Effect of heat treatment on the bacterial load and sensory attributes of different types of milk

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Abstract

The main objective of this study was to assess the effect of different heat treatments (Low pasteurized at 68°C, flash pasteurized at 72°C, boiling at 100°C and sterilize at 121°C) on four types of milk (Cow, Camel, Sheep and Goat) and hence evaluate the quality of the produced milk. Raw milk samples were obtained from different farms in Khartoum, Sudan. They were collected in sterile bottles and transported to the laboratories in ice boxes. The microbiological content (total bacteria count, *coliform*, *thermophilic* and *thermoduric bacteria*, in addition to sensory evaluation were determined before and after heat treatment. Unlike the total bacteria count and *coliform*, the results showed a significant heat treatment impact ($P \leq 0.05$) on the thermo-bacteria (*thermophilic* and *thermoduric*). Heat treatment reduced the microbiological count in most samples. Results showed that higher score of general acceptability evaluation after heating was found to be for sterilized cow milk, but the lower score was noted for sterilized sheep milk, whereas the remnant heat-treated samples fell in the middle.

Keywords: Milk, microorganism, sensory, heat treatments, low pasteurized, flash pasteurized.

1. Introduction

Based on the reservoir and most usual mode of transmission of the pathogens, the main source of milk contamination is characterized as either contagious or environmental. The udder of sick animals is the principal reservoir for infectious infections, and the primary route of transmission is through contact with contaminated milking equipment, the hands of the milking operator, or towels used to clean numerous teats during milking. *Staphylococcus aureus* and *Streptococcus agalactiae* are the most prevalent infectious pathogens. Water, manure, and soil in the environment serve as reservoirs for environmental infections. In their walks or dwelling spaces, animals frequently come into touch with environmental diseases (Murphy 2001). Large quantities of these bacteria have the opportunity to infect the udder when the teats and udder become wet and unclean. *Coliform* bacteria (such as *Escherichia coli* and *Klebsiella spp.)* and environmental *streptococci* are the most frequent environmental pathogens (such as *Streptococcus uberis* and *Streptococcus dysgalactia*). When the Standard Plate Count (SPC) results are high, the laboratory pasteurized count (LPC) is frequently used as a diagnostic test. The LPC is an

SPC that is performed on milk that has been heated to 145 F (62.8^{0}C) and held for 30 minutes (low temperature-long time pasteurization) (Reinemann 1999). The LPC's goal is to find species that can resist pasteurization (thermoduric bacteria). Unclean equipment, poor sanitizing practices, and milk stone deposits are all linked to high LPC. Pasteurization kills off the bacteria that cause mastitis infection. *Micrococcus, Microbacterium, Lactobacillus, Bacillus, Clostridium*, and *Streptococci* are examples of thermophilic bacteria. Pasteurized milk deterioration is frequently linked to thermoduric microbes. *Coliform* counts and SPC can be elevated with LPC near normal due to poor milking hygiene. The LPC of less than 100 to 200 cfu/ml suggests great equipment hygiene, while an LPC of less than 10 cfu/ml indicates poor equipment hygiene. In New York State, 60% of 855 bulk tank samples contained less than 200 cfu/ml, and 40% contained less than 80 cfu/ml (Boor et al., 1998). *Coliform* counts are made by growing raw milk dilutions on selective media such violet red bile agar. The plates are incubated for 24 hours at 90°F (32°C). The udders of animals or improper milking procedures are the sources of *coliform* bacteria in bulk tank milk. The *coliform* count is a measure of how well animal preparation protocols work during milking and how clean the animals' environment is. *Coliforms* can also incubate on milking equipment residue films. A *coliform* count of less than 10 cfu/ml is required. A *coliform* value of 100 to 1000 indicates inadequate milking hygiene, while a *coliform* level of 1000 or more shows bacterial growth on milk handling equipment. In 855 samples from New York State, 33% of *coliform* counts were less than 10 cfu/ml, but 20% of bulk milk samples surpassed 100 cfu/ml (Reinemann et al 1999).

