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Microbiological and Physicochemical Characteristics of Sheep Milk Heated with Charcoal, Gas and Microwave

Mohamed Osman M. Abdalla[1*], Zubida Elsayed Abdalla Elsayed[1] and Abdelazim Ahmed Mohamed Nour[2]

[1]Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Shambat, P.O.Box 32, Postal Code 13314, Khartoum North, Sudan.
[2]Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. Author MOMA designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author ZEAE wrote the protocol and managed the analyses of the study. Author AAMN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This investigation was conducted to study the effect of heat treatment on the physicochemical and microbiological characteristics of sheep milk.
Methodology: Samples were collected from different farms in Shambat, Khartoum State. Raw sheep milk was heated using charcoal, gas and microwave at 99℃/12 min, then cooled to 4℃ and stored for 10 days. Physicochemical and microbiological characteristics were determined at 1, 3, 7 and 10-day intervals.
Results: Milk treated with charcoal had higher values for all physicochemical characteristics except lactose and acidity which were higher in milk treated with microwave. The storage period significantly affected all physicochemical characteristics except protein and ash contents. Total

**Corresponding author: E-mail: abutahany1960@yahoo.com, abutahany@yahoo.com;*

viable bacteria (TVB) and lactobacilli counts were significantly higher in milk treated with charcoal and gas. During the storage period, total viable bacterial and lactobacilli counts significantly increased towards the end. The identification of bacteria of the genus level revealed that *Streptococcus*, *Enterococcus*, *Staphylococcus and Bacillus* were found during storage.
Conclusion: The method of heating milk significantly affected fat, protein, total solids [TS] contents, TVB and lactobacilli counts, while the storage period significantly affected all physicochemical and microbiological characteristics of milk except protein and ash contents. The genera *Staphylococcus*, *Bacillus* and *Enterococcus* were identified. The results indicated that gas and microwave heating of milk resulted in the reduction of the nutritive value of milk, although milk from these two methods was safer to the consumer.

Keywords: Heat treatment; microbiological; physicochemical; sheep milk; storage period.

1. INTRODUCTION

Milk is a secretion of the mammalian gland with varying physical characteristics and composition between species. It is a complex oil-in-water emulsion that contains fat, proteins, lactose, minerals, enzymes, cells, hormones, immunoglobulins, and vitamins [1]. The microbiological quality of milk may be affected by adulteration of milk, contamination during and after milking, mastitis, milking method, animal health, stage of lactation, season, feeding and the hygiene in the farm [2]. Information on physicochemical characteristics of sheep milk is essential for successful development of dairy industry in addition to marketing the products [3]. Sheep milk coagulates firmer than cow milk because it contains higher dry matter content [4]. Temperature and heat treatment time are very important factors that must be specified in relation to the quality and shelf-life of the milk [5], heat treatment of milk (holding method of pasteurization, 63℃ for >30 minutes; or high-temperature-short-time method of pasteurization, 71.7℃ for 15 seconds) effectively destroys most of the microbial population including pathogenic bacteria [6]. It was indicated that the major nutrients are left unchanged by pasteurization and that thiamin, folate, B_{12} and riboflavin experienced losses from zero to 10% [7].

Because of concerns that some potentially dangerous microorganisms may survive conventional pasteurization of milk and because the heat needed to sterilize milk affects marketability, the ability to efficiently cold pasteurize milk may become more desirable in order to efficiently inactivate microorganisms without causing deleterious effects on the nutrients of milk [8]. Methods of heat treatment include thermization, low temperature long time, high temperature short time, sterilization and ultra-high temperature [9]; however, in many rural areas traditional methods such as boiling with charcoal are the methods of choice [10,11]. Recently, many methods other than heat treatment were used to improve the quality of fresh milk which include ultraviolet treatment [12,13], pulsed UV laser light [14], microwave [11,15], membrane processing [16], microfiltration [17] and gas [10,11]. This study is conducted to evaluate the effect of heat treatment method and storage period on physicochemical and microbiological characteristics of sheep milk.

2. MATERIALS AND METHODS

2.1 Source of Milk

Fresh raw sheep milk (500 ml in each replicate) was obtained from different farms in Shambat area, Khartoum North, Sudan.

