

Pasteurization of milk through direct heating up to 75 °C over a kitchen stove at home

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Abstract

Pasteurization remains an important process that makes milk safe from pathogens and increases milk's shelf life without altering the taste and the nutritional value. But, still many people drink unpasteurized milk either because people do not know how to pasteurize the raw milk at home, or because they prefer minimally processed food. Herein, we demonstrated that once the temperature of milk raised to 65°C through simply heating over a kitchen stove can produce pasteurized quality of milk by reducing the total number of bacteria within the limit (<20,000 CFU.mL⁻¹) of U.S. Grade "A" Pasteurized Milk Ordinance, killing pathogens like coliforms, *E. coli*, *Vibrio*, *Salmonella*, *Lactobacillus spp.*, *Pseudomonas* and *Staphylococcus aureus* etc., inactivating milk's endogenous heat-resistant alkaline phosphatase (ALP), and extending milk's shelf life similar to pasteurized milk. However, heating milk up to 75°C showed greater effectiveness in killing pathogens than that of standard pasteurization (62.5°C for 30 min). Importantly, the key nutritional elements of milk including total protein, lipid, lactose, vitamin B2 and calcium remained protected even at 75°C. Thus, simply heating milk on a kitchen stove up to 65°C-75°C has been shown quick, cost-effective, an energy-saving in-home pasteurization technique to produce safe and nourishing milk with increased shelf life.

Significance | Pasteurization of milk at home

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Abbreviations: ALP, alkaline phosphatase; UHT, Ultra-heat-treatment; TSA, Tryptone Soya Agar; SMAC, Sorbitol MacConkey; TCBS, Thiosulfate citratebile salt sucrose agar; BSA, Bismuth sulfite agar; TSB, Tryptic Soy Broth; PBS, phosphate buffer saline; MSA, Mannitol Salt Agar; PDA, Potato Dextrose Agar

1. Introduction

Dairy milk remains an important source of nutrients for human being since ancient times (Nirwal S, 2013). Nearly 85% of all dairy milk is produced from cows (Gerosa & Skoet, 2012), and about 40% of the people in the world including a large number of children are thought to consume cow milk regularly (HILL, 2015). However, raw milk may contain many normal flora as well as pathogens that not only decrease milk's shelf life but also may cause zoonotic tuberculosis, brucellosis, diphtheria, scarlet fever, Q-fever, staphylococcal food poisoning, salmonellosis, typhoid fever, cholera, and gastroenteritis etc. (Cizek, Dolejska, Novotna, Haas, & Vyskocil, 2008; Leedom, 2006; Ngasala, Nonga, & Mtambo, 2015). Pasteurization of raw milk remains the most common method that applies controlled heating system like heating milk at 72°C for 15 sec (HTST: high-temperature, short-time), 138°C for 2 sec (UHT: Ultra-heat-treatment) or 62.5 °C for 30 min (Holder pasteurization) that destroy most pathogens and spoiling bacteria without causing significant loss of nutritional elements (Cappozzo, Koutchma, & Barnes, 2015; Peila et al., 2016). Unlike sterilization, the prime concern in pasteurization process is to keep the nutritional value of milk intact as much as possible rather than killing all microbes in the milk (Antunes et al., 2014). Indeed, pasteurization is aimed to reduce the number

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Figure 1 | In-home pasteurization through simply direct heating of milk in a pan over a kitchen gas stove. (Milk was stirred continuously with a clean spoon and the temperature was monitored by a digital thermometer).

that is unlikely to cause disease as well as to extend the shelf life of milk without altering the taste and nutritional value of milk (Antunes et al., 2014; Hassan Ammara, Imran, & Shahid, 2009; Myer et al., 2016).

However, pasteurization needs special devices to hold the milk at a particular temperature for certain period of time which is not convenient to perform at home with our regular household materials as temperature increases continuously while milk is heated over a cooking stove. Previously, domestic microwave oven was used as an appliance for pasteurization at home (Sieber, Eberhard, & Gallmann, 1996; Thompson & Thompson, 1990). However, oven is also not common, particularly, in developing countries where many individuals or small-scale traders own milk-producing animals at home. They often sell this raw milk in the local market and thus unpasteurized milk reaches to other people who even do not have milk-producing animals at home. More than 75% of milk in many developing countries is sold unpasteurized through informal channels (Ngasala et al., 2015). In the absence of pasteurization facilities, people usually boil the raw milk over a stove that makes the milk safer but also reduces its nutritional value (Asadullah et al., 2010; Bahman et al., 2017). Heating milk to lower temperature might be quick, cost-effective, energy-saving, and may retain better nutritional quality.

