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### **Preliminary assessment of microbiological quality of raw buffalo milk commercially produced in Thailand**

*Wira Duangpan<sup>1,2</sup> and Orasa Suriyaphan<sup>2\*</sup>*

<sup>1</sup>Faculty of Agro-Industry, Sakaeo College of Agriculture and Technology, Aranyaprathet, Sakaeo, 27120, Thailand.

<sup>2</sup>Department of food science, Faculty of science, Burapha university, Bangsaen, Chonburi 20131, Thailand.

\* Author to whom correspondence should be addressed, email: [orasa@buu.ac.th](mailto:orasa@buu.ac.th)

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#### **Abstract**

Recently, buffalo milk has been commercially produced and sold as pasteurized milk and Mozzarella cheese in the market. However, there is a lack of data on microbiological properties of raw buffalo milk produced in Thailand. Therefore, the objectives of this study were to investigate hygiene status of a buffalo farm selected for this study (Chachoengsao, Thailand) and to assess the microbiological quality of raw buffalo milk stored at 3 different conditions prior to HTST pasteurization. Swabbing technique was applied to collect the micro-organisms adhering to the surfaces of teat cups, milk cans and palms of workers involved in milking process. The microbiological enumeration techniques used in this study revealed that workers' hands was the major contamination source due to the highest count of mesophilic bacteria (510-546 cfu/in<sup>2</sup>), coliform (29-40 cfu/in<sup>2</sup>) and *E. coli* (4-11 cfu/in<sup>2</sup>). Moreover, raw buffalo milk contained high numbers of mesophilic bacteria (4.18-4.38 log cfu/mL), psychrotrophic bacteria (2.52-5.76 log cfu/mL), coliform (2.83-3.17 log cfu/mL), and *E. coli* (0.67-1.73 log cfu/mL). These results indicated the need for corrective measures to be taken in personnel hygiene. In order to simulate the conditions of transportation from farm to dairy plant, raw milk (pH 7.33-7.67, 0.14-0.16 % Titratable acidity) was kept for 3 hours (A) at ambient temperature (29±1 °C) (B) at ambient temperature after being cooled to 7±1 °C and (C) at cold storage (7±1 °C). Regardless of storage conditions, the increase in total count of mesophilic bacteria was less than 1 log cycle. On the basis of the methylene blue reduction test, the raw milk kept in cold storage was judged as first grade while the others were second grade.

**Keywords:** raw buffalo milk; mesophilic bacteria count; psychrotroph bacteria count ; *E. coli* count; methylene blue reduction test

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## Introduction

Buffalo milk has recently gained more interested from health concerned consumers due to its higher content of fat, vitamin and mineral compared to cow milk (Han *et al.*, 2007). The Buffalo Research and Development Section has its primary duty in support of Thai farmers to establish buffalo farms in Thailand. Nowadays, there are many large size buffalo farms and scattered households located in several provinces such as Chachoengsao, Songkla, Chaingrai, and Kanchanaburi. Up to date, buffalo milk has been commercially produced and sold as pasteurized milk and Mozzarella cheese in the market. However, there is a lack of data on microbiological properties of raw buffalo milk produced in Thailand. Therefore, the aims of this study were to investigate hygiene status of a buffalo farm selected for this study and to assess the microbiological quality of raw buffalo milk stored at 3 different conditions prior to HTST pasteurization. The better understanding of microbiological change during transportation would provide the guideline of good hygiene practices in locally small and medium size buffalo farms.

## Materials and Methods

The experiment was done at Murah Farm (Chachoengsao, Thailand) during March-May 2009. Murah farm is considered as a large buffalo farm according to its high milk production (average yield = 100-200 kg. per day). Buffalo herd (n = 200) was comprised of typical breeds in Thailand including Murrah buffalo, Swamp buffalo and their crossbreeds. Milking was done manually twice a day at 6 am and 3 pm. At each milking (n = 3), samples of ca.1,500ml milk was taken aseptically from bulk milk container and kept in 3 of 500 ml sterile glass bottles. Each milk sample was kept for 3 hours at three different conditions as following: (A) at ambient temperature ( $29\pm 1$  °C) (B) at ambient temperature after being cooled to  $7\pm 1$  °C and (C) at cold storage ( $7\pm 1$  °C). Every hour of each storage condition, milk was sampled and analyzed to determine its quality as the following.