The ability of any microorganism to grow in food products depends on a number of limiting factors such as temperature, redox potential, pH, water activity, added preservatives and competitive *microflora* (Gaze, 1992). Milk-borne pathogens that cause food poisoning among human population are *Yersinia, enteropathogenicE.coli and Salmonella* (Shewmake and Dillon, 1998). Thermoduric bacteria such as *micrococcus, microbacterium, lactobacillus, bacillus, Clostridium and occasionally streptococci* retain their activity and can affect quality of post pasteurized product (Ruegg and Reinemann, 2002). Bacterial quality of raw milk is important for both industry and consumers, since high bacteria count on the farm contributes to poor keeping quality and inferior product. The number of bacteria depends upon the cleanliness and health of animal and milkers, cleanliness and sanitation of milking utensils and the age and storage temperature of collected milk (Ruegg and Reinemann, 2002).

2. Materials and methods

2.1. Materials

Four types of raw milk samples (Cow, Camel, Goat and Sheep) were obtained from deferent farms in Khartoum state. They were collected in sterilized bottles and transported to the laboratories in the morning in ice boxes.

2.2. Pasteurization of milk

A 500 ml from all the four samples of milk was put in four different sterilized glass bottles from each sample of milk, and heated at 68°C (till the temperature reach 68^o C) for 30 minute, 72 ºC (till the temperature reach 72^0 C) for 15 seconds, and 100 ºC for 1 minute in thermostatically controlled oil bath followed by rapid cooling in ice-bath to 5ºC. In another experiment the different four samples of milk was heated to 60 C^o (to avoid milk browning) and then followed by sterilization at 121 C^o for 1 minute by autoclave, followed by rapid cooling (Grant, *et. al.,* 1996 and Anonymous, 1997).

2.3. Microbiological Analysis

2.3.1. Media preparation

The microbiological analysis was done according to Harrigan (1998) and Barrow & Feltham (1993). Quarter strength Ringer solution was prepared by dissolving 1 tablet in 500 ml distill water and for proper dilutions 9.0 ml solution was added to test tubes and covered with tops. The solution was sterilized at 121^{o}C for 15 minutes. Agar (39.5 g) was suspended in 1.0 lit distilled water, mixed well and poured in plates. The medium was made fresh at 47

°C and used within 3 hr. The plate count agar was prepared by dissolving 23.5 g in 1 lit distilled water and heated to a boiling point with frequent stirring and sterilized at 121°C for 15 min in autoclave.

2.3.2. Milk sample preparation

Aseptically, 1.0 ml of each milk sample was pipetted into a test tube containing 9.0 ml of Ringer solution to make the first serial dilution. Subsequent dilutions will be made at this point in a petri dish which depends on the expected count. Milk samples 1.0 ml was pipetted into a petri-dish. Approximately 12-15 ml of the VRBA agar for coliform analysis and PCA/plate count skim milk agar for TVC at 44 ºC to 47 ºC were added into the petri-dishes containing milk samples, and gently swirl the agar to disperse the sample evenly. Large bubbles were formed in the agar at this stage, they were burst using a sterilized straight wire before the agar sets. A control plate for each media and for each batch of agar was poured and allowed to solidify and level. After complete solidification, about 4.0 ml VRBA medium was added at 44 ºC to 47 ºC on the surface of the inculcated medium and allowed to solidify as described above. Only in the case where it is suspected that the product under examination contains microorganisms, whose colonies will grow on the surface of the medium, pour about 4.0 ml of the overlay medium at 44 ºC to47 ºC on the surface of the inculcated medium. Allow to solidify as described above. Incubate the plates at 32 °C$\pm$1.0 °C for 24–48 hrs. A continuous plating operation was conducted by the team so that the time between the initial transfer of the test portion to the diluents or directly in the plate and the pouring of the last sample is not more than 20 min.

2.3.3. Examination of the plates

A white background behind the plates was used when counting VRBA plates, and black background for total viable count (TVC). Violet red bile agar (VRBA) count of the colonies which are dark red and 0.5 mm to 2 mm in diameter, usually are surrounded by a reddish zone. Due to the nutrient broth medium (N.B), it is possible for moulds to grow giving a cloudy tiny appearance. Plate count agar (PCA) count colonies which can take any size between pinpoint and spreader colonies which should not be mistaken for particles of undissolved or precipitated matter in dishes for pinpoint colonies. Doubtful objects were examined carefully using higher magnifier, when required, in order to distinguish colonies from foreign materials and the number and type was identified. The N.B spreader type colonies may interfere with examining plates showing spreader were interpreted with caution.