2.2 Sampling of Milk

Milk samples were aseptically transferred into sterile glass bottles and transported in ice box at ≤4℃ to the laboratory, and heat-treated on arrival to the laboratory. During analysis, the samples were first aseptically drawn for microbiological examination, and then samples for physicochemical analysis were drawn.

2.3 Method of Heat Treatment

Milk was heat-treated by charcoal, gas and microwave at 99℃/12 min, followed by cooling to 4℃ (using ice water) and stored immediately at this temperature for 10 days. It took 20 min to reach the temperature of 99℃ for microwave and gas and 30 min for charcoal, the temperature was monitored by thermometer during the 12 min. Physicochemical and microbiological characteristics of milk were determined for raw milk and milk heat-treated at

1, 3, 7 and 10-day intervals. Three replicates were done.

2.4 Determination of Physicochemical Characteristics of Milk

Physicochemical characteristics (fat, protein, TS, solids-non-fat [SNF] and density) of milk samples were determined using Lactoscan 90 milk analyzer (Aple Industries Service-La Roche Sur Foron, France). Milk samples were mixed gently 4-5 times to avoid any air enclosure in the milk, then 5 ml of the sample were taken in the sample-holder, one at a time and put in the sample holder with the analyzer in the recess position. The starting button was inactivated, the analyzer sucked the milk, the measurements were taken and the results were shown on the digital display.

2.5 Determination of Ash Content, Titratable Acidity and pH

The ash content and titratable acidity were determined according to AOAC [18], while the pH was determined using pH meter (Hanna-instrument model 98107, Mauritium). Before determination, pH meter was calibrated using buffer solutions No. 4 and 7.

2.6 Microbiological Examination

2.6.1 Preparation of sample dilutions

Ten milliliters of the sample were added to 90 ml of sterile 0.1% peptone water at 45℃ in a clean sterile flask, then shacked until a homogenous solution was obtained to make 10^{-1} dilution. One milliliter from the above-mentioned dilution (10^{-1}) was aseptically transferred to 9 ml sterile distilled water. This procedure was repeated to make serial dilutions of 10^{-2}, 10^{-3}, 10^{-4} ,10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}.

2.6.2 Total viable bacteria count

The total viable bacteria count was determined according to Houghtby et al. [19] using standard plate count agar, and the plates were incubated at 32℃/48 h.

2.6.3 Lactobacilli count

The lactobacilli count was determined according to Harrigan [20]. One milliliter was transferred to Petri- dishes (duplicate) and the culture medium (M17 agar) was poured aseptically into each Petri-dish, mixed gently and incubated at 37℃/72 hr under anaerobic conditions using anaerobic jars.

2.6.4 Identification of bacteria

Bacteria were isolated to the genus level by Gram stain, catalase, oxidase, motility, oxidation fermentation (O/F) and endospore staining tests [21].

2.7 Statistical Analysis

The data were analyzed using Statistical Analysis Systems (SAS, ver. 9). General linear model (GLM) procedure was used to determine the effect of source of heat and storage period on the physicochemical and microbiological characteristics of milk. Mean separation was carried out by Duncan multiple range test ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Physicochemical and Microbiological Characteristics of Milk Heated with Different Sources of Heat

The results in Table 1 show that the source of heat applied to milk significantly affected ($P<0.01$) the fat content being higher (6.97±0.84%) in milk treated with charcoal than milk treated with microwave (6.16±0.84%). The protein content was high ($P<0.01$) in milk heated with charcoal (4.28±0.23%) and low (3.85±0.23%) in milk heated by gas. TS content in this study was higher ($p<0.01$) in milk heated by charcoal (17.16±0.15%) and decreased to 16.63±0.15% and 16.58±0.15% in milk heated by gas and microwave respectively. The above results disagree with those reported by Abdalla and Daffalla [10] who reported that the protein and total solids contents showed great losses when milk was heated with charcoal. Iliana et al. [22] reported a decreasing concentration of milk proteins with the increase of microwave exposure. In the current study milk heated by charcoal had slightly higher SNF content (10.06±0.57%), while acidity was higher in milk heated by microwave (0.31±0.02%). Slightly higher ash content (0.71±0.02%) was reported in milk heated by charcoal, and the pH was high (6.29±0.44%) in milk heated by gas. The results disagree with Abdalla and Daffalla [10] who found that the ash content was not significantly affected by the source of heat, although milk heated by charcoal had higher ash content (0.84±0.25%), compared to milk heated by gas (0.78±0.09) and solar energy (0.81±0.17%). The total viable bacteria count was significantly (p<0.001) affected by the source of heat treatment with the higher count being in milk heated by charcoal (log10 2.35±1.32) and gas ($\log_{10}$ 2.34±1.32) and low in milk heated by microwave (log10 2.29 cfu/ml). The results are in accord with the findings of Abdalla and Daffalla [10] who found the total viable bacteria count to decrease after heat treatment of sheep milk with charcoal, gas and solar energy with the lowest decrease being in milk heated by charcoal. The count of lactobacilli was significantly (p<0.001) lower in milk heated by microwave compared to other sources. This means that microwave has a detrimental effect on lactobacilli. The results indicated that the increase in the chemical composition of milk by charcoal heating compared to other methods is the results of water evaporation from the milk leading to the concentration of solids, while charcoal heating is not satisfactory to reduce the bacterial load of milk which is important to produce a safe product for the consumer. The heat treatment applied to the food should achieve two goals; to produce a safe product without reducing the nutritive value.

