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Microbiological Quality of Cows' Milk Butter Processed in Khartoum State, Sudan

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Authors' contributions

This work was carried out in collaboration between all authors. Author SSJA wrote the protocol and the first draft of the manuscript. Authors MOMA and SAR managed the analysis of the study and literature search and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This investigation was carried out to study the microbiological quality of butter produced in Khartoum State, Sudan.
Methodology: Butter was manufactured traditionally by farmers in Khartoum north (T1) and Omdurman (T2), in addition to butter manufactured commercially in a dairy plant (T3) and butter manufactured by researchers in the laboratory (T4). Microbiological characteristics were evaluated at 1, 15, 30, 45 and 60- day intervals for samples stored at 5.0±1.0℃, and at 1, 7, 14, 21 and 28-day intervals for samples stored at 25±2.0℃.
Results: The results showed that total viable bacteria count (TVBC) significantly increased from the beginning of storage period to the end in butter of all treatments, with the increase being remarkable for samples stored at 25℃. Coliform bacter ia increased to a lesser extent compared to TVBC, with the increase being crucial in samples stored at 25℃. The storage temperature significantly affected the counts of yeasts and moulds, *Pseudomonas aeruginosa*, proteolytic and

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lipolytic bacteria, however, the difference in the count of lipolytic bacteria between the two temperatures was not of importance.
Conclusion: In this study, the microbiological quality of butter is mainly affected by the method of butter manufacture and storage conditions during the storage period.

Keywords: Commercial; cow's butter; microbiological; traditional.

1. INTRODUCTION

Butter is a traditional food which is widely consumed all over the world, directly or as ingredient in processed food such as pastries and convenience dishes. Its nutritional value (due to high content of fats, vitamins and minerals), and unique and pleasant flavour make butter practically appreciated by consumers. Butter can be made directly from milk or by separation of milk and subsequent churning of the cream. The microflora in butter may be affected by the chemical composition of milk, differences in traditional processing methods, packaging material, storage conditions and post processing handling [1].

Undesirable microbes that cause the spoilage of dairy products are Gram-negative psychrotrophs, coliform bacteria, lactic acid bacteria, yeasts and moulds. In addition, various bacteria of public health concern such as *Salmonella spp., Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica,* pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphylococcus aureus* may also be found in milk and dairy products [2]. The primary spoilage factors in butter are moulds, the majority of species being Thamnidium, Cladosporium and Aspergillus. However, proper heat treatment can destroy moulds in the cream, meaning that any mould contamination thereafter indicates contamination of water and air after production, and there is a protective effect of salt added to butter against moulds [3,4].

Although butter is not a perishable food, it undergoes spoilage by moulds and bacteria, and the main source of microorganisms being the sweet, sour, raw or pasteurized cream. The important spoilage microorganisms are yeasts and moulds which result in surface discolouration and off-flavour. Lactic acid bacteria cause a too strong acidity, while lipolytic bacteria destroy and oxidize fat content leading to the rancidity of butter, and the proteolytic bacteria degrade casein of butter causing cheesy taste. Coliform bacteria, enterobacteria and other bacteria are responsible for colouring and undesirable tastes in the butter [5,6]. Suryavanshi and Ghosh [7] reported that psycrophilic Pseudomonas aeruginosa NCIM 2036 produces extracellular lipase which is one of the important enzymes secreted by organisms involved in the spoilage of dairy products like white unsalted butter when stored at low temperature (2-5℃) resulting in flavour defects.

Idoui et al. [6] reported the presence of lactic acid bacteria, psychotrophic bacteria, moulds and yeasts, lipolytic bacteria, total coliforms and the absence of Staphylococcus and Salmonella in traditional goat butter. Yilma et al. [8] identified the genera Klebsiella, Escherichia, Enterobacter and Klyuvera in traditional butter in the order of abundance, with *Escherichia coli* being the dominant isolate identified. Microbial enzymes are an indirect cause of spoilage in dairy products, such as proteases, phosphatases and lipases, some of which may remain active in the food after the enzyme-producing microbes have been destroyed. Psychrotophic bacteria can produce sufficient extracellular enzymes to cause defects in butter [9].

Butter making in Sudan is practiced by women who utilize the surplus milk during the rainy season, when milk is plentiful, women churn the soured milk (*laben rayeb or birkib*) into buttermilk (*rob)* and butter (*furssah*). There are different processing techniques and methods in different regions. While in western region "*bukhsa*" made of gourd/calabash is used, in the northern and central regions "*si'in*" made of goat's skin is used [10]. In the world earlier attempts were made to investigate butter making, however, in Sudan few studies have been conducted to evaluate the chemical and microbiological characteristics of butter quality.

The objective of this study is to study the microbiological characteristics of cows' milk butter (traditional and commercial) produced in Khartoum State, Sudan.

2. MATERIALS AND METHODS

2.1 Butter Manufacture

Butter was manufactured in this study by four methods.