2.3.4. Sensory evaluation

Sensory evaluation test determined according to 7.0 point hedonic scale. 7-point hedonic scale designed was employed to elucidate panelists' acceptance of color, taste, odor, texture and overall acceptability.

2.4. Statistical analysis

All Data were subjected to statically analysis using Statistical Analysis System (SAS). Significant differences between means were determined by Duncan's Multiple Range Test (DMRT) at $p < 0.05$, as reported by Montgomery, (2001).

3. Results and discussion

3.1. Microbial Content

The total microbial count is calculated using the $\log^{10}$cfu/mL (cfu=colony forming unit). The results in Table 1 showed that the total bacteria count of raw milk was 11050 in cow milk, >11050 in camel, sheep and goat milk. Heat treatment significantly ($p<0.05$) reduced the bacterial load of all raw milk, although the reduction in goat milk was a lot less compare to the other types of milk. Different heat treatments reduced the total bacteria count differently, where sterilized at 121 °C exhibited the least load for all 4 milk types. The effect can be ranked as: sterilized < boiled < flash pasteurized < low pasteurized. Overall, goat milk appeared to maintain more bacterial load after heat processing compared to the other milk types. Ammara *et. al.*, (2009) stated that the total bacteria count

was zero after ultra-high pasteurization at 138°C for a fraction of a second of cow milk compared to 121°C for 1.0 min used in this study. Hassan *et. al*., (2009) said that the total bacteria count (log10 cfu/ml) of Milk heated at UHT (Ultra High Temperature), HP (High Pasteurized), LP (Low Pasteurized) was found to be (1.06 – 1.60), (2.35 – 4.50) and (2.04 – 6.91), respectively. Verraes *et. al*., (2014) reported a range of total bacteria count (log10 cfu/ml) for different milks was 5.28, 1.90–5.13, (2.20–7.60) and (4.05–4.31) for camel, sheep, goat and cow, respectively. This variation of total bacteria count (log10 cfu/L) in raw milk may be attributed to the animal, hygienic practice, state of the animal, equipment, stencils, milking environment. Therefore, the decrease in total bacteria count (log10 cfu/mL) in Milk samples is accredited to the effect of sterilization heat on microorganism. It is apparent that raw cow milk exhibited low bacterial count compared to the other animals. The data showed that flash pasteurization, boiling at 100 °C and sterilization are equally effective in reducing the bacterial load of all tested milk types used in this study.

Table (1): Effect of heat treatment on the total bacterial count (log^{10}cfu/mL)

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Raw milk	11050.00^{a}±26.85	$>11050^{a}$	$>11050^{a}$	$>11050^{a}$
Low pasteurized at 68°C	960.00^{b}±6.32	60.50^{c}±4.62	100.00^{b}±1.46	1000^{b}±15.47
Flash pasteurized at 72°C	60.00^{c}±5.04	80.00^{b}±3.67	54.50^{c}±9.85	188.00^{c}±8.52
Boiled at 100°C	69.00^{c}±1.25	30.00^{c}±2.39	41.00^{c}±6.43	105.00^{c}±7.46
Sterilized at 121°C	0.00^{d}±0.0	5.00^{d}±0.36	26.00^{d}±2.19	59.00^{d}±1.63

Mean(s) sharing same superscript (s) in columns are not significantly different (P>0.05)

3.2. Coliforms (MPN/L)

As shown in Table (2), raw goat milk coliform count was > camel > sheep > cow. After heat treatment, cow and camel milk showed zero coliform count, whereas sheep and very little count. Once again, heat treatments were equally effective in reducing coliform count in all milk type. The results showed that there is no significant difference (P≤ 0.05) between raw milk samples of goat, sheep and camel, in addition significantly low count in cow milk (Table2). The variation of Coliforms *(MPN/L)* in raw milk may be due to hygienic practice and milking conditions. The presence of organisms was not identified in this investigation, despite reports in the literature that there may be contamination of bacterial enzymes in raw milk that is being processed, causing lipolysis and proteolysis. Although pasteurization or other heat treatments kill microorganisms, spore germination or recontamination can still degrade milk quality; moreover, heat-resistant extracellular proteinases and lipases produced by some bacteria before processing are a key spoiling factor in stored milk (Sorhaug and Stepaniak 1997).