Previously, we have demonstrated that a heating method as simple as heating the human expressed breast milk in a pan over a stove to 65°C inactivated inoculated HIV type 1 (HIV-1) without destroying milk's nutritional key elements, e.g. total protein, IgG, IgA and vitamin B12 etc. (Hoque et al., 2013). It

suggested that transient heating of milk over a kitchen stove up to some particular temperature could kill pathogens without reducing milk's nutritional benefits. In this study, we examined whether simply heating dairy milk over a kitchen stove to 65°C or 75°C could kill milk's pathogens, and extend its shelf life, yet protecting milk's nutritional value as efficiently as in pasteurization so that people could perform adequate pasteurization at home using regular household materials.

2. Materials and Methods

2.1 Milk samples

Fresh cow milk was collected from farmers just after milking in ordinary PET bottles and transferred into the laboratory of Centre for Advanced Research in Sciences (CARS) in University of Dhaka within 2h. The milk was then subjected to microbiological and nutritional analyses before and after heat treatment.

2.2 Heat treatment

Cow milk was poured into a 16-cm of aluminum pan and heated over a kitchen gas stove (RobiGazi Company, Dhaka, Bangladesh) with a medium to high range of heating. As shown in Figure 1, the milk was stirred continuously with a clean spoon and the temperature was monitored by a digital thermometer (O-207WT; Dretec Co. Ltd., Japan). The thermometer beeped when the temperature of milk reached to our target temperature. The milk was then immediately transferred into a sterile Falcon tube or glass bottle, and allowed to cool to room temperature. No holding time at a particular temperature was maintained. For pasteurization, 30 mL to 500 mL of raw milk was poured into a Falcon tube or glass bottle and heated in a water bath so that the milk could remain at 62.5°C for 30 min. Unheated milk was also kept in a sterile tube or bottle on the bench at room temperature during this time. All milk samples were cooled to room temperature and used for microbiological or nutritional analyses.

2.3 Microbiological analyses

Hundred micro liters of raw milk was spread on Tryptone Soya Agar (TSA) (Oxoid Ltd., UK), Chromocult Coliform agar, Sorbitol MacConkey (SMAC) agar (Sigma-Aldrich Corp., St. Louis, MO), Cetrimide agar (Oxoid Ltd., UK), MRS agar (Sigma-Aldrich Corp., St. Louis, MO), Thiosulfate citratebile salt sucrose agar (TCBS) (Nissui Co. Ltd., Japan) or Bismuth sulfite agar (BSA) (Sigma-Aldrich Corp., St. Louis, MO) and incubated at 37°C for 24 h to detect total aerobic bacteria, coliforms, *Escherichia. coli*, *Pseudomonas aeruginosa*, *Lactobacillus spp.*, *Vibrio spp* or *Salmo-*

nella, respectively.

2.4 Spiked assay using *Staphylococcus aureus* and *E. coli*

A single colony of *Staphylococcus aureus* as well as *E. coli* were streaked onto TSA plates. Two loopfulls of each bacterial colonies were inoculated into 5 mL of Tryptic Soy Broth (TSB) (Merck, Germany) supplemented with 10 µL of rifampicin (Wako, Japan) and incubated at 37°C for 24 h. Cells were then centrifuged at 3000 rpm for 10 min, pellets were washed once with phosphate buffer saline (PBS) and suspended in 15 mL of fresh milk. Three milliliters of it was diluted into 30 mL of raw milk and subjected to heat treatment. About 100 µL of heated or unheated milk were spread on selective media, Mannitol Salt Agar (MSA) (Merck, Germany) for *Staphylococcus aureus* and SMAC for *E. coli*, and incubated at 37°C for 24 h. Colony count was performed to detect bacterial load.

