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Effect of Source of Heat and Storage Period on Physicochemical and Microbiological Characteristics of Goat Milk

Review Article

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Abstract

This investigation was conducted to evaluate the effect of heat treatment of goat milk with charcoal, gas and microwave on the physicochemical and microbiological characteristics during the storage period. Raw goat milk was heated with charcoal, gas and microwave at 99°C for 12 min followed by storage at 4°C for 10 days. Physicochemical and microbiological characteristics were determined at 1, 3, 7 and 10-day intervals. The results showed a significant effect of source of heat on the physicochemical characteristics under study except fat content and pH. The highest protein (3.62%), lactose (4.97%) and solids-non-fat (SNF) (9.31%) contents were high in milk heated with charcoal, while fat (5.68%) and total solids (TS) (14.26%) contents were high in milk treated with microwave and the acidity (0.19%) was high in milk treated with gas. The total viable bacteria (TVB) and lactic acid bacteria (LAB) were significantly ($P<0.001$) higher (log 2.31 cfu/ml and log 2.26cfu/ml, respectively) in milk heated with charcoal. The protein, TS, lactose, SNF and acidity increased during the storage period, while fat and ash decreased. TVB and LAB increased during the storage period. Bacillus and Staphylococcus genera survived the heat treatment and were isolated in heated milk till day 7 of the storage period, and then Staphylococcus was not detected at day 10.

Keywords: Goat milk, Heat, Microbiological, Physicochemical, Storage priod.

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Introduction

Goat milk and their products are nutritionally versatile having gained a wide demand due to their potential nutraceutical properties. Goat milk can get contaminated by various pathogenic or spoilage microorganisms (mainly bacteria) during various stages of processing and storage from farm to table. Presence of high microbial load in milk can pose major economical loss for local farmers and small hold dairies, as milk price is calculated based on the bacteria count [1]. Goat milk is more sensitive to high heat treatments than bovine milk. The pH, micelle hydration, genetic polymorphism of α−S1 casein, non-protein nitrogen, salt balance and ionic calcium could be directly or indirectly involved in the heat sensitivity of milk [2]. Goats play a significant role in the food chain and overall livelihood of rural households, where they are largely the property of women and their children. These animals can be reared for various reasons such as income generation, religious purposes, household consumption and as security against crop failure [3]. The main objective of milk heat treatment is to eliminate pathogenic microorganisms, or reduce them to a level safe for human consumption and to increase the shelf-life by inactivating spoilage microorganisms without affecting the nutritive value of milk [4].

When milk is heated some changes such as appearance of scum take place on the surface, with prolonged heating complete coagulation of milk takes place, and on continuous heating some proteins form a flocculent precipitate which collects on the bottom of the pan and causes scorching [4]. On heating milk for extended period of time, it is subjected to browning reaction caused by the union of free amino groups with milk reducing sugar [5]. In modern dairy industry, milk heat treatment is the major method for milk preservation and extending the shelf life [4]. Heat treatment methods include thermization, low temperature long time, high temperature short time, sterilization and ultra high temperature [6]; 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 [7, 8], microwave [9] and gas [4]. The quality of most dairy products is closely related to the microbial status of raw milk from which they are manufactured.

Managing the safety of milk involves controlling the various endogenous and exogenous sources of contamination. Diseases such as salmonellosis, tuberculosis, brucellosis, listeriosis, Q fever, toxoplasmosis, streptococcal and staphylococcal infections and campylobacter infections can be transmitted to humans from milk. Endogenous contamination of mastitic agents in goats include coagulase-negative staphylococcus species; Staphylococcus aureus and streptococcus species [10]. 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 [11].

The heat stability of milk is reduced when it is first homogenized and then heated, but not when homogenization follows heating [12]. The reason for this seems to be that heat-induced interactions of serum protein with fat surface-adsorbed casein promote heat coagulation. In Sudan, the major milk heat treatment method is the boiling by charcoal in the rural areas and gas in the urban areas. However, recently some methods have been tried utilizing the solar energy for milk heating [4]. In addition, microwave technology is recently being introduced into Sudan as a method of heat treatment. Therefore, this study is conducted to assess the effect of different heat treatment and storage periods on the physicochemical and microbial characteristics of goat milk.

Materials and Methods

Source of milk

Fresh raw goat milk was obtained from a dairy farm in Shambat area, Khartoum North.