Table (2): Effect of heat treatment and type of milk on *coliforms* (MPN/L)

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Raw milk	760.00^{a}±24.37	2110.00^{a}±22.15	1600.00^{a}±29.37	2396.00^{a}±41.25
Low pasteurized (68°C)	0.00^{b}±0.00	0.00^{b}±0.00	2.50^{b}±0.03	2.00^{b}±0.01
Flash pasteurized (72°C)	0.50^{b}±0.01	0.00^{b}±0.00	0.00^{b}±0.00	9.00^{b}±0.07
Boiled (100 °C)	0.00^{b}±0.00	0.00^{b}±0.00	2.50^{b}±0.03	6.00^{b}±0.04
Sterilized (121°C)	0.00^{b}±0.00	0.00^{b}±0.00	14.00^{b}±0.13	4.00^{b}±0.03

Mean(s) sharing same superscript (s) in columns are not significantly different (P>0.05)

3.3. Thermoduric Bacteria

As shown in Table (3) the data showed that the Thermoduric Bacteria of raw milk was higher in Goat > Camel > Sheep > Cow. The results showed that there was no significant difference between raw sheep and camel milk samples, but there was a significant difference between Goat milk and the other milk sources (Table 3). Heat

treatment reduced Thermoduric Bacteria in all samples but it did not reach zero count as it was for coliform count. The same heat treatment was equally successful in suppressing thermoduric bacteria, but heat treatment had no influence on the count within milk types except for the sterilization treatment, where low and flash pasteurization, as well as boiling at 100 °C, had similar effects on the count (Table 3).

Table (3): Effect of heat treatment and type of milk on *thermoduric bacteria*

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Raw milk	50.00^{a}±0.21	1090.00^{a}±38.26	910.00^{a}±18.52	4000.00^{a}±0.51.39
Low pasteurized at 68°C	13.00^{b}±0.10	36.00^{b}±0.17	11.00^{b}±0.09	14.50^{b}±0.16
Flash pasteurized at 72°C	9.50^{b}±0.08	24.00^{b}±0.13	8.50^{b}±0.00	9.00^{b}±0.07
Boiled at 100°C	9.00^{b}±0.07	26.00^{b}±0.15	7.50^{b}±0.03	11.50^{b}±0.13
Sterilized at 121°C	2.00^{c}±0.01	13.50^{c}±0.10	5.00^{b}±0.02	10.00^{b}±0.11

Mean(s) sharing same superscript(s) in columns are not significantly different (P>0.05)

3.4. Thermophilic Bacteria

As shown in Table (4) the results showed that the thermophilic bacteria count of raw of milk was 3300, 1590, 1450 and 600 in camel, goat, cow and sheep, respectively. The data showed that the low and flash pasteurization treatment was more effective in reducing the thermophilic bacteria of cow milk, but overall the load was significantly lower than raw milk of all milk type. Consequently, the data showed that there was a little difference between the load of raw cow and goat milk samples, in addition major difference between sheep and camel (Table 4). It is obvious the extremely high thermophilic bacterial load of camel milk. The effect of boiling at 100 °C and heating at 121 °C exhibited the least effect on the bacterial count where the count was higher compared to the other two heat treatments (Table 4). Stulova et. al., (2011) reported the range of Thermophilic Bacteria count in UHT treated milk was between 0.05 and 16.02 which is in agreement with the data reported here. Despite the fact that heat greatly lowered the bacterium count to an acceptable level, it did not reach zero.