### Chemical analysis

The titratable acidity of sample was measured by titrating mixture of 17.6 ml of a sample and 17.6 ml of distilled water with 0.1 N NaOH with 2 drops of 1% phenolphthalein as an indicator until pink color persisted at least 15 s. The acidity was expressed as grams of lactic acid per 100 g milk (APHA,1992). The pH of each sample was measured by Orion pH meter model 250A.

### Methylene blue reduction tests (APHA, 1992)

Ten milliliters of raw milk was added into a test tube containing 1 ml of methylene blue reagent and homogenized by slowly inverting test tube a couple times. Tubes were placed in the water bath (35°C). Reduction time was recorded in whole hours between last inversion and complete de-colorization when four-fifths of the color has disappeared.

### Microbiological analysis of raw milk

Representative 25 g portions of raw milk were aseptically weighed, mixed with 225 ml peptone water, and homogenized by shaking. Subsequent decimal dilutions were prepared with the same diluent, and in all cases, duplicate counting Petrifilm were prepared of appropriate dilutions. Total count of mesophilic bacteria (TPC) and total count of psychrotrophic bacteria count were enumerated using 3M Petrifilm™ Total Plate count, after incubation at 37 °C for 2 day and at 7 °C for 10 days, respectively. Coliform bacteria count and *E. Coli* count were enumerated using 3M Petrifilm™ E.coli/coliform plates (3M, USA), after incubation at 37 °C

for 1 day and *E.coli* for 2 days. Typical colony of Coliform was indicated as a red colony while *E.coli* colony was blue.

### Swabbing Test

In this study, 27 samples from 9 different surfaces located in the milking area were examined such teat cups, milk cans and palms of workers. Microbiological samples were swabbed from the surface, using 2x2 (4 in<sup>2</sup>) sterile templates and sterile cotton buds. All samples were immediately performed microbiological analysis as aforementioned.

### Results and Discussion

The initial microbial loads of raw buffalo milk were shown in Table 1. Microbiological analysis of raw buffalo milk sampled immediately right after milking revealed that raw milk contained high amount of mesophilic bacteria (4.18-4.32 log cfu/ml). According to the EU specification (EU Directive 92/46/EEC), this bacterial count was slightly lower than the maximum level ( $5 \times 10^5$  cfu/ml) allowed in raw buffalo milk. Coliform bacteria count of raw milk was also found in the high level (2.64-3.17 log cfu/ml). The presence of coliform bacteria in raw milk generally indicates contaminations occurred during manually milking due to poor hygienic practices. Psychrotroph are known as major spoilage bacteria during cold storage prior to heat treatment of fluid milk. These microbial can produce heat resistance lipolytic enzyme which cause off-flavor due to lipid peroxidation (Wasatral et al, 1999). In Thailand, the considerable amount of HTST pasteurized milk is manufactured in small-scale dairy plants located in academic institutes, for example, Sakaeo College of Agriculture and Technology (SCAT, Aranyaprathet, Sakaeo). SCAT and other small scale dairy plants in remote area have encountered low supply of milk for operating. In order to have enough milk to cost-effectively run the HTST unit, dairy plant at SCAT operates 2-3 times a week. Hence, the cold storage of milk before subject to heat treatment is very crucial step to obtain the high quality of HTST pasteurized milk. In our study, the average psychrotrophic count of raw buffalo milk was less than 1000 cfu/ml which was about half of mesophilic count (Table 1). Traditionally, household or medium size cow farms in rural area generally delivery milk to dairy plant or milk collecting center after milking without cooling the milk temperature down to 8 °C as recommended in GMP. Therefore, the better understanding on growth of microorganism during transportation from farm to dairy plant would elucidate the cause of low quality of pasteurized milk product and subsequently the problem would be minimized.

