butter and *datshi* respectively (NSB 2022). However, the country relies mostly on the import of dairy products to meet the national demand. To address the increasing demand for milk and milk products and to reduce imports, the Department of Livestock has supported the formation of Dairy Farmers Groups and establishment of dairy infrastructures such as Milk Collection Centers (MCCs), Milk Processing Units (MPUs), dairy processing plants and dairy sales outlets.

With the establishment of dairy infrastructures, the groups and cooperatives have initiated product diversification into yogurt, ice cream and varieties of cheese. However, local *datshi* has seen minimal improvement in its production process limiting to traditional knowledge passed through generations despite access to modern dairy equipment. The production process of *datshi* mainly involves fermentation of milk over a few days at room temperature without the pasteurization process.

Milk is an ideal medium for growth of various microbes such as spoilage and pathogenic bacteria. Globally, foodborne illness has been associated with consumption of raw milk and its products, under pasteurized products and due to post-production contamination. It is therefore essential to initiate improvement of *datshi* production process to incorporate food safety and good management practices. The process standardization using pasteurization and cheese cultures is expected to improve the quality and shelf life of *datshi* thereby reducing the probability of causing food-borne illness.

The current processing practices of *datshi* involves application of traditional knowledge with modern processing equipment. This process lacks milk pasteurization which is crucial in minimizing pathogenic bacteria rendering milk and dairy products safe for human consumption. The scientific literature pertaining to manufacture of *datshi* is limited and in order to close the gap of scientific knowledge, such traditional product necessitates a standardized process for commercial purposes. Thus, introduction of pasteurization process and utilization of cottage cheese cultures in the traditional cheese making process is expected to improve the quality and shelf life of cheese. To derive quality dairy products, it is essential to standardized the *datshi* making process through incorporation of mandatory pasteurization inclusion of culture and enzyme. Therefore, these study is conducted with the objective to standardized the *datshi* making process following good manufacturing process and assess the quality of raw milk used for production of *datshi* and its sensory attributes, microbial and compositional quality.

2. MATERIALS AND METHODS

2.1 Sample collection

The study was carried out at Laykha MPU, Lamgong and Shari MCCs in Paro and Tshaluna and Taba MCCs in Thimphus Dzongkhags. The selection of MPUs/MCCs was based on purposive sampling.

A total of 20 samples each of raw milk, pasteurized skimmed milk and *datshi* were collected during each standardization process. The samples were analysed for milk composition, microbial count and microbiological contamination. The samples were stored in cool box with ice packs during sampling and transported to National Centre for Animal Health at Serbithang for laboratory analysis. The study was conducted during October 2023.

2.2 Analysis of milk composition

The raw milk and pasteurized skimmed milk were subjected to milk compositional analysis using a milk analyzer (Lactoscan FarmEco, Milkotronic LTD, Bulgaria). The parameters analyzed were fat, SNF, protein, density, lactose, water, ash and freezing point.

2.3 Microbial count and microbiological identification

The raw milk, pasteurized skimmed milk and *datshi* produced from standardized process were analyzed for microbial load using pour plate technique (NCAH SoP version 2018.1). The samples were also assessed for microbiological contamination using bacteriological culture and identification

(NCAH SoP version 2018.1). The *datshi* samples were tested for yeast and mold count at National Food Testing Laboratory (NFTL) in Yusipang, (IS 5403:1999).

2.4 Statistical Analysis

The data were statistically analyzed using one-way ANOVA in SPSS version 23.

4. RESULTS AND DISCUSSION

4.1 Compositional analysis of raw milk and pasteurized skimmed milk

Table 1 represents the mean milk compositional quality of raw milk. The mean fat content was highest in Tshaluna MCC with 4.62% and lowest in Lamgong MCC (4.21%). Shari MCC recorded the highest mean protein content (3.23%) and lowest in Tshaluna MCC (2.81%). Overall, a significant difference was observed in milk composition among the study sites with the exception to fat and water content (p>0.05). Kunda et al. 2015 reported the low mean fat and protein contents and high mean SNF content when compared to this study. The result of mean fat, protein and SNF percent of raw milk in the current study was lower than the finding of Negash et al. (2012).

