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RESEARCH ARTICLE

Effect of Heat Treatments and Containers on Physiochemical Properties and Microbial Content of Cow Milk

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Abstract

This study was conducted to evaluate the influence of heat treatments by water path without cover (A), water path with cover (B), direct flame without cover (C) and direct flame with cover (D) in two containers Steel (S) and Aluminum (M) on chemical, physical and microbial content of cow milk sample. Proximate analysis for milk sample was performed and physicochemical properties (pH, SNF and density), minerals content (P, Ca, Mg, Na and K) and microbiological analysis (Total viable count of bacteria, Total coli forms and *E. coli*) were examined for heated milk samples. Heating was increased for all the proximate analysis of milk. The results showed that there were significant differences ($p \leq 0.05$) between raw milk sample and all heated milk samples in the two types of containers. Method of heat treatment and containers type significantly ($p \leq 0.05$) increased the SNF and density, but decreased pH. Heating also significantly increased Ca, Mg, Na and K but decreased P. The microbiological content of raw milk was significantly decreased by the four methods of heating in two types of containers. In the sensory evaluation of heated milk samples, the panelists preferred the colour, taste, texture and generally accepted the sample that was heated in aluminum container by direct flame with cover and the odour of sample that heated in steel container by direct flame without cover. The sensory evaluation results showed there were no significant differences ($P \leq 0.05$) between milk samples heated in the two types of containers.

Keywords: Heat treatments, cow milk, physicochemical properties, containers, aluminium.

Introduction

The main objective of milk heat treatment is to eliminate pathogenic microorganisms or reduce them to safe level for human consumption. In modern dairy industry, milk heat treatment is the major method for milk preservation and extending the shelf-life. Heat treatment methods include thermization, low temperature long time, high temperature short time, sterilization and ultra high temperature (Gedam *et al.,* 2007). However, in many rural areas, traditional methods such as boiling are the methods of choice. Recently, many methods other than heat treatment were used to improve the quality of fresh milk including ultraviolet treatment (Reinemann *et al.,* 2006) and microwave. The quality of most dairy products is closely related to the microbial status of raw milk from which they are manufactured. Depending on the temperature, conditions and length of milk storage, various groups of microorganisms can undergo a period of intensive growth producing high concentrations of enzymes, particularly lipases and proteinases. Although the microorganisms are destroyed by sterilization, the enzymes produced may remain active in sterilized products (Mani and Huber, 1997). The heat stability of milk is reduced when it is first homogenized and heated, but nowadays homogenization is followed before heating (Walstra *et al*., 2006). Considering the above factors in view, the objective of this study is to evaluate the effect of heat treatments and container type on physiochemical properties and microbial content of cow milk.

Materials and methods

Source of milk: Raw cow milk samples were obtained from University of Khartoum farm in Shambat, Khartoum North, Sudan. Milk was collected in sterilized bottles and transported to the laboratory in the morning in ice boxes.

Methods of heat treatment: Milk samples were heated at $100^{o}C$ in Steel and Aluminum containers with four treatments namely water bath without cover (A), water bath with cover (B), direct flame without cover (C) and direct flame with cover (D).

Proximate analysis: The chemical composition was examined for raw and treated milk for the following parameters.

Determination of fat, protein and lactose: Each sample were tested using Lacto scan (90) Milk Analyzer (ALPS, industries services–La Roche Sur Foron, France). The apparatus reads automatically the following constituents: fat, protein and lactose. The milk (20 mL) was decanted in a special plastic tube of 25 mL capacity. The apparatus pull up the tube and fix it, 40 sec later; the results were printed out as % composition of the pre-mentioned components. Each sample was monitored three times and the mean values were calculated.

Ash content determination: Ash content was determined according to (AOAC, 1990). Five mL of milk was taken into suitable clean and dry crucibles and then evaporated on steam bath until dryness. The crucibles were then placed in a muffle furnace at 550^{0}C for 3 h until ashes carbon free were obtained, then cooled in a desiccator and weighed. The ash content was calculated using the following equation:
Ash content (%) = $w_1/w_0 \times 100$
Where W_1 = weight of ash and W_0 = weight of sample.