Table 1. Effect of source of heat on physicochemical and microbiological characteristics of sheep milk

Parameter	Raw milk	Source of heat			SE	*P*
		Charcoal	Gas	Microwave		
Fat (%)	6.58	6.97^{a}	6.61^{b}	6.16^{c}	0.84	<0.0001
Protein (%)	3.92	4.28^{a}	3.85^{b}	3.94^{b}	0.23	0.0012
Total solids (%)	16.79	17.16^{a}	16.63^{b}	16.58^{b}	0.15	0.0038
Lactose (%)	5.22	5.30^{a}	5.07^{a}	5.30^{a}	0.30	1.456
Solids-non-fat (%)	9.95	10.06^{a}	9.77^{a}	10.03^{a}	0.57	0.9367
Ash (%)	0.86	0.71^{a}	0.67^{a}	0.70^{a}	0.02	1.185
Titratable acidity[1]	0.17	0.29^{a}	0.23^{a}	0.31^{a}	0.02	1.790
pH	6.28	6.28^{a}	6.29^{a}	6.26^{a}	0.44	2.431
Total bacterial count[2]	8.19	2.35^{a}	2.34^{a}	2.29^{b}	1.32	0.0002
Lactobacilli count[2]	7.00	2.24^{b}	2.26^{a}	2.22^{c}	1.36	<0.0001

Means in a row bearing the same superscript are not significantly different at P>0.05.
SE= Standard error of means.
[1] expressed as % lactic acid
[2] Log cfu/ml

Table 2. Effect of storage period on the physicochemical and microbiological characteristics of sheep milk heated with charcoal, gas and microwave

Parameter	Storage period (days)				SE	*p*
	1	3	7	10		
Fat (%)	7.26^{a}	7.16^{a}	6.22^{b}	5.68^{c}	0.26	0.0024
Protein (%)	3.85^{a}	3.91^{a}	3.94^{a}	3.98^{a}	0.02	0.9234
Total Solids (%)	16.07^{b}	16.33^{b}	17.67^{a}	17.10^{ab}	0.65	0.0342
Lactose (%)	5.00^{bc}	5.20^{ab}	5.30^{a}	5.39^{a}	0.10	0.0015
Solids-non-fat (%)	9.72^{b}	9.90^{ab}	10.03^{ab}	10.17^{a}	0.16	0.0092
Ash (%)	0.69^{a}	0.67^{a}	0.72^{a}	0.70^{a}	0.01	0.5689
Titratable acidity[1]	0.28^{ab}	0.22^{b}	0.28^{ab}	0.32^{a}	0.02	<0.0001
pH	6.95^{a}	6.03^{b}	6.15^{b}	5.99^{b}	0.03	0.0392
Total bacterial count[2]	2.03^{c}	2.51^{a}	2.26^{b}	2.62^{a}	1.32	<0.0001
Lactobacilli count[2]	1.66^{c}	1.86^{bc}	2.27^{b}	2.51^{a}	1.30	0.0002

Means in a row bearing the same superscript are not significantly different at P>0.05
SE= Standard error of means.
[1] expressed as % lactic acid
[2]Log cfu/ml