Table 2 represents the mean milk composition of pasteurized skimmed milk in the study. The overall mean milk composition for pasteurized skimmed milk was 1.11% fat, 9.65% SNF, 35.62% density, 3.55% protein, 5.31% lactose, 1.22% water, -0.51°C freezing point and 0.81% ash. The mean fat composition was highest in Tshaluna MCC (4.72%) and lowest in Laykha MPU (0.01%). All the other MCCs recorded fat content within the range of 0.01±0.01 to 0.43±0.32 except for Tshaluna MCC. This was because the raw milk in Tshaluna MCC was not cream separated. Likewise, the protein content was found highest in Laykha (6.44%) and lowest in Tshaluna (2.77%). Overall, a significant difference was observed for all the milk composition of pasteurized skimmed milk except for freezing point (p>0.05).

Table 1: Mean±SD milk composition of raw milk

MCC	Fat	SNF	Density	Protein	Water	Lactose	FP	Ash
Tshaluna	4.62±0.220^{a}	7.69±0.480^{a}	25.13±1.983^{a}	2.81±0.179^{a}	5.33±5.949^{a}	4.24±0.626^{a}	0.527±0.008a	0.64±0.044a
Laykha	4.40±0.012^{a}	8.28±0.119^{ab}	27.61±0.554^{ab}	3.02±0.059^{ab}	0.00^{a}	4.57±0.079^{ab}	0.534±0.011a	0.68±0.010ac
Lamgong	4.21±0.379^{a}	8.48±0.061^{b}	28.47±0.525^{b}	3.10±0.025^{b}	0.00^{a}	4.67±0.031^{b}	0.546±0.003a	0.70±0.006ad
Taba	4.53±0.061^{a}	8.58±0.085^{b}	28.32±0.590^{b}	3.10±0.051^{b}	0.00^{a}	4.67±0.042^{b}	0.550±0.010ac	0.70±0.010ae
Shari	4.39±0.030^{a}	8.86±0.187^{b}	29.74±0.729^{b}	3.23±0.068^{b}	0.00^{a}	4.88±0.042^{b}	0.574±0.013bc	0.73±0.012bcde
Mean±SD	4.43±0.221	8.37±0.454	27.85±1.808	3.05±0.165	1.07±3.152	4.61±0.245	0.546±0.019	0.69±0.035

*Different superscript within the column significantly differs at 95% confidence interval

Table 2: Mean±SD milk composition of pasteurized skimmed milk

MCC	Fat	SNF	Protein	Density	Lactose	Water	FP	Ash
Tshaluna	4.72±0.10^{a}	7.60±0.24^{acde}	2.77±0.09a	24.71±0.83^{a}	4.19±0.13^{a}	6.15±3.29^{a}	-0.19±0.66^{a}	0.74±0.03^{a}
Laykha	0.01±0.01^{b}	11.72±2.13^{b}	4.33±0.79bc	44.49±8.20^{b}	6.44±1.17^{bcd}	0.00±0.00^{bf}	-0.58±0.02^{a}	0.95±0.18^{a}
Lamgong	0.29±0.06^{b}	8.97±0.17^{bc}	3.30±0.06ac	33.67±0.70^{acd}	4.93±0.96^{ab}	0.00±0.00^{cf}	-0.55±0.01^{a}	.73±.012^{a}
Taba	0.43±0.32^{b}	9.74±0.16^{bd}	3.60±0.06ac	36.65±0.58^{bc}	5.36±0.09^{ac}	0.00±0.00^{df}	-0.61±0.01^{a}	0.79±0.02^{a}
Shari	0.11±0.46^{b}	10.22±0.14^{be}	3.77±0.05ac	38.59±0.56^{bd}	5.62±0.08^{ad}	0.00±0.00^{ef}	-0.64±0.01^{a}	0.83±0.01^{a}
Mean±SD	1.11±1.88	9.65±1.63	3.55±0.61	35.62±7.43	5.31±0.90	1.22±2.83	-0.51±0.30a	0.81±0.11^{a}

*Different superscript within the column significantly differs at 95% confidence interval

4.2 Total Bacterial Count of raw milk, pasteurized skimmed milk and *datshi*

The mean microbial count of raw milk, pasteurized skimmed milk and *datshi* were 6.20±0.61, 4.79±1.06 and 6.31±0.39 log10cfu/ml respectively (Table 3). The mean bacterial count of raw milk in Paro was 6.40±0.34 log10 cfu/ml and 5.85±0.31 log10cfu/ml in Thimphu.