Physicochemical properties: The pH was determined using pH meter (Pulp model 98107, Mauritius). Before determination, pH meter was calibrated using buffer solutions No. 4 and 7. Solids not fat (SNF) and density were determined by lacto scan (90) Milk Analyzer (ALPS, industries services–La Roche Sur Foron, France) by using same procedure stated above.

Minerals analysis: Mineral content of samples were determined according to the dry-ashing method (Pearson, 1981). Two grams from each sample were placed in Porcelain dish, burnt in muffle furnace at 550°C and placed in a sand bath for 10 min after addition of 5 N HCl. Then the solution was carefully filtered in a 100 mL volumetric flask and finally distilled water was added up to mark. From this extract, the elements Calcium, Sodium, Potassium and Magnesium were determined using Perkin Elmer Atomic Absorption Spectroscopy. Ammonium vanadate was used to determine phosphorus by the ammonium molybdate method of Chapman and Pratt (1982).

Microbiological examinations: Total viable count of bacteria was carried out using the pour plate count method as described by Harrigan (1998). A serial dilution (10^{-1} to 10^{-6}) from each sample was prepared by using sterile diluents (0.1 peptone water). One mL of each dilution was transferred into sterile petri dishes, to each plate, 15 mL of sterile melted plate count agar were added. The inoculums were mixed with medium and allowed to solidify. The plates were incubated at 37°C for 48 h. A colony counter was used to count the viable bacterial colonies after incubation and the results were expressed as colony forming units (CFU) per gram. Coli form bacteria were determined by using the most probable number (MPN) technique.

One mL of each of the three first dilutions (10^{-1}, 10^{-2} and 10^{-3}) was inoculated in triplicates of MacConkey broth test tubes containing Durham tubes, the tubes were incubated at 37°C for 48 h. The production of acid together with sufficient gas to fill the concave of the Durham tube is recorded as positive presumptive test. *Escherichia coli* test was carried out; every tube showing positive result in the presumptive test was inoculated into a tube of EC broth containing Durham tube. The tubes were incubated at 44.5°C for 24 h. Tubes showing any amount of gas were considered positive and then the most probable number was recorded. For further confirmation of *E. coli* tubes, for EC showing positive result at 44.5°C for 24 h were streaked on Eosin methylene blue (EMB) agar plates, the plates were incubated at 37°C for 48 h. Colonies of *E. coli* are usually small with metallic green sheen on EMB agar.

Sensory evaluation: The tests were conducted using conventional profiling by semi-trained panelists. Twenty judges who had successfully passed standardized tests for olfactory and taste sensitivities as well as verbal abilities and creativity were selected. The panelists were given a hedonic questionnaire of coded samples. They were scored on a scale of 1-5 (1= poor, 2=fair, 3=good, 4=very good, 5=excellent).

Statistical analysis: All Data were subjected to statistical analysis using Statistical Analysis System (SAS). Two-factor Randomized Complete Design (RCD) was performed, where factor A = samples (4) and factor B = type of container (2). Significant differences between means were determined by Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$, as reported by Montgomery (2001).

Results and discussion

Proximate composition (%) of milk samples: The effect of heating methods and container type on a proximate analysis were found:

Protein content (%): As shown in Table 1, the protein content (%) of raw milk sample was found to be 3.54, the protein content (%) of milk heated in Steel (S) containers: for samples: A (water path without cover), B (water path with cover), C (direct flame without cover) and D (direct flame with cover) were found to be 3.81, 3.70, 3.77 and 3.72 respectively. On the other hand, the protein content (%) of milk heated in Aluminum (M) containers for samples: A, B, C and D were found to be 3.83, 3.73, 3.71 and 3.65 respectively. These results were within the range 3.50-3.70% reported by Ayman (2011). These results showed that there were significant differences ($p \leq 0.05$) between raw milk sample and heated milk samples in the two types of containers by all methods of heating. These results showed that heating increased the protein content (%) in milk, this may be attributed to increase milk concentration, the variation of protein content (%) of heated milk attributed to containers and heating methods.

Table 1. Proximate composition (%) of raw and treated milk samples.