Sampling of milk

Milk samples were aseptically transferred into sterile glass bottles and transported to the laboratory inside ice box (≤ 4°C). During analysis the samples were first aseptically drawn for microbiological examination, and then samples for chemical analysis were drawn. The samples were heat-treated immediately on arrival to the laboratory.

Method of heat treatment

Milk was heat-treated by charcoal, gas and microwave at 99°C and held on this temperature during 12min. The milk was then cooled to 4°C and stored for 10 days. Physicochemical and microbiological characteristics of milk were determined for raw milk and milk heat-treated at 1, 3, 7 and 10 day-intervals.

Determination of chemical composition of milk

Chemical analyses (fat, protein, SNF and density) of milk samples were determined by 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 5ml 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. Before used Lactoscan, it was calibrated in accordance with the standard method.

Determination of ash content, titrable acidity and pH

The ash content and titrable acidity were determined according to AOAC [13] and pH meter (Hanna-instrument model 98107, Mauritium) were used for determining pH. Before determination, pH meter was calibrated by using buffer solutions No. 4 and 7.

Microbiological examination

Preparation of sample dilutions: Ten millilitres of the sample were added to 90ml of sterile 0.1% peptone water at 45°C in a clean sterile flask, then shacked until a homogenous solution was obtained to make 10^{-1} dilution. One ml from the above-mentioned dilution (10^{-1}) was aseptically transferred to 9ml sterile distilled water. This procedure was repeated to make serial dilutions of 10^{-2} - 10^{-8}.

Total viable bacteria count: The total viable bacteria count was determined according to Houghtby et al. [14] using standard plate count agar. The plates were incubated at 32°C for 48hrs and colonies were counted.

Lactic acid bacteria count: The lactic acid bacteria count was determined according to Harrigan [15]. The appropriate sample dilution (0.1ml) was aseptically transferred to pre-solidified M17 agar medium, spread plated and incubated at 37°C for 72hr under anaerobic conditions using anaerobic jar, and the typical colonies were counted.

Identification of bacteria (biochemical tests): The following primary tests were used to identify bacterial isolates to the genus level [16] Gram stain, catalase test, oxidase test, motility test, oxidation fermentation test (O/F) and endospore staining test.

Statistical analysis

The data were analyzed using Statistical Analysis Systems (SAS, ver. 9). General Linear Models (GLM) was used to study the effect of source of heat and storage period on the chemical composition and microbiological quality of milk. Means were separated by Duncan multiple range test ($P \leq 0.05$).

Results and Discussions

Physicochemical and microbiological characteristics of goat milk heated with different sources of heat

The physicochemical and microbioligical characteristics of raw goat milk was as follows: fat 4.35%, protein 3.53%, TS 13.45%, lactose 4.83%, SNF 9.07%, ash 0.65%, titratable acidity 0.17%, pH 6.19, TVB count log 7.69 cfu/ml, lactic acid bacteria count log 6.51 cfu/ml. Table 1 shows that fat content was significantly ($p<0.001$) higher when milk was heated by microwave (5.68%), and lower when milk was heated by gas (3.41%). The protein content was found to be higher ($p<0.05$) in milk heated by charcoal (3.62%), while TS content was higher ($p<0.05$) when milk was heated with microwave (14.26%). Lactose ($p<0.001$) and SNF ($p<0.001$) contents were higher in milk heated with charcoal (4.97% and 9.31% respectively), while acidity was higher ($p<0.01$) in milk heated by

Table 1. Effect of source of heat on physicochemical and microbiological characteristics quality of goat milk heated by charcoal, gas and microwave.

Parameter	Source of heat			SE	SL
	Charcoal	Gas	Microwave		
Fat (%)	3.97[b]	3.41[c]	5.68[a]	0.38	***
Protein (%)	3.62[a]	3.41[b]	3.54[ab]	0.03	*
Total Solids (%)	13.11[b]	12.97[b]	14.26[a]	0.75	*
Lactose (%)	4.97[a]	4.76[b]	4.66[ab]	0.14	***
Solids-non-fat (%)	9.31[a]	8.88[b]	9.02[ab]	0.24	**
Ash (%)	0.47[a]	0.45[a]	0.49[a]	0.02	NS
Titratable acidity (% lactic acid)	0.16[b]	0.19[a]	0.18[a]	0.01	**
pH	6.21[a]	6.24[a]	6.12[a]	0.13	NS
Total bacterial count1	2.31[a]	2.30[a]	2.19[b]	1.30	***
Lactobacilli count1	2.26[a]	2.24[a]	2.21[b]	1.39	***

