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Microbiological Quality of Heat-Treated Milk During Storage

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Abstract: This study was carried out to evaluate the microbial load of heat treated milk during storage. Collected raw milk samples were subjected to heat treatment at 98°C for 1.87 min (High Pasteurization, HP) and 85°C for 40 min (Low Pasteurization, LP) in addition to Ultra High Temperature Treated (UHT) milk samples which were purchased from local market. Milk samples were subjected to microbial examination, titratable acidity and pH at 1, 10, 20 and 30 day intervals. Results indicated that total bacteria and lactic acid bacteria count significantly increased with time during storage period, together with increase in titratable acidity of all samples tested, while pH gradually decreased towards the end. The following genera were isolated from milk samples: *Bacillus, Micrococcus, Saphylococcus, Enterobacter, Pseudomonas, Streptococcus, Pediococcus* and *Lactobacillus*.

Key words: UHT milk, HP milk, LP milk, microbiological examination, storage period

INTRODUCTION

From farm to fork, milk shelf life was a responsibility shared by producers, processors, retailers and consumers. Extension of shelf life from hours to months has been a prime objective of the dairy industry for many years to meet the demands for increasing distribution times and distances (Goff and Griffiths, 2006). Fluid milk processors would like to achieve 60-90 days of refrigerated shelf life for High Temperature Short Time (HTST) pasteurized milk to allow more efficient marketing and distribution of product. Ultra pasteurization is one approach to do this but consumers do not like heat induced off flavors associated with high heat treatment and would prefer high temp short time treated milk (Champan and Boor, 2001).

During cold storage after milk collection, psycrotrophic bacterial populations dominate the microflora and their extracellular enzymes, mainly proteases and lipases contribute to the spoilage of dairy products (Hantsis-Zacharov and Halpern, 2007). The numbers of psychrotrophs that develop after milk collection depends on the storage temperature and time. Under sanitary conditions <10% of the total microflora is psychrotrophs in contrast to >75% under unsanitary conditions (Cousin, 1982).

Pathogens involved in foodborne outbreaks associated with consumption of milk include *Salmonella*, *Listeria monocytogens*, *Campylobacter*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum*, beside other most common contagious mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis* (Ryser, 1998; Zadoks, 2003).

Extended shelf life products, with shelf life of 21-28 days at refrigerated temperature, through normal pasteurization and shelf stable milk products through Ultra High Temperature (UHT) continuous flow sterilization, are available (Goff and Griffiths, 2006). The conditions of heat treatment used for pasteurization depend on the final product, lower temperatures are used for refrigerated products and higher heat treatments are used for products stored at room temperature (USCFR, 2006).

The main objective of milk heat treatment is to eliminate inborn pathogenic organisms or reduce them to a level safe for human consumption during extended-shelf life. The effectiveness of heat treatment depends on type of microorganisms present and concentration of each (CFDRA, 1992).

Heat treatment systems are vat treatment (85°C for 10-40 min), high temperature short time (HTST; 98°C for 0.5-1.87 min) and ultra high temperature (UHT; 140°C for 2-8 sec) (Parnell-Cluniess *et al*., 1986).

This investigation was carried out to evaluate the microbiological quality of heat treated milk during storage and isolate some microorganisms.

MATERIALS AND METHODS

Source of milk: Raw milk samples were obtained from Khartoum University farm and two private farms, one in Omdurman and the second in Khartoum North. Sixty four cow milk samples were collected during February-March, kept in sterilized screw-capped bottles (100 ml) and transported to the laboratory in ice boxes at <6°C. Ultra High Temperature Treated (UHT) samples were purchased from Khartoum supermarkets.

Heat treatment of milk samples: Milk samples were heat treated in glass containers, using temperature-

adjusted water bath. Heat treatment involved two time-temperature combinations: 85°C for 40 min (Low Pasteurization, LP) and 98°C for 1.87 min (High Pasteurization, HP). Samples were cooled immediately and stored at 4°C and microbiological examination was carried out at day 1, 10, 20 and 30.

Microbiological examination: Milk samples were microbiologically examined for total bacteria count and lactic acid bacteria count as follows:

Total bacteria count was determined using Nutrient agar medium (Houghtby *et al*., 1992). The liquefied medium (45-46°C) was poured into each Petri dish and mixed thoroughly and dishes were inverted and incubated at 32°C for 48 h.

Lactic acid bacteria count was determined using MRS agar medium. Plates were incubated at 37°C for 72 h under anaerobic conditions using anaerobic jars. Plates containing 30-300 colonies were then enumerated (Harrigan, 1998).

Identification of bacteria (Biochemical tests): Gram staining, Catalase test, Oxidase test, Motility test, Oxidation Fermentation (O/F) test and Endospore staining were used as primary tests to identify bacterial isolates to the genus level (Barrow and Feltham, 1993): Titrable acidity test was determined according to AOAC (1990), while pH of milk was determined using pH meter (Hanna-instrument).