A significant difference was observed in mean bacterial count of raw milk between the Paro and Thimphu ($p<0.05$). In Thimphu, Tshaluna MCC showed higher bacterial count than Taba MCC and there was a significant difference ($p<0.05$). This might be due to poor hygiene practices in milking and lack of proper handling, washing and sterilization of utensils/equipment. As per Hossain et al. (2011) the high bacterial count in raw milk is attributed to poor cleaning of milking system and due to milking dirty udders, maintaining an unclean milking and housing environment and failing to rapidly cool milk to less than 40°F.

In Paro, Lamgong MCC showed the highest bacterial count followed by Laykha MPU and least in Shari MCC. The total bacterial count in raw milk varied significantly among the three study sites ($p<0.05$) in Paro. As per Khairunnisak et al. (2017) the acceptable total bacterial count limit in raw milk is $\leq 1.0x10^6$ cfu/ml but this study recorded mean total bacterial count of $3.0x10^6 \pm 2.8x10^6$ which is higher than the acceptable limit in Malaysia. However, the data on acceptable limit for total bacterial count in raw milk in Bhutan was not available at the time of this study. The overall mean total bacteria count of raw milk is similar to the findings reported by Aysheshim et al. (2018). All the raw milk in the study showed total bacterial load ranging from $1.2x10^5$ cfu/ml to 9.7 $x10^6$ cfu/ml. Sameera et al. (2020) reported total microbial count in raw milk between $1.06x10^8$ to $1.62x10^8$ cfu/ml which was higher compared to this study. According to Sameera and team, the quality of water used for washing utensils could be the reasons leading to poor milk quality owing to high microbiological quality in the milk samples. Oladipo et al. (2016) in Nigeria reported total bacterial counts in raw milk ranging from $0.2x10^6$ cfu/ml to $4.2x10^6$ cfu/ml which is similar to this study. Kunda et al. (2015) reported total bacterial count in raw milk ranging from 4.45 $x10^2$-$2.6x10^6$ cfu/ml which is lower than our current findings. Hossain et al. (2011) reported higher bacterial load ranging from $1.75x10^6$ to $1.22x10^8$ cfu/ml than our current findings.

The mean total bacterial count in pasteurized skimmed milk in the study was 4.79±1.06 log10cfu/ml. The mean total bacterial count in Thimphu and Paro were 4.99±1.40 and 4.66±0.80 log10cfu/ml respectively. No

Table 3: Mean±SD total plate count of raw milk, pasteurized skimmed milk and *datshi*

Study Site	Raw milk (Log10 cfu/ml)	Pasteurized skimmed Milk (Log10 cfu/ml)	Datshi (Log10 cfu/gm)
Tshaluna	6.74 ± 0.06^{a}	5.83 ± 0.36^{a}	6.18 ± 0.18^{ac}
Laykha	6.35 ± 0.18^{b}	4.15 ± 0.33^{a}	6.40 ± 0.25^{ab}
Lamgong	6.78 ± 0.18^{a}	4.26 ± 0.40^{a}	5.76 ± 0.25^{cd}
Taba	5.19 ± 0.09^{c}	4.15 ± 1.61^{a}	6.38 ± 0.09^{ae}
Shari	6.08 ± 0.14^{b}	5.59 ± 0.61^{a}	6.80 ± 0.12^{be}
Mean±SD	6.20±0.61	4.79±1.06	6.31±0.39

*Different superscript within the column significantly differs at 95% confidence interval significant difference was observed in total mean bacterial count of pasteurized skimmed milk between Thimphu and Paro ($p>0.05$). In Thimphu, Tshaluna MCC recorded higher bacterial count in pasteurized skimmed milk than Taba MCC. However, no significant difference was found between these two MCCs ($p>0.05$). In Paro, the total bacterial count of pasteurized skimmed milk in Shari MCC was significantly higher than Laykha MPU and Lamgong MCC ($p<0.05$). This was attributed to contamination of utensils/equipment during pasteurization process. The total bacterial count in pasteurized skimmed milk was higher compared to the study conducted by Anderson et al. (2011).