Means in each row bearing similar superscripts are not significantly different (P>0.05)

*=P<0.05

**=P<0.01

***=P<0.001

NS=Not significant

SE = Standard error of means

SL =Significance level

[1] Log cfu/ml

gas and microwave (0.19 and 0.18% respectively). Although the ash content and pH of milk were not significantly affected by the source of heat, the ash content was high in milk heated by microwave (0.49%), and the pH was high in milk heated with gas (6.24). Microbiological results of milk heated with the three sources of heat showed that microwave heat treatment had more lethal effect on total bacteria count resulting in reduction of the number to log 2.19cfu/ml compared to gas (log 2.30cfu/ml) and charcoal (log 2.31cfu/ml). The same effect of heat was noticed in lactic acid bacteria count when microwave application lead to a slight reduction in the number of lactic bacteria to log 2.21cfu/ml compared to log 2.24cfu/ml for gas and log 2.26cfu/ml for charcoal heat treatment. From the results it is obvious that microwave treatment caused increase in fat, TS and ash contents, while charcoal heat treatment resulted in increased protein, lactose and SNF contents, and gas heat treatment resulted in increased acidity of milk. The results are in accordance with the findings of Bora and Goyal [17] who reported high fat and TS contents of milk heated by microwave and in disagreement with the finding of Sahan *et al.* [18] who reported no significant effect of pasteurization on the fat and protein contents of milk. However, these results contradict those reported by Abdalla and Daffalla [4] who reported high fat, TS and ash contents of milk heated with gas compared to charcoal and solar energy, while protein content was high when milk was heated with solar energy. Although time/temperature combination of heat treatment was similar to that used by Abdalla and Daffalla [4], gas treatment reduced protein content more. Hassan [19] reported that UHT resulted in a decreased fat, protein and TS contents of milk compared to low heat treatment (85°C/40 min) Matak *et al.* [8] reported that there is no significant change in fatty acid profile of goat milk treated with UV light. The results of microbiological examination indicate that microwave treatment had more detrimental effect on total viable bacteria (TVB) and lactic acid bacteria counts. These results are in line with the findings of Abdalla and Daffalla [4] who reported high TBC in conventional method (charcoal) compared to gas and solar energy, and Clare *et al.* [9] who reported that UHT and microwave processing eliminated all bacterial growth in the milk as evidenced by the lack of colony formation using various microbiological media. The results of TVB count in this investigation are lower than those reported by Elzubeir *et al.* [20] who reported a mean TBC of log 5.99±4.47 cfu/ml for pasteurized milk, and Aggad *et al.* [21] who reported a mean aerobic mesophilic flora of log 5.21±5.19 cfu/ml for pasteurized milk.

Effect of storage period on the quality of goat milk heated with charcoal, gas and microwave

Table 2 presents the effect of storage period on the chemical composition and microbiological characteristics of goat milk which was heated by charcoal, gas and microwave. In this research, fat content and pH for 1 and 10 day storage period were found to be 5.92%, 6.69 and 4.70%, 6.04 respectively. Fat content and pH values were decreased for 10 days period. In addition, protein, TS, lactose, SNF and ash contents steadily were increased as the storage period progressed (1, 3, 7, 10 days).

However, the acidity fluctuated being high (0.23%) at day 1, then decreased at day 3 (0.21%) before gradually increasing to 0.24% at the end. The TVB and lactobacilli count gradually increased from log 1.39cfu/ml and log 1.04 cfu/ml respectively at the beginning to log 1.99cfu/ml and log 1.95 cfu/ml respectively at the end. The fat, TS, acidity and pH of goat milk treated with gas decreased with the advancement of storage period, while protein, lactose, SNF and ash contents increased. For goat milk treated with charcoal, all chemical components increased towards the end of storage period except the acidity and pH which slightly decreased. When milk was treated with microwave, only fat content and pH decreased as storage period progressed and the other components steadily increased. For microbiological quality it was noticed that TVB and lactic acid bacteria counts increased drastically towards the end of storage period in milk treated with all heat sources, it

Table 2. Effect of storage period on the physicochemical and microbiological characteristics of goat milk.