2.5 Examination of Alkaline Phosphatase (ALP) activity

Different volumes (1, 0.5, and 0.25 L) of raw milk was heated up to 65°C or 75°C. Namely, once the temperature of milk reached to 65°C or 75°C, it was transferred into sterile tubes/bottles, and allowed to cool to room temperature. The activity of Alkaline Phosphatase (ALP) was examined in Institute of Food Science and Technology (IFST), Bangladesh Council of Science and Industrial Research (BCSIR), Dhaka, according to their developed protocol (BDS method 1083:2006). Unheated milk and pasteurized milk were used as positive and negative control, respectively. In brief, 1 mL of the milk was mixed with 5 mL of sterile buffer containing sodium carbonate, sodium bicarbonate, and disodium p-nitrophenyl phosphate, and kept in the water bath at 30°C for 2 h. Qualitative assessment was made by comparing the developed color with that of positive and negative controls.

2.6 Chemicals analyses

The total content of protein, lipid, and lactose of heated and unheated milk was determined by Kjeldahl, Rose-Gottlieb and Lane and Eynon's methods, respectively, at the IFST laboratory, BCSIR, Dhaka, Bangladesh. Calcium content was determined in the flame photometer and vitamin B2 content was estimated using the High-Performance Liquid Chromatography (HPLC).

2.6 Shelf life study

To examine shelf life, heated and unheated milk was transferred into autoclaved bottles inside a laminar flow and allowed to cool to room temperature. It was then kept in refrigerator (4°C) for up to 4 weeks. Sensory quality and pH was evaluated every week.

2.7 Sensory evaluation

For sensory evaluation, every time nearly 30 mL of milk was withdrawn aseptically from stored refrigerated milk bottles into disposable glasses. Total 10 independent adult panelist (5 female and 5 male), selected randomly from the teachers/researchers and students of the University of Dhaka, were asked to evaluate

the sensory quality in terms of color, smell, texture and overall acceptability using a 9-point hedonic scale, in which a score of 1 referred to 'dislike extremely' and a score of 9 referred to 'like extremely'.

3. Results

3.1 Reducing total number of bacteria in milk

About thirty milliliters of raw cow milk was poured into a pan and heated over a kitchen stove as shown in Figure 1 until the temperature of milk reached to 65°C (66±1°C) or 75°C (76±1°C). For pasteurization, thirty milliliters of raw milk was poured into a Falcon tube and kept in water bath at 62.5°C for 30 min. After cooling, heated and unheated milk were serially diluted and spread on TSA plate to determine the total bacterial count.

Numerous bacterial counts, 5 to 7 log CFU.mL⁻¹, were detected in unheated milk samples (Table 1). As shown in Table 1, every time heating milk to 75°C was found more efficient than heating to 65°C or holding pasteurization in reducing total number bacteria. Nevertheless, heating to 65°C reduced the total number of bacteria within the standard limit (≤20,000 CFU.mL⁻¹) of U.S. Grade "A" pasteurized milk quality (U.S. Department of Health and Human Services, 2015). Thus, our data suggest that although heating milk up to 65°C is less effective than that of holding pasteurization, it still can produce Grade "A" pasteurized quality of milk. The result remained consistent for as large as 1 L of milk was examined.

Table 1 | **Total bacterial count in milk after direct heating.** Total number of aerobic bacteria was determined through spread plate technique on Tryptone Soya Agar (TSA) plate. Colony count (CFU) per milliliter has been shown after 24 h of incubation.

Milk Samples	CFU.mL ⁻¹			
	Unheated	Pasteurization at 62.5°C for 30 min	Heated on stove 65°C	Heated on stove 75°C
1	2 x 10 ⁵	20	160	20
2	5 x 10 ⁶	730	2200	200
3	1 x 10 ⁷	90	1720	40
4	2 x 10 ⁷	70	300	50
5	2 x 10 ⁵	118	500	30
6	5 x 10 ⁶	90	130	20

3.2 Destruction of pathogenic bacteria through transient heating

Next, we examined whether target pathogenic bacteria in milk were killed after these heat treatments. For this, unheated raw milk, pasteurized milk (62.5°C for 30 min), or heated milk (up to 65°C or 75°C) was spread on selective/differential agar plates like Chromocult Coliform, SMAC, Cetrimide, MRS, TCBS, and BSA to enhance the selective growth of total coliform, *E. coli*, *Pseudomonas*, *Lactobacilli*, *Vibrio*, and *Salmonella*, respectively.