Statistical analysis: Data were analyzed using Statistical Analysis Systems (SAS). Significant differences between means were determined by Duncan's Multiple Range Test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Heat treatment had significant ($p<0.01$) effect in reducing the bcaterial load, however, no significant differences were observed at the beginning of storage period, but later with progressing storage period a significant difference was noted. Total bacterial count increased from Log_{10} 1.06±0.00, 2.35±1.17 and 2.04±1.52 at day one to Log_{10} 2.9±0.14 and 6.41±0.35, 7.17±0.07 at day 30 and lactic acid bacteria increased from Log_{10} 1.09±0.00, 1.53±1.26 and 1.77±1.07 at day one to Log_{10} 3.26±0.38, 4.84± 1.94 and 7.00± 0.19 at day 30 for UHT, HP and LP respectively.

The above results are in agreement with the findings of Sahan *et al.* (1996) who found that heat treatment had significant ($p<0.01$) effect on the total bacterial count. Korhonen *et al.* (1998) reported that heat sterilization of milk is essential to ensure total microbial safety and stability of enzymic activity. Our data are also in accord with Holsinger *et al.* (1997) who concluded that standard pasteurization is effective for the destruction of pathogens in raw milk, however microbial population counted significant beyond day 20 indicating that injury and recovery time preceded growth.

This is asserted by Buchanan and Klawitter (1991) who reported that, the smaller the degree of difference between the old and new environment, the shorter the period of lag phase, during which microbial cells adjust to new environments prior to growth.

Our results are in full agreement with the findings of Ziarno *et al.* (2005) who concluded that, the changes in total bacterial count in milk were not observed before 7-14 days of storage at 4°C and then a significant change was observed on day 21 of storage at the same conditions.

Table 2 shows that titratable acidity was 0.15±0.00, 0.16±0.00 and 0.17±0.00 at day one and then increased to 0.19±0.00, 0.22±0.00 and 0.25±0.00 at day 30. The significant increase ($p<0.01$) in acidity corresponded by significant lowering in pH from 6.69±0.00, 6.69±0.00 and 6.68±0.02 at day one to 6.64±0.01, 6.21±0.13 and 5.83±0.14 at day 30 for UHT, HP and LP respectively. It was evident that, acidity was higher at the end of the storage and this was attributed to increased lactic acid

Table 1: Effect of heat treatment and storage conditions on total bacteria count and lactic acid bacteria count (Log cfu/ml)

Type of milk	Total bacteria count				Lactic acid bacteria count			
	d1	d10	d20	d30	d1	d10	d20	d30
UHT	1.06 ± 0.00^{bB}	1.29 ± 0.74^{bB}	1.60 ± 0.05^{bC}	2.90 ± 0.14^{aC}	1.09 ± 0.00^{bA}	1.33 ± 0.00^{bB}	1.92 ± 1.11^{bB}	3.26 ± 0.38^{aB}
HP	2.35 ± 1.17^{bA}	3.36 ± 0.40^{abA}	4.50 ± 0.96^{abB}	6.41 ± 0.35^{aB}	1.53 ± 1.26^{aA}	1.57 ± 1.57^{aB}	2.87 ± 1.87^{bB}	4.84 ± 1.94^{bB}
LP	2.04 ± 1.5^{bA}	4.77 ± 0.06^{abA}	6.91 ± 0.14^{abA}	7.17 ± 0.07^{aA}	1.77 ± 1.07^{cA}	4.06 ± 0.08^{bA}	7.13 ± 0.48^{aA}	7.00 ± 0.19^{aA}

Means bearing same superscripts in row (lower case) and column (upper case) are not significantly different ($p>0.05$); UHT = Ultra High Temperature, HP = High Pasteurized (98°C for 1.87 min), LP = Low Pasteurized (85°C for 40 min), d = day, cuf/ml = colony forming unit per milliliter

Table 2: Effect of heat treatment and storage conditions on titratable acidity and pH value of milk

Type of milk	Titratable acidity (% lactic acid)				pH			
	d1	d10	d20	d30	d1	d10	d20	d30
UHT	0.15 ± 0.00^{cA}	0.17 ± 0.00^{bB}	0.18 ± 0.00^{bC}	0.19 ± 0.00^{aC}	6.69 ± 0.00^{aA}	6.68 ± 0.00^{aA}	6.66 ± 0.00^{bA}	6.64 ± 0.01^{bA}
HP	0.16 ± 0.00^{dA}	0.19 ± 0.00^{cA}	0.20 ± 0.01^{bB}	0.22 ± 0.00^{aB}	6.69 ± 0.00^{aA}	6.61 ± 0.01^{abB}	6.42 ± 0.13^{abB}	6.21 ± 0.13^{bB}
LP	0.17 ± 0.00^{dA}	0.20 ± 0.00^{cA}	0.23 ± 0.01^{bA}	0.25 ± 0.00^{aA}	6.68 ± 0.02^{aA}	6.57 ± 0.02^{abC}	6.30 ± 0.14^{bC}	5.83 ± 0.14^{cC}

Means bearing same superscripts in row (lower case) and column (upper case) are not significantly different ($p>0.05$); UHT = Ultra High Temperature, HP = High Pasteurized (98°C for 1.87 min), LP = Low Pasteurized (85°C for 40 min), d = day