Table 3. Effect of storage period on the physicochemical and microbiological characteristics of sheep's milk heated by gas, charcoal and microwave

Parameter	Gas						Charcoal						Microwave					
	1	**3**	**7**	**10**	***p***	**SE**	**1**	**3**	**7**	**10**	***p***	**SE**	**1**	**3**	**7**	**10**	***p***	**SE**
Fat %	5.75^{ab}	4.74^{c}	5.15^{bc}	4.40^{c}	<0.0001	0.28	5.79^{ab}	6.45^{a}	5.20^{bc}	4.45^{c}	<0.0001	0.28	6.22^{a}	6.20^{a}	6.00^{ab}	5.24^{bc}	0.00012	0.28
Protein %	3.54^{b}	3.53^{b}	3.76^{ab}	3.69^{ab}	0.0021	0.02	3.72^{ab}	3.74^{ab}	3.83^{a}	3.90^{a}	0.0234	0.02	3.66^{ab}	3.74^{ab}	3.76^{ab}	3.81^{a}	0.0201	0.02
TS %	14.67^{bcd}	14.48^{cd}	15.16^{abc}	14.90^{abcd}	0.0002	0.63	14.63^{bcd}	15.26^{abc}	15.26^{abc}	15.40^{abc}	0.0341	0.63	12.96^{d}	15.30^{abc}	16.67^{ab}	16.76^{ab}	<0.0001	0.63
Lactose %	4.68^{d}	4.81^{cd}	5.11^{abc}	5.06^{abc}	<0.0001	0.10	5.01^{bcd}	5.00^{bcd}	5.20^{ab}	5.35^{a}	0.0034	0.10	5.02^{abc}	5.04^{abc}	4.91^{bcd}	5.17^{ab}	0.0039	0.10
SNF %	9.15^{cd}	9.04^{d}	9.61^{abcd}	9.49^{abcd}	<0.0001	0.18	9.46^{abcd}	9.47^{abcd}	9.79^{ab}	10.03^{a}	0.0046	0.18	9.51^{abcd}	9.55^{abcd}	9.30^{bcd}	9.73^{abc}	0.0210	0.18
Acidity %	0.20^{ab}	0.22^{ab}	0.19^{ab}	0.22^{ab}	0.9023	0.01	0.18^{b}	0.20^{ab}	0.23^{ab}	0.29^{ab}	0.0210	0.01	0.20^{ab}	0.20^{ab}	0.28^{ab}	0.31^{a}	0.0230	0.01
Ash %	0.46^{d}	0.52^{bcd}	0.63^{abc}	0.63^{abc}	0.0014	0.01	0.58^{abcd}	0.49^{dc}	0.68^{a}	0.60^{abcd}	0.0040	0.01	0.58^{abcd}	0.66^{ab}	0.53^{bcd}	0.61^{abc}	0.00014	0.01
pH	6.61^{abc}	6.16^{abc}	6.18^{abc}	6.11^{abc}	NS	0.03	6.74^{a}	6.07^{c}	6.15^{abc}	6.04^{c}	<0.0001	0.03	6.72^{ab}	6.01^{c}	6.08^{bc}	5.96^{c}	0.0400	0.03
TBC Log_{10}	1.98^{f}	2.13^{def}	2.25^{cd}	2.63^{a}	<0.0001	1.19	2.05^{ef}	2.13^{def}	2.33^{c}	2.60^{a}	<0.0001	1.19	1.94^{f}	2.13^{def}	2.33^{c}	2.54^{b}	<0.0001	1.19
Lacto Log_{10}	1.65^{de}	1.92^{de}	2.28^{c}	2.59^{a}	0.0003	1.24	1.70^{de}	1.98^{d}	2.34^{c}	2.55^{ab}	<0.0001	1.24	1.57^{e}	1.91^{de}	2.32^{c}	2.52^{b}	<0.0001	1.24

Means in a row bearing the same superscript are not significantly different at P>0.05, SE. = Standard error of means

Table 4. Identification of bacteria in sheep's milk heated by gas, charcoal and microwave