Pathogens detected in unheated milk were not detected in pasteurized or heated milk samples, suggesting that heating milk up to 65°C is sufficient to kill these pathogenic bacteria that are

commonly found in milk (Table 2). For further confirmation, pasteurized and heat treated milk samples were enriched by mixing 10 mL of milk with 100 mL of TSB, incubated overnight at 37°C, and then examined again on selective media. Again, no bacterial growth was detected. Our data reveal that a number of pathogenic bacteria like coliforms, *E. coli*, *Pseudomonas*, *Lactobacilli*, *Vibrio*, and *Salmonella* cannot survive after heating the milk up to 65°C.

Table 2 | Bacterial growth on selective media plate after 48 h of incubation. Representative data of six samples have been shown. ^aWhen no bacterial colony was detected on spread plate, its titer was calculated to be <10 CFU.mL⁻¹.

Media	CFU.mL ⁻¹			
	Unheated	Pasteurization at 62.5°C for 30 min	Heated on stove 65°C	75°C
Chromocult agar	8.2 x 10 ²	<10 ^a	<10	<10
SMAC	8.5 x 10 ³	<10	<10	<10
Cetrimide agar	1.7 x 10 ³	<10	<10	<10
MRS	2.5 x 10 ²	<10	<10	<10
TCBS	20	<10	<10	<10
BSA	60	<10	<10	<10

3.3 Destruction of inoculated pathogens

Next, we compared the effectiveness of transient heating to that of holding pasteurization in terms of killing pathogenic bacteria. Milk was inoculated with two most common contaminants of dairy milk (Knight-Jones, Hang'ombe, Songe, Sinkala, & Grace, 2016), Gram-positive *Staphylococcus aureus* (> 5 log CFU.mL⁻¹) or Gram-negative *E. coli* (> 6 log CFU.mL⁻¹) and subjected to heat treatment, either through pasteurization or direct heating over a stove up to 65°C or 75°C. Bacterial load of *Staphylococcus aureus* and *E. coli* were determined by spread plate technique on selective Mannitol Salt Agar (MSA) and SMAC plates, respectively.

No *Staphylococcus aureus* was detected in milk that was heated up to 75°C, suggesting that >5log CFU.mL⁻¹ *Staphylococcus aureus* was reduced due to heating milk up to 75°C, while around 3 log CFU.mL⁻¹ *Staphylococcus aureus* survived even after pasteurization or heating to 65°C (Table 3). Gram-negative *E. coli* was found even more susceptible to heat treatment and reduced by 6 log CFU.mL⁻¹ through pasteurization as well as by heating to 65°C. All heat treated milk samples in which bacterial growth was not detected were enriched in TSB and spread on TSA plates. No bacterial growth was detected even after enrichment of samples. Thus, the efficiency of heating milk up to 65°C seemed less potent but still remained comparable with that of holding pasteurization method in killing Gram-positive and Gram-negative bacteria, while heating the milk up to 75°C was more effective and safer.

Table 3 | Enumeration of inoculated bacteria in milk with or without heat treatment. Bacterial load of *Staphylococcus aureus* and *E. coli* were determined by spread plate technique on selective Mannitol Salt Agar (MSA) and Sorbitol-MacConkey agar (SMAC) plate, respectively. Representative data of two independent experiments have been shown. ^aWhen no bacterial colony was detected on spread plate, its titer was calculated to be <10 CFU.mL⁻¹.

Bacteria	CFU.mL ⁻¹			
	Unheated	Pasteurization at 62.5°C for 30 min	Heated on stove 65°C	75°C
<i>Staphylococcus aureus</i>	6 x 10 ⁵	9.2 x 10 ²	2.1 x 10 ³	<10 ^a
<i>E. coli</i>	3.5 x 10 ⁶	<10	<10	<10