Table 3: Identification of bacterial isolates from High Pasteurized (HP) samples

Samples	No. of isolates	Gram staining	Shape	Endospore staining	Motility	Growth in air	Catalase test	Oxidase test	O/F test	Genus
HP/d1	3	+	Rod	+	+	+	+	+	F	Bacillus
		+	Sphere	-	-	+	-	-	F	Streptococcus
		+	Sphere	-	-	+	+	-	O	Micrococcus
HP /d10	3	+	Sphere	-	-	+	+	-	O	Micrococcus
		+	Rod	+	+	+	+	+	F	Bacillus
		+	Sphere	-	-	+	+	-	F	Staphylococcus
HP/ d20	3	+	Sphere	-	-	+	-	-	F	Streptococcus
		+	Rod	+	+	+	+	+	F	Bacillus
		+	Sphere	-	-	+	+	-	O	Micrococcus
HP/d30	3	+	Rod	+	+	+	+	+	F	Bacillus
		+	Sphere	-	-	+	-	-	F	Streptococcus
		-	Rod	-	+	+	+	+	O	Pseudomonas

Table 4: Identification of bacterial isolates from Low Pasteurized (LP) samples

Samples	No. of isolates	Gram staining	Shape	Endospore staining	Motility	Growth in air	Catalase test	Oxidase test	O/F test	Genus
LP/d1	3	+	Cocci	-	-	+	+	-	O	Micrococcus
		+	Cocci	-	-	+	-	-	F	Streptococcus
		+	Cocci	-	-	+	+	-	F	Staphylococcus
LP /d10	3	+	Cocci	-	-	+	-	-	F	Streptococcus
		+	Rod	+	+	+	+	+	F	Bacillus
		-	Rod	-	+	+	+	-	F	Enterobacter
LP/ d20	3	+	Rod	+	+	+	+	+	F	Bacillus
		+	Cocci	-	-	+	-	-	F	Streptococcus
		+	Cocci	-	-	+	+	-	O	Micrococcus
LP/d30	3	+	Cocci	-	-	+	+	-	F	Staphylococcus
		+	Rod	+	+	+	+	+	F	Bacillus
		+	Cocci	+	-	+	-	-	F	Streptococcus

Table 5: Identification of lactic acid bacteria isolates

Samples	No. of isolates	Gram staining	Shape	Spore forming	Motility	Growth (Anaerobes)	Castalase test	Oxidase test	O/F test	Genus
LP/d1	1	+	Rod	-	-	+	-	-	F	Lactobacillus
LP/d10	1	+	Cocci	-	-	+	-	-	F	Pediococcus
LP/d20	1	+	Cocci	-	-	+	-	-	F	Streptococcus
LP/d30	1	+	Rod	-	-	+	-	-	F	Lactobacillus
HP/d1	1	+	Rod	-	-	+	-	-	F	Lactobacillus
HP/d10	1	+	Cocci	-	-	+	-	-	F	Streptococcus
HP/d20	1	+	Rod	-	-	+	-	-	F	Lactobacillus
HP/d30	1	+	Rod	-	-	+	-	-	F	Lactobacillus

+ = Postitive; - = Negative; O = Oxidative; F = Fermentative

producing bacteria, particularly streptococci and lactobacilli, which ferment lactose into lactic acid.

The above results are in agreement with the study of Parky (1991) who showed that titrable acidity was similar when milk was subjected to different heat treatments. Kuippers *et al.* (2000) reported that, the antimicrobial effect of lactic acid bacteria was mainly due to their lactic acid production, causing the pH of the growth environment to decrease. Moreover, defects were detected when microbial concentration reached 5 x 10^5-10^7 cfu/ml (Vyletelova *et al.*, 2000).

Bacteria were isolated post heat treatments, then identified (only detected colonies using primary biochemical tests. *Bacillus, Staphylococcus, Micrococcus, Enterobacter, Pseudomonas, Streptococcus, Pediococcus* and *Lactobacillus* were isolated from heat treated milk (Table 3, 4 and 5). Our findings are in agreement with the findings of Ruegg and Reinemann (2002) who concluded that thermoduric bacteria such as *Bacillus*, *Clostridium*, *Micrococcus*, *Microbacterium*, *Lactobacillus* and occasionally streptococci, can retain their activity and affect the quality of post pasteurized products.

O'connor (1999) reported that, the species of bacteria found in milk as it comes from the udder are limited to few genera, the micrococci are generally present in the greatest proportion followed by streptococci and rods.

Cousin (1982) reported that psychrotrophic bacteria from numerous genera have been isolated from milk such as *Pseudomonas*, *Bacillus*, *Mmicrococcus* and *Lactobacillus*.

Our results are also in agreement with the findings of Anonymous (1994) who concluded that, pasteurization does not destroy all pathogenic microorganisms, but reduces the number to a level at which they don't constitute a significant health hazard.

The study concludes that heat treatment of milk did not significantly affect the number of total bacteria as well as lactic acid bacteria. However, during shelf life the count significantly increased. Different bacteria including lactic acid, spoilage as well as pathogenic were isolated following heat treatment of milk indicating unhygienic conditions during production, processing and storage.

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