The overall mean total bacterial count of pasteurized skimmed milk was comparatively lower than the study conducted at Ethopia by Desye et al. (2023). The total bacterial count in pasteurized skimmed milk ranged from 4.15±0.33 to 5.83±0.36 log10cfu/ml which is higher than the findings of Kunda et al. (2015). However, the range of total bacterial count of pasteurized milk reported by Sameera et al. (2020) was higher than this study. According to the above authors, the higher range of total bacterial count was due to poor quality of milk, defects in processing techniques, inadequate pasteurization, and poor compliance to best management practices. A study conducted by Nur et al. (2021) at Dhaka observed low total bacterial load in pasteurized milk compared to our current finding.

The total mean bacterial count of *datshi* in Thimphu and Paro were 6.28±0.17 and 6.32±0.49 log10cfu/ml respectively. However, no significant difference was observed ($p<0.05$). In Thimphu, Taba MCC (6.38±0.90) recorded a slightly higher bacterial count than Tshaluna MCC (6.18±0.18). In Paro, Shari MCC (6.80±0.12) recorded the highest mean total bacterial count followed by Laykha MPU (6.40±0.25) and Lamgong MCC (5.76±0.25) which is statistically significant ($p<0.05$). The variation in total mean bacterial count in *datshi* of three MCCs in Paro might be due to poor management practices during processing of *datshi*. The mean total bacterial count in datshi was highest in Shari MCC (6.80±0.12) followed by Laykha MCC (6.40±0.25) and Lamgong MCC (5.76±0.25). As per Esho et al. (2013), the fermented products like cheese generally show high number of Standard Plate Count (SPC) because of presence of beneficial microorganisms to ferment the food properly. According to the same authors, high level of SPC in natural cheese samples seems to be obvious, since some lactic bacteria and mold are known to grow on SPC agar.

Nair et al. (2021) reported higher total bacteria count in *datshi* than the finding of this study. This may be due to *datshi* produced from pasteurized milk attributing to reduction in microbial load compared *datshi* processed through boiling of raw milk. However, the *datshi* produced at a controlled condition by the above authors had lower microbial load compared to our study. A study conducted in Italy by Costanzo et al. (2020) recorded total bacterial count of hard cheese of raw milk above 6.0 log10cfu/gm. In south west of Ethopia, Birhanu et al. (2013) recorded 8.844 log10cfu/gm total bacterial count in traditional cottage cheese The study conducted in Bhutan by Shangpliang et al. (2017) recorded 3.9 $X10^{8}$ cfu/ml microbial load in *datshi* and isolated lactic acid bacteria.

4.3 Bacteria Identified

The bacterial culture and identification in raw milk, pasteurized skimmed milk and *datshi* showed 25% positive to both pathogenic and non-pathogenic bacteria (n=60). The detection rate was 15%, 0% and 10% in raw milk, pasteurized skimmed milk and *datshi* respectively. Out of 15 positive samples, six were positive in Thimphu and nine in Paro. The bacteria identified in *datshi* sample from Tshaluna MCC were *E. faecalis*, *E. faecium*, *E. gallinarum*, *S. lentus* and *K. kristinae*. *S. aureus* and *S. chromogenes* was identified in raw milk of Taba MCC. In Laykha MPU, *S. uberis*, *K. kristinae* and *L. lactis* were identified from raw milk and *S. hominis*, *L. garvieae*, *S. epidermidis*, and *L. lactis* were detected in *datshi* of Lamgong MCC. The study did not detect pathogenic salmonella and *E. coli* in all the samples, however, the detection of *Staphylococcus aureus* in raw milk of Taba MCC is a concern. The presence of *S. aureus* in raw milk may be due to contamination as a result of unhygienic practices during the processing and use of unsterilized equipment. According to Fadaei (2014) the most common pathogens involved in milk borne diseases include *Salmonella* spp., *Staphylococcus aureus*, and *E. coli*. Vahedi et al. (2013) detected *S. aureus* in 42% of cow's raw milk. *Staphylococcus aureus* is often found in milk, and has also been reported to be isolated from skin of udders and teats, wounds and mucosa, milking equipment and shelves, floor, door knobs, etc. (Jorgensen et al. 2005). Harmiroune et al. (2016) reported isolation of *Staphylococcus aureus* from the water used at the different stages of milking (50.9%), from samples taken from the hands of milkers (39.6%) and from udders (28.9%).