Sample	Protein content (%)		Fat content (%)		Ash content (%)		Lactose (%)	
	Steel	Aluminum	Steel	Aluminum	Steel	Aluminum	Steel	Aluminum
Raw milk	3.54±0.02^{d}		4.56±0.01^{f}		0.20±0.01^{g}		4.82±0.03^{e}	
A	3.81±0.03^{a}	3.83±0.01^{a}	5.14±0.01^{d}	5.36±0.06^{c}	0.61±0.01^{f}	0.66±0.01^{d}	5.18±0.04^{a}	5.19±0.01^{a}
B	3.70±0.04^{bc}	3.73±0.01^{bc}	4.96±0.02^{e}	5.48±0.08^{b}	0.76±0.01^{c}	0.66±0.01^{e}	5.03±0.05^{bc}	5.03±0.00^{c}
C	3.77±0.01^{ab}	3.71±0.05^{bc}	5.57±0.03^{b}	5.93±0.04^{a}	0.16±0.01^{h}	0.13±0.01^{i}	5.09±0.01^{b}	4.98±0.06^{cd}
D	3.72±0.02^{bc}	3.65±0.03^{c}	5.37±0.00^{c}	5.87±0.02^{a}	1.50±0.02^{a}	0.96±0.02^{b}	5.03±0.03^{bc}	4.19±0.04^{d}

Values are mean of three replicates ±SD; Any two mean value(s) bearing different superscript letter(s) are differ significantly (p≤0.05) according to DMRT; A ≡ water path without cover and B ≡ water path with cover; C ≡ direct flame without cover and D ≡ direct flame with cover.

Table 2. Physiochemical properties of raw and treated milk samples.

Sample	pH value		SNF		Bulk density	
	Steel	Aluminum	Steel	Aluminum	Steel	Aluminum
Raw milk	4.44±0.02^{a}		9.05±0.04^{e}		1.034±0.00^{c}	
A	4.44±0.01^{a}	4.36±0.02^{c}	9.75±0.08^{a}	4.44±0.01^{a}	4.36±0.02^{c}	9.75±0.08^{a}
B	3.91±0.02^{i}	3.92±0.03^{h}	9.45±0.08^{bc}	3.91±0.02^{i}	3.92±0.03^{h}	9.45±0.08^{bc}
C	4.03±0.02^{g}	4.08±0.01^{f}	9.60±0.01^{b}	4.03±0.02^{g}	4.08±0.01^{f}	9.60±0.01^{b}
D	4.13±0.03^{e}	4.21±0.02^{d}	9.48±0.05^{bc}	4.13±0.03^{e}	4.21±0.02^{d}	9.48±0.05^{bc}

Fat content (%): As shown in Table 1; the fat content (%) of raw milk sample was found to be 4.56, the fat content (%) of milk heated in S container for samples A, B, C and D were found to be 5.14, 4.96, 5.57 and 5.37 respectively. The fat content (%) of milk samples heated in M container for samples A, B, C and D were found to be 5.36, 5.48, 5.93 and 5.87 respectively. These results were within the range 4.16-4.97% reported by Ayman (2011). There were significant differences (p≤0.05) between raw milk sample and heated milk samples in the two types of containers by all methods of heating.

Ash content (%): As shown in Table 1; the ash content (%) of raw milk sample was found to be 0.20, the ash content (%) of milk samples heated in S container for samples: A, B, C and D were found to be 0.61, 0.76, 0.16 and 1.50. The ash content (%) of milk samples heated in M containers for samples: A, B, C and D were found to be 0.66, 0.66, 0.13 and 0.96. These results showed that there were significant differences (p≤0.05) between raw milk sample and milk samples heated in the two types of containers by all methods of heating and these results were within the range 0.66-0.78% reported by Ayman (2011). These results showed that heating increased the Ash content (%) in milk except CS and CM method decreased Ash content, this may be attributed to increase in milk concentration and heating induced evaporation of water, the variation of fat content (%) of heated milk attributed to containers, heating methods and lab conditions.