Parameter	Storage period (days)				SE	SL
	1	3	7	10		
Fat (%)	5.92^{a}	5.79^{a}	5.45^{a}	4.70^{b}	0.28	*
Protein (%)	3.67^{b}	3.68^{b}	3.75^{ab}	3.80^{a}	0.05	*
Total Solids (%)	14.09^{b}	15.01^{a}	15.70^{a}	15.68^{a}	0.47	*
Lactose (%)	4.90^{b}	4.95^{b}	5.07^{ab}	5.19^{a}	0.09	*
Aolids-non-fat (%)	9.37^{b}	9.35^{b}	9.57^{ab}	9.75^{a}	0.14	*
Ash (%)	0.55^{b}	0.85^{a}	0.64^{ab}	0.61^{ab}	0.02	**
Titratable acidity (% lactic acid)	0.21^{a}	0.23^{a}	0.24^{a}	0.26^{a}	0.14	NS
pH	6.69^{a}	6.08^{b}	6.14^{b}	6.04^{b}	0.04	*
Total bacterial count1	1.39^{d}	1.51^{c}	1.66^{b}	1.99^{a}	1.11	***
Lactobacilli count1	1.04^{d}	1.66^{c}	1.71^{b}	1.95^{a}	1.19	***

Means in each row bearing similar superscripts are not significantly different (P>0.05)
*=P<0.05
**=P<0.01
***=P<0.001
NS=Not significant
SE= Standard error of means
SL = Significance level
[1] Log cfu/ml

Table 3. Effect of storage period and method of heating on the physicochemical characteristics and microbiological quality of goats milk.

Parameter	Source of heat																	
	Gas						Charcoal						Microwave					
	1	3	7	10	SL	SE	1	3	7	10	SL	SE	1	3	7	10	SL	SE
Fat (%)	3.69^{b}	3.05^{c}	3.83^{a}	3.07^{c}	***	0.15	3.97^{b}	4.23^{a}	4.19^{a}	3.52^{d}	***	0.15	4.09^{a}	4.02^{a}	4.03^{a}	3.56^{b}	*	0.15
Protein (%)	3.37^{ab}	3.19^{b}	3.51^{ab}	3.58^{a}	***	0.02	3.56^{ab}	3.60^{a}	3.63^{a}	3.69^{a}	*	0.02	3.52^{ab}	3.53^{ab}	3.53^{ab}	3.60^{a}	*	0.02
TS (%)	12.65^{d}	12.65^{d}	12.78^{cd}	12.81^{cd}	***	0.56	12.81^{bcd}	13.15^{bcd}	13.00^{bcd}	13.50^{bcd}	NS	0.56	12.86^{c}	15.30^{abc}	15.41^{ab}	16.50^{a}	***	0.12
Lactose (%)	4.81^{ab}	4.42^{b}	4.84^{ab}	4.97^{a}	**	0.12	4.89^{a}	4.93^{a}	4.98^{a}	5.10^{a}	NS	0.12	4.72^{ab}	4.74^{ab}	4.74^{ab}	4.91^{ab}	NS	0.56
SNF (%)	8.99^{ab}	8.24^{b}	9.11^{ab}	9.26^{a}	*	0.2	9.15^{ab}	9.24^{a}	9.33^{a}	9.52^{a}	*	0.2	8.93^{ab}	8.97^{ab}	8.95^{ab}	9.22^{a}	*	0.2
Acidity1	0.20^{b}	0.23^{a}	0.16^{c}	0.15^{ef}	***	0.01	0.18^{bcde}	0.17^{bcde}	0.14^{f}	0.17^{bcde}	*	0.01	0.16^{def}	0.19^{bcd}	0.17^{bcd}	0.19^{bc}	***	0.01
Ash (%)	0.30^{c}	0.48^{a}	0.50^{ab}	0.55^{ab}	**	0.01	0.44^{abc}	0.32^{bc}	0.57^{a}	0.53^{ab}	***	0.01	0.46^{abc}	0.54^{ab}	0.48^{abc}	0.49^{abc}	*	0.01
pH	6.35^{a}	6.30^{a}	6.12^{a}	6.13^{a}	NS	0.04	6.48^{a}	6.30^{a}	6.19^{a}	6.13^{a}	NS	0.04	6.46^{a}	6.01^{a}	6.04^{a}	6.00^{a}	NS	0.04
TBC (Log10)	1.93^{k}	2.03^{h}	2.30^{e}	2.62^{a}	***	1.25	1.10^{j}	2.12^{g}	2.31^{d}	2.59^{b}	***	1.19	1.90^{i}	2.03^{i}	2.18^{f}	2.47^{c}	***	1.19
LAB (Log10)	1.59^{i}	1.79^{i}	2.18^{f}	2.65^{a}	***	1.3	1.72^{j}	1.94^{g}	2.32^{d}	2.59^{b}	***	1.24	1.55^{i}	1.83^{h}	2.30^{e}	2.54^{c}	***	1.24