**Table 1** Microbiological changes of raw buffalo milk stored at different storage conditions.

Storage condition*	Traits	Mean± Standard deviation			
		Storage time (hr.)			
		0	1	2	3
A	Actual temperature (°C)	28±0.57	29±0.00	29±0.00	29±0.00
	Mesophilic count (log cfu/ml)	4.18±0.31 <sup>a</sup>	4.30±0.38 <sup>ab</sup>	4.56±0.40 <sup>ab</sup>	4.87±0.21 <sup>b</sup>
	Psychrotrophic count (log cfu/ml)	2.76±0.02 <sup>a</sup>	3.01±0.01 <sup>b</sup>	3.33±0.01 <sup>c</sup>	3.46±0.01 <sup>d</sup>
	Coliform count (log cfu/ml)	2.83±0.12 <sup>a</sup>	3.09±0.20 <sup>ab</sup>	3.48±0.23 <sup>b</sup>	4.05±0.35 <sup>c</sup>
B	Actual temperature (°C)	7±0.58	18±0.58	23±0.58	24±0.58
	Mesophilic count (log cfu/ml)	4.29±0.16	4.35±0.17	4.39±0.17	4.43±0.19
	Psychrotrophic count (log cfu/ml)	2.59±0.16 <sup>a</sup>	2.73±0.01 <sup>ab</sup>	2.81±0.04 <sup>ab</sup>	2.96±0.20 <sup>b</sup>
	Coliform count (log cfu/ml)	2.64±0.08 <sup>a</sup>	2.77±0.14 <sup>ab</sup>	2.88±0.19 <sup>ab</sup>	3.00±0.13 <sup>b</sup>
C	Actual temperature (°C)	7±0.00	4±2.89	3±3.21	4±2.65
	Mesophilic count (log cfu/ml)	4.32±0.10 <sup>a</sup>	4.40±0.07 <sup>a</sup>	4.42±0.08 <sup>a</sup>	4.47±0.10 <sup>a</sup>
	Psychrotrophic count (log cfu/ml)	2.52±0.04 <sup>a</sup>	2.62±0.03 <sup>a</sup>	2.94±0.06 <sup>b</sup>	3.03±0.08 <sup>b</sup>
	Coliform count (log cfu/ml)	3.17±0.06 <sup>a</sup>	3.22±0.07 <sup>ab</sup>	3.25±0.05 <sup>ab</sup>	3.31±0.06 <sup>b</sup>

\*Storage condition: A = stored at ambient temperature (29±1 °C); B = stored at ambient temperature after being cooled to 7±1 °C and C = stored at cold storage (7±1 °C)

Means bearing different superscripts in a row differed ( P <0.05)

Three conditions of milk transport were simulated to study changes during milk transportation for 3 hours including (A) at ambient temperature (29±1 °C), (B) at ambient temperature after being cooled to 7±1 °C and (C) at cold storage (7±1 °C). The acidity and microbial counts of milk stored at each treatment were presented in Table 1 and 2. As expected, the best raw buffalo milk handling was storage of milk at low temperature (7±1 °C). Within three hours, the temperature of pre-cooled milk was gradually close to ambient temperature (Table 1). However, the acidity of raw milk slightly changed by storage time. Pre-cooled milk (B) and raw milk (C) showed the increase in total count of mesophilic and psychrotrophic bacteria less than 1 log cycle. Nonetheless, the raw milk kept in cold storage was judged as first grade while the others were second grade according to the methylene blue reduction test (MBRT)