Table (4): Effect of heat treatment and type of milk on thermophilic bacteria

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Raw milk	1450.00^{a}±50.47	3300.00^{a}±246.13	600.00^{a}±20.95	1590.00^{a}±69.52
Low pasteurized at 68°C	4.00^{c}±0.05	1.00^{b}±0.01	32.00^{c}±0.02	7.00^{d}±0.01
Flash pasteurized at 72°C	7.50^{c}±0.08	3.00^{b}±0.01	11.00^{c}±0.04	8.00^{d}±0.02
Boiled at 100°C	30.00^{b}±0.07	8.50^{b}±0.01	80.00^{b}±22.26	52.50^{c}±0.19
Sterilized at 121°C	9.00^{c}±0.01	5.00^{b}±0.02	9.50^{c}±0.01	74.00^{b}±18.56

Mean(s) sharing same superscript(s) in columns are not significantly different (P>0.05)

3.5. Sensory Evaluation

3.5.1. Color

Color is an important factor that has a significant impact on customer acceptance. Changes in casein size and denaturation of whey protein both increase the amount of light scatter (reflectance) in milk after heat treatment, making it look whiter. Browning, on the other hand, counteracts this benefit by lowering the degree of reflectivity and resulting in a mild white color. As shown in Table (5) the color of the tested cow and camel milk was not significantly affected by the different heat treatments, but a slit reduction on the color was recorded, whereas sheep and goat milk was significantly affected by sterilization at 121 °C. Therefore, we can infer higher sensitivity of sheep and goat milk to heat treatment, especially higher temperatures, from the data presented here. Sheep milk exhibited the highest color value followed by goat milk. One of the most common causes of color loss is the Maillard reaction. Increases in heat treatment temperature and time resulted in lower color scores (Martel et.

al., 1987). The color score for sheep and goat milk was the lowest after sterilization, whereas the color score for cow milk was the highest after low pasteurization.

Table (5): Effect of heat treatment and type of milk on color

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Low pasteurized at 68°C	6.25^{a}±0.03	6.00^{a}±0.03	5.75^{a}±0.01	5.50^{a}±0.04
Flash pasteurized at 72°C	5.00^{a}±0.01	5.00^{a}±0.01	6.00^{a}±0.02	5.25^{a}±0.02
Boiled at 100°C	5.50^{a}±0.02	5.25^{a}±0.01	4.50^{a}±0.02	4.25^{a}±0.01
Sterilized at 121°C	5.00^{a}±0.01	2.50^{a}±0.00	2.00^{b}±0.00	2.25^{b}±0.01

Mean(s) sharing same superscript(s) in columns are not significantly different (P>0.05)

3.5.2. Odor

As indicated in Table (6), heat treatments had an impact on the odor value, which was reduced in most samples as the temperature climbed. Other milk types, with the exception of cow milk, showed a reduction in odor after being boiled at 100° C or sterilized. Pasteurized milk samples had the strongest odor. Cow milk had the strongest odor, whereas sheep milk had the weakest. Milk flavor is attributed to a decrease in flavorsome chemicals, such as Maillard browning, the creation of sulfhydryl compounds, and the formation of a variety of carbonyl and other flavorsome compounds (Datta et. al., 2002).

Table (6): Effect of heat treatment and type of milk on odor

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
(B) Low pasteurized at 68°C	5.00^{a}±0.01	4.75^{a}±0.02	4.00^{a}±0.03	4.25^{a}±0.04
(C) Flash pasteurized at 72°C	5.25^{a}±0.03	4.75^{a}±0.01	4.00^{a}±0.01	4.50^{a}±0.01
(D) Boiled at 100°C	5.00^{a}±0.02	4.25^{b}±0.01	2.50^{c}±0.02	4.00^{b}±0.02
(E) Sterilized at 121°C	5.25^{a}±0.01	3.75^{b}±0.03	1.00^{c}±0.00	2.50^{c}±0.02

Mean(s) sharing same superscript(s) in columns are not significantly different (P>0.05)

3.5.3. Taste

The taste score is presented in Table 7. The results showed that there was a significant difference (P≤ 0.05) between most samples treated at different heating process. Cow and camel milk were the least influenced by the heating procedure, whereas sheep milk tasted the most affected. Furthermore, after sterilization, the biggest change was noticed in sheep and goat milk. After heat processing, camel milk tasted the same, except after sterilization, when it scored much lower. This means that there aren't many volatile materials present. Cow milk exhibited the highest taste score regardless of heat treatment.