Means in each row bearing similar superscripts are not significantly different (P>0.05)
*=P<0.05; **=P<0.01; ***=P<0.001; NS=Not significant
SE= Standard error of means
SL = Significance level
[1] expressed as % lactic acid

was also noticed that storage period adversely affected fat content of milk leading to decrease in value as the storage period progressed, while other components were not substantially affected. As the acidity fluctuated or decreased the pH also followed the same trend, and this contradicts the results of microbiological examination, since the increase in lactic acid bacteria should lead to reduction in the pH [21]. The results are in agreement with the findings of Hassan [19] for fat of UHT milk, and in disagreement with the results of protein, TS, and lactose contents. Villamiel et al. [22] reported the denaturatoin of whey protein by heating with microwave treatment. The increase in protein content during storage might have resulted in increased TS content together with increase in ash content. Hassan [19] observed no significant change in fat, protein, SNF, acidity and pH of UHT treated milk during storage period of 12 weeks. Mohammed [23] found that total solids content gradually increased from the beginning of storage, and then decreased at the end of storage period. The results of microbiological count agree with those reported by Hassan *et al.* [24] who reported an increasing total bacterial count of milk treated with UHT, low pasteurization (85°C/40 min) and high pasteurization (98°C/1.87 min) when stored for 30 days. However, the results in this study are in disagreement with Clare *et al.* [9] who found that microwave treatment eliminated all microbial growth, and that the sterility of milk was maintained through a 1-year storage period indicating an effective aseptic packaging method. In microwaved goat's milk, there was on average a 5-log reduction of the bacterial contamination of l06cfu/mL [25]. Nakthong *et al.* [26] reported that all goat milk samples showed decrease in total microorganism count after the UV treatments, and during 20 days of storage, UV-treated milk samples also showed lower rates of

microorganism growth than the untreated milk.

Identification of bacteria in goat milk heated by gas, charcoal and microwave

Bacteria were isolated post heat treatment 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: *Staphylococcus* and *Bacillus*. Our results agree with the findings of Burgess *et al.* [27] who concluded that, pasteurization does not destroy all pathogenic microorganisms, but reduce the number to a level at which they don't constitute a significant health hazards.

The importance of this research is that the use of gas and microwave does not affect the nutritive value of milk compared to charcoal, in addition to improving the microbiological quality of milk. Therefore, it is important that people in the Sudan use these methods for heat treatment of milk instead of charcoal which leads to removal of vegetation cover of the Sudan.

Conclusion

The significance of this study stems from the fact that in rural areas of Sudan the main method of milk heat treatment is charcoal, a practice which leads to the removal of vegetation by cutting the trees to be used in charcoal manufacture. Therefore, the idea behind this study is to compare between charcoal, gas and microwave methods practically in order to convince the people in remote areas to use other methods to heat treat the milk without adversely affecting the environment.

Table 4. Identification of bacteria in goat 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 milk	1	+	Rod	-	+	+	-	F	Bacillus
Day 1									
Gas	1	+	Sphere	+	-	+	-	F	Staphylococcus
Charcoal	1	+	Rod	+	+	+	-	F	Bacillus
Microwave	1	+	Sphere	-	-	+	-	F	Staphylococcus
Day 3									
Gas	1	+	Rod	+	-	+	+	F	Bacillus
Charcoal	1	+	Rod	+	+	+	-	F	Bacillus
Microwave	1	+	Rod	-	+	-	+	OF	Bacillus
Day 7									
Gas	1	+	Sphere	-	-	+	-	F	Staphylococcus
Charcoal	1	+	Rod	+	+	+	-	F	Bacillus
Microwave	1	+	Sphere	-	+	-	-	F	Staphylococcus
Day 10									
Gas	1	+	Rod	+	+	+	-	F	Bacillus
Charcoal	1	+	Rod	+	+	+	-	F	Bacillus
Microwave	1	+	Rod	+	+	+	-	F	Bacillus

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