**Table 2** Acidity, reduction time and quality grade of raw buffalo milk stored at different storage conditions.

Storage condition*	Traits	Mean± Standard deviation			
		Storage time (hr.)			
		0	1	2	3
A	Actual temp.(°C)	28±0.57	29±0.00	29±0.00	29±0.00
	pH	6.74	6.71	6.68	6.65
	% TA	0.16±0.00 <sup>a</sup>	0.17±0.00 <sup>b</sup>	0.17±0.00 <sup>c</sup>	0.18±0.00 <sup>d</sup>
	Reduction time(hr)	8,7,7	7,6,6	6,5,5	5,4,5
	Quality grade**	1 <sup>st</sup> grade	2 <sup>nd</sup> grade	2 <sup>nd</sup> grade	2 <sup>nd</sup> grade
B	Actual temp.(°C)	7±0.58	18±0.58	23±0.58	24±0.58
	pH	6.81	6.76	6.72	6.71
	% TA	0.14±0.00 <sup>a</sup>	0.15±0.00 <sup>b</sup>	0.16±0.00 <sup>c</sup>	0.16±0.00 <sup>c</sup>
	Reduction time(hr)	8,8,7	7,7,6	6,6,5	5,5,5
	Quality grade**	1 <sup>st</sup> grade	1 <sup>st</sup> grade	2 <sup>nd</sup> grade	2 <sup>nd</sup> grade
C	Actual temp.(°C)	7±0.00	4±2.89	3±3.21	4±2.65
	pH	6.83	6.82	6.82	6.82
	% TA	0.14±0.00 <sup>a</sup>	0.15±0.00 <sup>ab</sup>	0.15±0.00 <sup>ab</sup>	0.15±0.00 <sup>b</sup>
	Reduction time(hr)	8,8,7	7,7,6	7,7,6	7,7,6
	Quality grade**	1 <sup>st</sup> grade	1 <sup>st</sup> grade	1 <sup>st</sup> grade	1 <sup>st</sup> grade

\*Storage condition: A = stored at ambient temperature (29±1 °C); B = stored at ambient temperature after being cooled to 7±1 °C and C = stored at cold storage (7±1 °C)

\*\* Based on reduction time. 1<sup>st</sup> grade = reduction time > 6 hr., 2<sup>nd</sup> grade = reduction time ≤ 6 hr..

Means bearing different superscripts in a row differed ( P <0.05)

Swabbing technique was applied to collect the micro-organisms adhering to the surfaces of teat cups, milk cans and palms of workers involved in milking process. The enumeration techniques used in this study revealed that workers' hands was the major contamination source due to the highest count of mesophilic bacteria (510-546 cfu/in<sup>2</sup>), coliform (29-40 cfu/in<sup>2</sup>) and *E. coli* (4-11 cfu/in<sup>2</sup>). These results indicated the need for corrective measures to be taken in personnel hygiene.

**Table 3** Microbiological loads of milking equipment and workers.

Surface	No.	Range (cfu/in <sup>2</sup> )			
		Mesophilic count	Psychrotrophic count	Coliform count	E. coli count
Teat cup	1	18-26	1-3	7-15	0-3
	2	24-26	1-2	9-13	1-2
	3	18-20	1-2	8-11	1-4
Milk tank	1	170-184	1-2	18-24	1-3
	2	170-192	1-3	20-28	0-4
	3	160-183	1-2	17-21	1-4
Worker's hand	1	520-562	4-7	20-40	4-9
	2	529-546	5-8	27-36	4-7
	3	510-530	2-6	29-36	7-11

## References

APHA.1992. Microbiological methods for dairy products. In *Standard methods for examination of dairy products*.16<sup>th</sup> edition. Marshall, R.T. (ed.). American public health association, Washington, Dc. P.287-307.

EU Directive 92/46/EEC (2004). *Health risk for the production and placing on the market of raw milk, heat-treated milk and milk based products*. EU:EU Directive.

Han, B.-Z, Meng, Y., Li, M., Yang, Y.-X.,Ren, F.-H., Zeng, Q.-K., Nout, M.J.R. (2007). A survey on the microbiological and chemical composition of buffalo milk in china. *Food Control*, 18,742-746

Walstra , P., Geurts, T.J., Noomen, A., Jellema, A., and van Boekel, M.A.J.S. 1999. *Dairy Technology*. Marcel and Dekker.