Lactose content (%): As shown in Table 1; the Lactose content (%) of raw milk sample was found to be 4.82, the lactose content (%) of milk samples heated in S containers for samples: A, B, C and D were found to be 5.18, 5.03, 5.09 and 5.03. On the other hand, lactose content (%) of milk samples heated in M containers for samples A, B, C and D were found to be 5.19, 5.03, 4.98 and 4.19 respectively. These results showed that there were significant differences (p≤0.05) between raw milk sample and heated milk samples in two types of containers by all methods of heating. These results were within the range 4.73-4.93% reported by Ayman (2011). These results showed that heating increased the lactose content (%) in milk may be attributed to increase in milk concentration and heating induced evaporation of water, the variation of lactose content (%) of heated milk attributed to containers, heating methods and lab conditions**.**

Physicochemical properties of milk samples: pH value of raw milk sample was found to be 4.44, the results showed that the pH value of milk samples heated in S containers for samples A, B, C and D were found to be 4.44, 3.91, 4.03 and 4.13. The results showed that the pH value of milk samples heated in M containers for samples A, B, C and D were found to be 4.36, 3.92, 4.08 and 4.21. There were a significant difference (p≤0.05) between raw milk sample and heated milk samples in the two type of containers by all methods of heating except AS, there was no significant difference (p≤0.05) between this and raw milk sample. Heating decreased the pH value in the milk, it may be due to Lactobacillus which may had a favourable condition for producing lactic acid and the variation of pH of heated milk attributed to containers and heating methods**.** As shown in Table 2, the SNF of raw milk sample was found to be 9.05, the SNF of milk samples heated in S containers for samples A, B, C and D were found to be 9.75, 9.45, 9.60 and 9.48. The SNF of milk samples heated in M containers for samples A, B, C and D were found to be 9.76, 9.49, 9.42 and 9.27. These results showed that there were a significant differences (p≤0.05) between raw milk sample and heated milk samples in two types of containers by all methods of heating, the results showed there were a significant differences (P≤ 0.05) between A and B, C, D, but there was no significant difference (p≤0.05) between B, C and D.

Table 3. Proximate composition (%) of raw and treated milk samples.

Sample	Phosphorous (g/100 mL)		Calcium (g/100 mL)		Magnesium (g/100 mL)		Sodium (g/100 mL)		Potassium (g/100 mL)	
	S	A	S	A	S	A	S	A	S	A
Raw milk	0.0350±0.00^{a}		0.0093±0.00^{i}		0.0040±0.00^{g}		0.0020±0.00^{g}		0.0010±0.00^{g}	
A	0.0260 ± 0.00^{bc}	0.0240 ± 0.00^{d}	0.0407 ± 0.00^{f}	0.1557 ± 0.00^{a}	0.0880 ± 0.00^{a}	0.0260 ± 0.00^{bc}	0.0240 ± 0.00^{d}	0.0407 ± 0.00^{f}	0.1557 ± 0.00^{a}	0.0880 ± 0.00^{a}
B	0.0263 ± 0.00^{b}	0.0217 ± 0.00^{f}	0.0463 ± 0.00^{e}	0.0610 ± 0.00^{c}	0.0830 ± 0.00^{c}	0.0263 ± 0.00^{b}	0.0217 ± 0.00^{f}	0.0463 ± 0.00^{e}	0.0610 ± 0.00^{c}	0.0830 ± 0.00^{c}
C	0.0263 ± 0.00^{b}	0.0233 ± 0.00^{e}	0.0347 ± 0.00^{h}	0.0523 ± 0.00^{d}	0.0533 ± 0.00^{d}	0.0263 ± 0.00^{b}	0.0233 ± 0.00^{e}	0.0347 ± 0.00^{h}	0.0523 ± 0.00^{d}	0.0533 ± 0.00^{d}
D	0.0257 ± 0.00^{c}	0.0237 ± 0.00^{de}	0.0960 ± 0.00^{b}	0.0357 ± 0.00^{g}	0.0450 ± 0.00^{e}	0.0257 ± 0.00^{c}	0.0237 ± 0.00^{de}	0.0960 ± 0.00^{b}	0.0357 ± 0.00^{g}	0.0450 ± 0.00^{e}

Values are mean of three replicates ±SD; Any two mean value(s) bearing different superscript letter(s) are differ significantly (p≤0.05) according to DMRT.

Table 4. Microbiological analysis of raw and treated milk samples.