Table (7): Effect of heat treatment and type of milk on taste

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Low pasteurized at 68°C	5.50^{b}±0.04	4.00^{a}±0.00	3.50^{a}±0.01	4.25^{a}±0.02
Flash pasteurized at 72°C	5.25^{b}±0.01	4.50^{a}±0.01	2.50^{b}±0.03	3.75^{b}±0.01
Boiled at 100°C	5.75^{b}±0.02	4.25^{a}±0.02	2.25^{b}±0.01	3.00^{b}±0.02
Sterilized at 121°C	6.50^{a}±0.01	2.50^{b}±0.02	1.00^{c}±0.00	1.50^{c}±0.03

Mean(s) sharing same superscript(s) in columns are not significantly different (P>0.05)

3.5.4. Texture

Within each milk sample and across heat treatments, the texture data revealed no significant differences (Table 8). Except for sheep milk, where the texture reduced after flash pasteurization, boiling, and sterilizing, the level of heat treatments had no effect on the texture value. Cow milk treated at boiling had the greatest texture score, whereas sterilized sheep milk received the lowest.

Table (8): Effect of heat treatment and type of milk on texture

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Low pasteurized at 68°C	5.50^{a}±0.02	5.75^{a}±0.01	4.25^{a}±0.02	5.25^{a}±0.01
Flash pasteurized at 72°C	5.25^{a}±0.01	4.50^{a}±0.01	4.00^{b}±0.01	4.75^{a}±0.02
Boiled at 100°C	6.00^{a}±0.01	5.00^{a}±0.00	2.00^{c}±0.03	4.50^{a}±0.02
Sterilized at 121°C	5.75^{a}±0.03	4.25^{a}±0.02	1.25^{c}±0.02	4.50^{a}±0.01

Mean(s) sharing same superscript(s) in columns are not significantly different (P>0.05)

3.5.5. General Acceptability

Heat treatment of all evaluated products resulted in a significant difference (P≤ 0.05) in general acceptance results. Heat treatment improved the general acceptability of the milk sample in some circumstances, such as cow milk (Table 9). Heat treatment, on the other hand, had a detrimental impact on the acceptability of camel, sheep, and goat milk. Heat treatment had the greatest impact on goat milk acceptance, with all heat treatments reducing acceptability except for low pasteurization. Heat processed sheep milk after sterilization was the least desirable milk, whereas cow milk after boiling and sterilization was the most acceptable. Hence, the overall acceptability can be ranked as: cow > camel > goat > sheep.

Table (9): Effect of heat treatment and type of milk on overall Acceptability

Treatment/milk type	**Cow**	**Camel**	**Sheep**	**Goat**
Low pasteurized at 68°C	5.75^{b}±0.03	5.50^{a}±0.02	4.00^{a}±0.02	5.25^{a}±0.05
Flash pasteurized at 72°C	5.25^{b}±0.01	4.50^{b}±0.00	4.25^{a}±0.01	4.75^{b}±0.03
Boiled at 100°C	6.00^{a}±0.02	4.50^{b}±0.01	3.00^{b}±0.03	4.00^{b}±0.01
Sterilized at 121°C	6.50^{a}±0.04	3.25^{c}±0.03	1.00^{c}±0.02	2.75^{c}±0.02

Mean(s) sharing same superscript(s) in columns are not significantly different (P>0.05)

4. Conclusion

Except for total bacteria count and Coliform, the results demonstrated a significant effect of heat treatment and raw milk source on the thermal bacteria (thermophilic and thermoduric) investigated in this study. Raw camel milk contained more thermophilic bacteria 3300.00 M/L, coliforms 2396 MPN/L, and thermoduric 4000.00 M/L than raw cow milk, but raw camel, sheep, and goat milk had higher value than raw cow milk. Heat treatment was found to be beneficial in modifying the qualities of milk, with greater heat lowering the microbiological count and improving the sensory evaluation of heat treated milk. Heat treated cow milk that had been sterilized by boiling at 100°C was the most widely accepted, whereas sterilized sheep milk was the least well accepted.

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