Sample	Isolate	Gram staining	Shape	Endospore staining	Motility test	Catalase test	Oxidase test	O/F test	Genus
Raw	1	+	Cocci	-	-	+	-	F	Staphylococcus
Day 1									
Gas	1	+	Cocci	-	+	+	-	F	Staphylococcus
Charcoal	1	+	Cocci	-	+	+	-	F	Staphylococcus
Microwave	1	+	Cocci	-	+	+	-	F	Staphylococcus
Day 3									
Gas	1	+	Rod	+	-	+	+	F	Bacillus
Charcoal	1	+	Rod	+	+	+	+	F	Bacillus
Microwave	1	+	Rod	+	+	+	+	F	Bacillus
Day 7									
Gas	1	+	Cocci	-	+	-	-	F	Staphylococcus
Charcoal	1	+	Rod	+	+	+	-	F	Bacillus
Microwave	1	+	Rod	+	+	+	-	F	Bacillus
Day 10									
Gas	1	+	Cocci	-	+	-	-	F	Enterococcus
Charcoal	1	+	Cocci	-	+	-	-	F	Enterococcus
Microwave	1	+	Cocci	-	+	-	-	F	Enterococcus

3.2 Effect of Storage Period on the Quality of Milk Heated with Charcoal, Gas and Microwave

The change has taken place during storage depending on the temperature of storage, extent of exposure of the milk to light and availability of oxygen. The effect of storage period on the physicochemical and microbiological characteristics is shown in Table 2. The fat content and pH decreased with the advancement of storage period, while protein, TS, lactose, SNF, ash and acidity increased as the storage period progressed from day 1 to day 10. The total viable bacteria and lactobacilli counts increased with storage period, reaching the highest at the end. Table 3 presents the physicochemical and microbiological characteristics of milk heated with the three sources of heat. Fat content decreased during the storage period in milk heated by all sources, while pH decreased in milk heated by gas and charcoal only. Protein and TS contents decreased in milk heated by gas only. Lactose content decreased in milk treated with charcoal, while SNF decreased in milk treated by gas and microwave. Acidity increased as the storage period progressed in all treatments. TVB and lactobacilli counts increased as the storage period progressed in milk treated with all sources of heat. The results also are in disagreement with those reported by Birginin et al. [23] who reported that pasteurization of milk resulted in an increase in fat content by 94% and in protein by 1.19%. Similar findings were obtained by Abo–Elnaga [24] who reported that standard plate count reduced after boiling, and Data et al. [25] who reported that, ultra high treatment processing of milk and its subsequent storage causes several changes, which affect the shelf life of milk although it remains commercially sterile. Abdalla et al. [11] reported that, as the storage period progressed, the acidity of milk heated with gas and charcoal decreased, while of that heated with microwave increased. However, Abdalla and Daffalla [10] reported no significant effect of heating on ash content of milk. In another study, Korhonen et al. [26] reported that heat sterilization of milk is essential to ensure total microbial safety and enzymatic stability. Microwave heat treatment of cow or human milk is effective in reducing bacterial counts [27]. Jaynes [28] performed standard plate and coliform counts on microwave and control pasteurized milk, and indicated comparable count reductions by the two treatments at all three rates of 200, 300 and 400 mL/min through the microwave heater. Al-Hilphy and Ali [29] reported that flash pasteurization of milk by the microwave reduced the total bacterial count from 47×10^5 before flash pasteurization to 23×10^1 after flash pasteurization.

3.3 Identification of Bacteria in Sheep Milk Heated by Gas, Charcoal and Microwave

Bacteria were isolated post heat treatments and every 3 days of storage. The identification of bacteria isolated from milk is shown in Table 4. The following genera were isolated and identified: Streptococcus, Enterococcus, Staphylococcus and Bacillus (Table 4). Abdalla et al. [11] identified the genus *Bacillus* from raw goat milk, and the genera *Staphylococcus* and *Bacillus* from goat milk heated by charcoal, gas and microwave. The presence of these genera in the milk after treatment indicates that the exposure time was not satisfactory therefore these bacteria could survive post treatment of milk, which may be hazardous to the consumer since these genera are pathogenic.

4. CONCLUSION

This study is designed to compare the conventional method of heating milk with the most advanced ones that do not harm the environment in order to convince the people in remote areas to use these methods as alternative to the conventional. The source of heat significantly affected the fat, protein, total solids contents and total bacteria and lactobacilli counts. In Third World countries, charcoal is used for heating all foods which results in destruction of vegetation leading to desertification, therefore, it was interesting to use alternative means of heat treatment that do not harm the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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