3.4 Destruction of fungi in milk

To determine the effect of transient heating on destruction of fungi in milk, raw milk with or without heat treatment was poured into Potato Dextrose Agar (PDA) plate and incubated at 30°C for a week. PDA medium is composed of dehydrated potato infusion and dextrose and supplemented with acid or antibiotics to inhibit bacterial growth and encourage luxuriant fungal growth, and thus used to enumerate yeasts and molds (Beuchat et al., 2001; Deak et al., 2001). About 58 CFU.mL⁻¹ yeasts or molds that were found in unheated raw milk was reduced to 2 CFU.mL⁻¹ due to heating to 75°C, while the number was reduced to 5 and 14 CFU.mL⁻¹ because of pasteurization and heating to 65°C, respectively. Thus, again, heating milk up to 75°C was found more potent in reducing fungal growth compare to holding pasteurization or transient heating to 65°C (Table 4).

Table 4 | Fungi content in milk with or without heat treatment. Fungal growth was detected through pour plate technique into Potato Dextrose Agar (PDA) after one week incubation at 30°C. Representative data of two independent experiments have been shown.

	CFU.mL ⁻¹			
	Unheated	Pasteurization at 62.5°C for 30 min	Heated on stove 65°C	75°C
<i>Fungi</i>	58	5	14	2

3.5 Inactivation of Alkaline Phosphatase (ALP)

Since endogenous ALP enzyme of milk is slightly more heat resistant than *Mycobacterium tuberculosis* and *Coxiella burnetii*, two most heat-resistant milk-borne pathogens, thermal inactivation of ALP is considered as the standard practice for indirect assessing of pasteurization status of milk and milk products (Rankin, Christiansen, Lee, Banavara, & Lopez-Hernandez, 2010). Different volumes of raw milk (1L, 0.5L, and 0.25L) were heated up to 65°C or 75°C and ALP-activity was examined in comparison with that of boiled milk (negative control) and unheated raw milk (positive control). ALP activity was negative for milk that was heated up to 65°C or 75°C (Table 5). It suggests that direct heating

of milk on a stove up to 65°C is as efficient as proper pasteurization in killing pathogens.

Table 5 | Inactivation of Alkaline Phosphatase (ALP) in milk. Different volumes of raw milks were heated and ALP activity was examined. Color change similar to unheated raw milk (positive control) was indicated as +ve, while color change similar to boiled milk (negative control) was shown as -ve.

	Unheated		Pasteurization at 62.5°C for 30 min			Heated to 65°C			Heated to 75°C		
			0.25L	0.5L	1L	0.25L	0.5L	1L	0.25L	0.5L	1L
ALP activity	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

3.6 Chemical analysis of milk after heating

Cow milk is an important dietary source of carbohydrate (lactose), proteins (essential amino acids), fat (unsaturated fatty acids), minerals (e.g., Ca) and vitamins (e.g., B12 and B2) (Wendie L. Claeysa, 2013). As shown in Table 6, the content of total protein, lipid, lactose, free calcium and vitamin B2 did not alter significantly ($p>0.05$) due to heating milk up to 75°C. It suggests that important nutrients in milk are preserved even after direct heating of milk to 75°C.

Table 6 | Chemical composition of milk. One liter of raw milk was heated directly over a stove to 75°C. Data are means \pm SD of two independent experiments.

Nutritional elements	Unheated	Heated up to 75°C
Total Protein (%)	3.58 \pm 0.30	3.28 \pm 0.26
Total Lipid (%)	3.56 \pm 0.8	3.17 \pm 0.93
Lactose (%)	3.74 \pm 0.16	3.88 \pm 0.10
Calcium (ppm)	98 \pm 2	95 \pm 3
Vitamin B ₂ (μ g/mL)	18.3 \pm 1	16.4 \pm 1.3

3.7 Extension of Shelf life

Another important objective of milk pasteurization is to extend milk's shelf life (Myer et al., 2016). Heated milk up to 65°C or 75°C, pasteurized milk as well as unheated raw milk were stored at refrigerator (4°C), and milk's pH and sensory quality for color, smell, texture, and overall acceptance were evaluated at the hedonic scale every week for up to 4 weeks. Unheated raw milk became acidic and lost its characteristic texture and aroma because of sour taste just after the first week (Table 7), whereas the quality of milk heated up to 65°C or 75°C lasted similar to pasteurized milk up to 4 weeks.