Sample	Total viable count of bacteria (cfu/mL)		Coli forms (MPN/mL)		Total viable count of bacteria (cfu/mL)	
	Steel	Aluminum	Steel	Aluminum	Steel	Aluminum
Raw milk	48670.00±14843.63^{a}		24.33±3.51^{a}		10.67±1.53^{a}	
A	700.00±100.00^{b}	80.00±10.00^{b}	9.33±3.21^{b}	700.00±100.00^{b}	80.00±10.00^{b}	9.33±3.21^{b}
B	50.00±10.00^{b}	50.00±10.00^{b}	0.00±0.00^{d}	50.00±10.00^{b}	50.00±10.00^{b}	0.00±0.00^{d}
C	70.00±10.00^{b}	43.00±5.77^{b}	0.00±0.00^{d}	70.00±10.00^{b}	43.00±5.77^{b}	0.00±0.00^{d}
D	23.00±20.82^{b}	10.00±17.32^{b}	0.00±0.00^{d}	23.00±20.82^{b}	10.00±17.32^{b}	0.00±0.00^{d}

Table 5. Sensory evaluation of raw and treated milk samples.

Sample	Colour		Taste		Odour		Texture		General acceptability	
	S	A	S	A	S	A	S	A	S	A
A	4.06 ± 0.56^{a}	4.06 ± 0.56^{a}	3.47 ± 0.80^{a}	3.53 ± 0.94^{a}	3.59 ± 1.06^{a}	3.65 ± 1.00^{a}	3.59 ± 1.00^{a}	3.65 ± 0.70^{a}	3.66 ± 0.56^{a}	3.72 ± 0.59^{a}
B	3.82 ± 0.88^{a}	4.00 ± 0.79^{a}	3.59 ± 0.87^{a}	3.47 ± 0.87^{a}	3.41 ± 0.87^{a}	3.53 ± 1.01^{a}	3.41 ± 0.94^{a}	3.47 ± 1.12^{a}	3.56 ± 0.60^{a}	3.62 ± 0.78^{a}
C	4.00 ± 0.79^{a}	3.88 ± 0.60^{a}	3.59 ± 0.87^{a}	3.53 ± 0.94^{a}	3.71 ± 1.21^{a}	3.47 ± 0.94^{a}	3.77 ± 0.90^{a}	3.77 ± 0.90^{a}	3.77 ± 0.73^{a}	3.66 ± 0.65^{a}
D	3.77 ± 0.97^{a}	4.24 ± 0.66^{a}	3.24 ± 0.83^{a}	3.65 ± 1.11^{a}	3.47 ± 0.94^{a}	3.65 ± 0.93^{a}	3.77 ± 0.90^{a}	4.00 ± 0.87^{a}	3.56 ± 0.72^{a}	3.88 ± 0.64^{a}

These results showed that there is a significant difference (p≤0.05) between AM and BM, CM, DM, but there is no significant difference (p≤0.05) between BM and CM. The findings also showed that there is no significant difference (p≤0.05) between AS and AM, no significant difference (p≤0.05) between BS, CS, DS and BM and no significant difference (p≤0.05) between BS, DS, BM and CM. These results were within the range 9.10-11.43% as reported by Ayman (2011). These results showed that heating has increased the SNF in milk, this may be attributed to increase in milk concentration and heating has evaporated some water.

As shown in Table 2, the results showed that the Bulk density of raw milk sample was found to be 1.034, the results showed that the bulk density of milk samples heated in S containers for samples A, B, C and D were found to be 1.037, 1.035, 1.036 and 1.035 respectively. The results showed that the bulk density of milk samples heated in M containers for samples A, B, C and D were found to be 1.037, 1.036, 1.035 and 1.033 respectively. These results showed that there were significant differences (p≤0.05) between raw milk sample and heated milk samples in the two types of containers by all methods of heating.