2.1.1 Butter traditionally manufactured by dairy farmers in Khartoum north and Omdurman (treatments 1 and 2 respectively)

Raw milk was left at room temperature (25±2℃) overnight to coagulate and convert into "*laben rayeb*". Next morning "*laben rayeb*" was churned in a skin bag from goat called "*siin*" until the coalescence of fat globules was formed indicating the conversion of milk into butter "*furssah*" and a by-product called "*rob*". Butter "*furssa*h" was stored at 4℃ till analysis.

2.1.2 Butter commercially manufactured in a dairy plant (treatment 3)

Sweet cream obtained by cream separation of fresh milk (30% fat) was cooled at 4℃ till used. The cream was pasteurized at 82℃ for 30 min, cooled to 10℃ and held at this temperature for two hours (sometimes overnight) before working into butter. The cream temperature was adjusted to 11.6℃ and churning was started by filling the churn with ⅔ volume followed by addition of cold water, and churning was continued until granules were formed. Butter was washed with cold water for 5 min repeating the washing twice, then the washed water was drained off and butter was wrapped, weighed, packed and stored at 4℃ till analysis.

2.1.3 Butter manufactured by the investigators (treatment 4)

Butter was manufactured in the dairy processing laboratory, Department of Dairy Production, Faculty of Animal Production, University of Khartoum. Raw milk was obtained from the University of Khartoum dairy farm. After fat determination, milk was warmed to 40℃ and the cream was separated by hand separator. The cream obtained was analyzed for fat content and kept overnight in the refrigerator (4℃). Next morning the cream was churned at 12℃ by hand churner till whipped cream became coarser and semi-solid butter granules were formed that rapidly increased in size and separated sharply from the liquid buttermilk. Butter was washed with cold water several times and the excess water was removed. Butter was filled in sterile disposable polyethylene bags and stored at 4℃ till analysis.

2.2 Sampling and Sample Preparation

Replicate samples were taken from T1, T2, T3 and T4. To ensure uniform distribution of water, the samples were warmed in unopened airtight containers in a water bath at 35℃, agitated to facilitate melting and the test sample was thoroughly mixed to a homogeneous form in aseptic conditions.

2.3 Sample Analysis

Microbiological characteristics (total viable bacteria, coliform bacteria, proteolytic bacteria, lipolytic bacteria, *Pseudomonas aeruginosa* spp. and yeasts and moulds counts) were determined at 1, 15, 30, 45 and 60-day intervals for samples stored in the refrigerator (5.0±1.0℃) and 1, 7, 14, 21 and 28-day intervals for samples stored at room temperature (25±2.0℃).

2.4 Microbiological Examination

2.4.1 Preparation of serial dilutions

After melting, the sample was warmed to 45^{o}C and 10 g sample was aseptically transferred to a flask containing 90 ml sterile peptone water to make 10^{-1} dilution. Further dilutions were prepared by transferring 1 ml from 10^{-1} dilution into a sterile test tube containing 9 ml sterile peptone water to make 10^{-2} dilution. The process was repeated to make 10^{-1}– 10^{-6} dilution.

2.4.2 Total Viable Bacterial Count (TVBC)

Total viable bacteria count was determined by the pour-plate method using plate count agar medium [11]. The plates were incubated (in an inverted position) at 30oC for 48 hr. The colonies were counted using a colony counter and the number of colonies was determined as colony forming units (cfu/g).

2.4.3 Coliform bacteria count

Coliform bacterial count was determined by the most probable number technique (MPN) using MacConkey broth medium for presumptive coliform test [12]. The five tube method was used and the tubes were incubated at 37ºC for 48 hr. All tubes showing positive results (production of acid and gas) were submitted to confirmatory tests as follows: a loopful from the presumptive tubes showing positive results were incubated in sterile tubes and incubated at 37ºC for 48 hr. Tubes showing positive results were recorded and the most probable number (MPN) table was used to determine the coliform number.

2.4.4 *Pseudomonas aeruginosa* count

From a suitable dilution, 0.1 ml was aseptically transferred into the surface of solidified sterile cetrimide agar plates, and the plates were incubated at 25℃ for 48 hr [11].

2.4.5 Proteolytic bacteria count

Proteolytic bacteria count was determined according to Frank et al. [13] using the plate count agar plus10% sterile skim milk. The plates were incubated at 37℃ for 3 days.

2.4.6 Lipolytic bacteria count

From a suitable dilution, 0.1 ml was aseptically transferred to pre-solidified plates containing nutrient agar with Tween 80. The plates were incubated at 30℃ for 3-5 days [14].

2.4.7 Yeast and moulds count

Yeasts and moulds count was determined by spread plate method using malt extract agar containing 0.1 g chloramphenical/L medium [15], and the plates were incubated at 25℃ for 7 days.

2.5 Statistical Analyses

The data were subjected to statistical analysis using Statistical Analysis Systems (SAS, ver.9). Factorial design (4x5) was used for microbiological analysis, and the means were separated by Duncan multiple range test at P≤0.05.