4. Discussion

The objective of this study was to demonstrate a simple and convenient pasteurization technique that people can easily perform at home using their very common kitchen appliance. It is because a special device that can hold the milk at a particular temperature for a given time is required for traditional pasteurization which is expensive and unavailable even in many milk

industries of underdeveloped countries (Ahmed M.M. Metwally, 2011). As a result, people of these countries readily get access to unpasteurized or post-pasteurization contaminated unsafe milk. Therefore, it is important to introduce a handy in-home pasteurization technique so that people can easily make milk safe by themselves prior to drinking.

Previously, we have demonstrated that double-boiler method i.e., indirect heating of milk in hot water is unnecessary, while simple direct heating of milk in a pan over a stove to 65°C can destroy both cell-free and cell-associated HIV-1 in expressed breast milk without causing significant loss of milk's nutrients (Hoque et al., 2013). Therefore, in this study, we tried to examine if direct heating of dairy milk to 65°C could produce a quality of pasteurized milk. We have considered the main objectives of pasteurization: (1) making the food safe (2) retaining nutritional value, and (3) extending shelf life.

According to United States (U.S.) Grade "A" Pasteurized Milk Ordinance (Grade "A" PMO), 2015 Revision, Grade "A" pasteurized milk should not exceed bacterial limits 20,000 CFU.mL⁻¹, coliform 10 CFU.mL⁻¹ and phosphatase activity 350 mU.L⁻¹ (U.S. Department of Health and Human Services, 2015). Here, we investigated milk samples that often contained bacteria number exceeding the U.S. standard limit (300,000 CFU.mL⁻¹) for comm-

Table 7 | Sensory quality and pH of unheated and heated raw cow milk.

Milk	Characteristics	Hedonic Scale				
		Day 0	Day 7	Day 14	Day 21	Day 28
Unheated	Color	8.0 \pm 0.3	7.8 \pm 0.3	7.0 \pm 1.3	5.4 \pm 0.8	4.5 \pm 0.5
	Smell	7.2 \pm 0.7	6.8 \pm 0.3	5.6 \pm 1	3.6 \pm 0.8	2.75 \pm 0.8
	Texture	7.8 \pm 0.4	7.2 \pm 0.8	6.2 \pm 0.4	4.2 \pm 1.2	2.5 \pm 0.9
	Acceptance	7.8 \pm 0.4	7.5 \pm 0.5	5.6 \pm 0.8	4.0 \pm 1.1	2.5 \pm 0.9
	pH	6.5	6.4	5.0	4.6	4.3
Pasteurized at 62.5°C for 30 min	Color	8.0 \pm 0.2	7.8 \pm 0.4	7.8 \pm 0.4	6.6 \pm 0.8	5.7 \pm 0.9
	Smell	7.4 \pm 0.8	7.5 \pm 0.5	7.0 \pm 0.1	6.2 \pm 0.8	5.5 \pm 1.2
	Texture	7.8 \pm 0.4	7.3 \pm 0.5	7.2 \pm 0.4	6.4 \pm 0.5	5.2 \pm 0.9
	Acceptance	7.8 \pm 0.4	7.6 \pm 0.5	7.1 \pm 0.4	6.6 \pm 0.8	5.0 \pm 1.1
	pH	6.5	6.5	6.4	5.6	5.0
Heated to 65°C	Color	8.0 \pm 0.2	7.8 \pm 0.4	7.4 \pm 0.8	6.8 \pm 0.8	5.7 \pm 0.9
	Smell	7.8 \pm 0.4	7.3 \pm 0.8	6.6 \pm 0.5	7.0 \pm 1	6.2 \pm 0.9
	Texture	7.8 \pm 0.4	7.6 \pm 0.5	6.6 \pm 0.5	6.6 \pm 0.8	5.5 \pm 1.7
	Acceptance	7.8 \pm 0.4	7.8 \pm 0.4	6.4 \pm 0.8	6.6 \pm 0.8	5.5 \pm 1.7
	pH	6.5	6.5	6.4	6.2	5.8
Heated to 75°C	Color	7.8 \pm 0.4	7.8 \pm 0.4	7.6 \pm 0.5	6.8 \pm 0.8	5.7 \pm 0.9
	Smell	7.8 \pm 0.3	7.5 \pm 0.5	7.4 \pm 0.5	6.8 \pm 0.8	6.2 \pm 0.9
	Texture	8.0 \pm 0.4	7.5 \pm 0.4	7.0 \pm 0.1	6.6 \pm 0.5	5.0 \pm 1.5
	Acceptance	7.8 \pm 0.4	7.6 \pm 0.5	7.0 \pm 0.2	6.6 \pm 0.5	5.0 \pm 1.1
	pH	6.5	6.5	6.5	6.4	5.8