As stated by Rosengren (2012), the microbiological status of cheese depends on the quality of the milk, possible contamination during processing and cheese type. The most common pathogens present in cheese *are Listeria monocytes*, *Salmonella* spp, *Staphylococcus aureus* and *Escherichia coli* (Turhan 2019). According to Hudson et al. (2003) these four bacterial pathogens are the predominant microorganisms that caused outbreaks of diseases as a result of contaminated traditional cheese. The main reason for the presence of these pathogens during the cheese making process in small-scale cheese production is the use of raw milk (Rola et al. 2016). The dominant bacterial pathogens isolated from traditional Ethopian cottage cheese were *E. coli*, *Staphylococcus aureus*, *Vibrio* spp., *Vibrio* and also *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococci* spp., *Shigella flexneri*, and *Proteus mirabilis* (Birhanu et al. 2013).

4.4 Yeast and Mold count

Overall, yeast count in *datshi* ranged from <1.0 x10 to $1.9x10^7$ cfu/gm and mold count ranged from <1.0x10 to 1.0xX10 cfu/gm. Taba MCC recorded the highest yeast count in *datshi* followed by Shari MCC and Laykha MCC. Similar study carried out by Choki et al. (2021) revealed the yeast count ranging from $1.0x10^4$ to $2.3x10^8$ cfu/gm and mold count ranging from $1.0x10^5$ to $1.2x10^9$ cfu/gm in *datshi* which is comparatively higher than the present study. The mold count in all the samples of *datshi* were found within the acceptable level of 10cfu/gm (Bhutan Standard Bureau, 2020). As per Choki et al. (2021) the growth of yeast and mold in *datshi* indicates the contamination and unhygienic processing conditions during packing and handling arising from traditional methods.

4.5 Sensory attributes

In this study, *datshi* samples were evaluated for flavor, texture, appearance and color. *Datshi* prepared with standardized method incorporating pasteurization, addition of starter culture and rennet had slightly different characteristics and sensory attributes. *Datshi* presented a natural white color with slight degree of acidity and flat/chewy taste. It had a rubbery kind of texture. The granules were firm and elastic type. Overall, *datshi* had a compact smooth texture and a bit of semi hard cheese structure. Upon observation, the shelf life of *datshi* is the same as that of normal traditional *datshi* while kept in refrigerated condition. However, exposing *datshi* to room temperature it gets spoiled overnight due to presence of culture and enzyme. A study conducted at Pakistan has use citric acid and acetic acid as coagulant in which they have revealed that the use of acetic acid has a bitter aftertaste and the use of citric acid at the level of 0.4% was found best at all aspects (Ali et al. 2022).

According to (BAFRA 2017), standard for *datshi* shall possess a pleasing and desirable flavor similar to fresh whole milk or cream. The product may possess a slight degree of acidity, flat, or salty flavor, but shall be free from chalky, utensil, fruity, yeasty, or other objectionable flavors. The flavor shall not be harsh or unnatural. It shall have a meaty texture. The texture shall be smooth and velvety and shall not be mealy, crumbly, pasty, sticky, mushy, watery, or slimy, or possess any other objectionable characteristics of body and texture. It shall present a clean, and natural creamy white color.

5. CONCLUSION

Traditional method of *datshi* processing involves natural souring/fermentation of milk over a few days at room temperature without pasteurization process. The standardized process involves pasteurization, addition of starter culture for acid production and ripening and addition of rennet for the coagulation of milk. The standardized processed *datshi* was bit a different from the traditionally produced in terms of texture, appearance and flavor. No pathogenic bacteria were detected in any of the samples of *datshi* and pasteurized skimmed milk except in three raw milk samples of Taba MCC, which detected *Staphylococcus aureus* and is a concern for public health safety. The result revealed that the pasteurization plays a substantial effect in decreasing the microbial load. This study concludes that the raw milk produced from the study areas are of poor quality due to high bacterial count in all the milk and *datshi* samples. Therefore, it is recommended to follow pasteurization process in any of the traditionally produced dairy products. The study also emphasizes on incorporating adequate sanitary measures and adoption of best management practices at all levels of production to enhance quality and the shelf life of dairy products. Moreover, there is further a need to study on standardization process for all the traditionally processed dairy products incorporating pasteurization process at the collection and processing units to improve the quality of milk and dairy products and prevent foodborne illnesses. Since the present study was confined to Thimphu and Paro only, it warrants a higher representative sample size covering collection centre and processing units across the country to devise an appropriate intervention for improving the quality of milk and dairy products.

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