Mineral content of milk samples: As shown in Table 3, the results showed that the Phosphorus (P) content of raw milk sample was found to be 0.0350 g/100 mL, the P content of milk samples heated in S containers for samples A, B, C and D were found to be 0.0260, 0.0263, 0.0263 and 0.0257 g/100 mL respectively. The results showed that the P content of milk samples heated in M containers for samples A, B, C and D were found to be 0.0240, 0.0217, 0.0233 and 0.0237 g/100 mL respectively. Table 3 showed that the Ca content of raw milk sample was found to be 0.0093 g/100 mL, the results showed that the Ca content of milk samples heated in S containers for samples A, B, C and D were found to be 0.0407, 0.0463, 0.0347 and 0.0960 g/100 mL respectively. Mineral results showed there were significant differences ($p \leq 0.05$) between raw milk sample and milk samples that heated in the two types of containers by all methods of heating. The results also showed that there were significant differences ($p \leq 0.05$) between some heated milk samples that heated in steel container with that heated in aluminum container. The variation of mineral content of heated milk attributed to containers, heating methods and lab conditions.

Microbiological analysis of milk samples: As shown in Table 4, the total viable count of bacteria of raw milk sample was found to be 48670.00 cfu/mL, the total viable count of bacteria of milk heated in S containers for samples A, B, C and D were found to be 70.00, 50.00, 70.00 and 23.00 cfu/mL respectively. The total viable count of bacteria of milk heated in M containers for samples A, B, C and D were found to 80.00, 50.00, 43.00 and 10.00 cfu/mL respectively. The results showed there were significant differences ($p \leq 0.05$) between raw milk sample and heated milk samples, but there is no significant difference ($p \leq 0.05$) between all heated milk samples the two types of containers. The results were less than that reported by Ayman (2011) in raw milk which has been found 561240 cfu/mL, but in heated milk samples within the range 1.2811-8.69133. The results showed that heating has decreased the total viable count of bacteria in milk, the variation of total viable count of bacteria of heated milk attributed to containers and heating methods. The total coli forms of raw milk sample were found to be 24.33 MPN/mL and that of milk samples heated in S or M containers for samples B, C and D were found to be 0.00 MPN/mL. The total coli forms of heated milk sample A in containers S and M were found to be 9.33 and 5.67 MPN/mL respectively, both results showed significant difference ($p \leq 0.05$) from each other and from that obtained for raw milk sample. The *Escherichia coli* of raw milk sample were found to be 10.67 MPN/mL and that of milk samples either heated in S or M containers for samples B, C and D were found to be 0.00 MPN/mL. The results showed that heating has decreased the *E. coli* in milk, the variation of *E. coli* of heating milk attributed to containers, heating methods and lab conditions.

Sensory evaluation of milk samples: As shown in Table 5, the Colour of milk heated in S containers for samples A, B, C and D were found to be 4.06, 3.82, 4.00 and 3.77 respectively. The colour of milk heated in M containers for samples A, B, C and D were found to be 4.06, 4.00, 3.88 and 4.24 respectively. The taste of milk samples heated in S containers for samples A, B, C and D were found to be 3.47, 3.59, 3.59 and 3.24 respectively. The taste of milk heated in M containers for samples A, B, C and D were found to be 3.53, 3.47, 3.53 and 3.65 respectively. The odour of milk heated in S containers for samples A, B, C and D were found to be 3.59, 3.41, 3.71 and 3.47 respectively. The odour of milk heated in M containers for samples A, B, C and D were found to be 3.65, 3.53, 3.47 and 3.65 respectively. The texture of milk samples heated in S containers for samples A, B, C and D were found to be 3.59, 3.41, 3.77 and 3.77 respectively. The texture of milk samples heated in M containers for samples A, B, C and D were found to be 3.65, 3.47, 3.77 and 4.00 respectively. The general acceptability of milk samples heated in S containers for samples A, B, C and D were found to be 3.66, 3.56, 3.77 and 3.56 respectively. The results showed that the general acceptability of milk samples heated in M containers for samples A, B, C and D were found to be 3.72, 3.62, 3.66 and 3.88 respectively. The sensory evaluation results showed that there was no significant difference ($p \leq 0.05$) between all methods used in the two types of containers.

Conclusion

Proximate analysis of heated milk samples showed difference with the raw milk sample. The highest percentage of protein content (%) was found in samples heated either in Steel or Aluminum containers. All mineral content, microbial analysis and most physicochemical parameters of heated milk samples showed difference from raw milk sample. On the other hand, sensory evaluation showed no significant difference between milk samples heated in Steel and Aluminum containers.

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