-ingled milk (U.S. Department of Health and Human Services, 2015). Even so, every time total bacterial count was reduced within the limit ($<20,000$ CFU.mL⁻¹) of Grade “A” pasteurized milk after direct heating over a stove up to 65°C (Table 1). Importantly, it killed pathogens like coliforms (<10 CFU.mL⁻¹), *E. coli*, *Pseudomonas*, *Lactobacillus spp.*, *Vibrio*, *Salmonella*, *Staphylococcus aureus* and fungi etc. (Table 2, 3 and 4), and also inactivated ALP enzyme of milk (Table 5). Thus, we have shown that heating milk up to 65°C is sufficient to produce Grade “A” pasteurized quality of milk.

However, heating milk up to 75°C was found more efficient than holding pasteurization in reducing total bacteria (Table 1), killing both Gram-negative and positive pathogens (Table 2 and 3) and fungi (Table 4). Importantly, the key nutritional elements of milk like total protein, lipid, lactose, calcium and vitamin B2 were not destroyed significantly due to direct heating of milk up to 75°C (Table 6). Thus, heating milk up to 75°C seems safer yet equally healthful.

Previously, heating milk in a microwave oven at 65°C for 30 min had been shown to reduce aerobic bacterial count up to 6 log cycles although its effectiveness in killing pathogens and increasing shelf life remained unknown (Thompson & Thompson, 1990). In this study, we used appliance which is more common, and the technique is very similar to one's daily practice of cooking. In addition, we have manifested the efficiency of our technique in reducing pathogens, increasing shelf life yet protecting nutritional value. It took only 4 to 5 min to heat one liter of milk to 75°C (data not shown). The milk remained at peak temperature (65°C or 75°C) for less than 5 sec. Thus, it resembles the HTST (72°C for 15 sec) method which has been shown better in preserving nutritional food value than holder pasteurization (Baro et al. 2011). The merits of direct heating in-home pasteurization method include (1) quick, (2) handy, (3) can be performed with household equipment similarly like day-to-day art of cooking, (4) cost-effective in terms of saving fuel energy that was spent for unnecessary boiling, and last but not least (5) heating to 75°C ensures better safety than that of holding pasteurization.

This report will mainly assist people of developing countries, who receive raw milk but have no access to traditional pasteurization. Even so, people of developed countries who are used to drink pasteurized packet/bottle milk can heat the milk before drinking to ensure safety from post-pasteurization contamination because outbreaks for drinking not only unpasteurized milk but also for post-pasteurization contaminated milk have been reported even in developed counties in recent past (David, 2012; Langer et al., 2012; Sharp, 1987). In spite of that, the demand for drinking raw milk appears to be increasing day by day (Costard, Espejo, Groenendaal, & Zagmutt, 2017; Mendelson, 2011). While better taste or easier digestion is often cited as the primary

reason, the respondents mainly choose raw milk because they believe that raw milk is more natural and healthful (Mullin & Belkoff, 2014). Indeed, consumers have a demand for minimally processed foods (White & McCarthy, 1982)).

5. Conclusions

In this study, we have shown how minimally heated milk can assure better protection against milk-borne pathogens while increasing the shelf life of milk and ensuring maximum nutritional benefits. Thus, this complete study would be helpful to increase one's confidence on heat treatment towards making this method acceptable and popular. Not only raw milk, but also many other home-made foods like juice, wine, cider, and vinegar etc. are often needed to be pasteurized to make it safe and increase shelf life. Simply heating food to 65°C or 75°C over a kitchen stove could be the best choice to meet the purposes.

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Author Contributions

SA Hoque, UN Sultana, T Hossain were contributed equally to conduct the microbiological and analytical experiments.

Competing financial interests

The author(s) declare no competing financial interests.

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