3. RESULTS AND DISCUSSION

3.1 Total Viable Bacteria

TVBC in samples stored at refrigerator temperature (5℃) significantly increased during the storage period in all treatments from $\log_{10}$ 3.47 cfu/g, $\log_{10}$ 3.52 cfu/g, $\log_{10}$ 2.70 cfu/g and $\log_{10}$ 2.99 at the beginning to $\log_{10}$ 4.29 cfu/g, $\log_{10}$ 4.40 cfu/g, $\log_{10}$ 3.96 cfu/g and $\log_{10}$ 3.95 cfu/g at the end of storage period in T1, T2, T3 and T4 respectively (Table 1). At room temperature (25℃), TVBC increased from $\log_{10}$ 3.47 cfu/g, $\log_{10}$ 3.52 cfu/g, $\log_{10}$ 2.70 cfu/g and log10 2.99 cfu/g in T1, T2, T3 and T4 to $\log_{10}$ 7.24 cfu/g, $\log_{10}$ 5.71 cfu/g, $\log_{10}$ 7.68 cfu/g and log10 7.89 cfu/g, respectively at the end of storage period. These results are in agreement with Samet-Bali et al. [16] who reported total microbial count of log 4.70±0.05 in traditional Tunisian butter, Idoui et al. [6,17] who reported total bacteria count of log 5.18 – 6.83 in traditional Algerian cow's and goat's milk respectively, and Gökce et al. [3] who reported a mean total bacteria count of log 5.18 – 6.08 cfu/g in Karın traditional butter from Turkey. Kacem and Karam [1] reported that the mean plate counts of different microbial groups of butter from camel milk (shemn) collected from four regions of Algerian Sahar were log 3.55±0.12, 2.76±0.19, 3.88±0.23 and 3.54±0.19 cfu/g, respectively. The high count of bacteria in this study may be attributed to the absence of heat treatment (pasteurization) and salt. High total bacteria count in butter may be attributed to high microbial load initially present in the milk, absence of pasteurization and salt, and the effect of both separation and churning processes on the breaking up of bacterial clumps which increases their number [6,17,18]. Butter was classified according to its total aerobic mesophilic bacteria as very good quality (< 1.0×10^6 cfu/g), good quality (1.0×10^6 - 2.0×10^6 cfu/g) and low quality (> 2.0×10^6 cfu/g) [3]. The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used in the manufacture of butter and the environmental and sanitary conditions during packaging and handling. It is advisable to adopt strict hygienic measures during milk handling to prevent contamination and improve its quality, in addition to proper heat treatment of milk [5].

3.2 Total Coliform Bacteria

A significant increasing trend of total coliform bacteria count was observed during the storage period of T1 and T2 samples stored 5℃ (from $\log_{10}$ 0.73 cfu/g and $\log_{10}$ 0.70 cfu/g at the beginning to $\log_{10}$ 0.81 cfu/g and $\log_{10}$ 0.93 cfu/g at the end respectively), while there was no significant variation between samples of T3 and T4. The samples in treatments stored at 25℃ showed a significant steady increase from $\log_{10}$ 0.73 cfu/g, $\log_{10}$ 0.70 cfu/g, $\log_{10}$ 0.86 cfu/g and $\log_{10}$ 0.97 cfu/g at day 1 to $\log_{10}$ 2.14 cfu/g, $\log_{10}$ 2.51 cfu/g, $\log_{10}$ 2.38 cfu/g and $\log_{10}$ 2.41 cfu/g for T1, T2, T3 and T4 respectively (Table 2). Coliforms are indicators of cleanliness of handling of milk and cream, premises and equipment [6]. In the present study, total coliform count ranged between $\log_{10}$ 0.70-0.97 cfu/g at 5℃ and $\log_{10}$ 0.70-2.51 cfu/g at 25℃. The results in this study are in agreement with those reported by Gökce et al. [3], and lower than those reported by Meshref [5], Idoui et al. [6,17] and Asresie et al. [2]. Kacem and Karam [1] reported coliform bacteria count of 0.90-1.66 log

cfu/g at refrigerator temperature. Karaozlu and Eronul [19] reported that coliform and total fecal coliform count of the samples were found between <3->1400 cfu/g. Elkhidir [20] observed that 41.71% of butter samples examined in Khartoum State had total coliforms in the range of ≥10 to ≤1400 MPN/g. Saad et al. [21] reported that 17 out of 30 examined butter samples (56.67%) were positive for Escherichia spp. on both E.M.B. agar and H&L agar. *Escherichia spp*. and *E. coli* contamination of milk during or after milking is probably of fecal origin, and improper washing and treatment of the udder with unsuitable disinfectant or contact of the milking pails with the floor may result in high level of contamination [21]. The existence of coliform bacteria in food material is of greatest importance because it indicates that the food product is exposed to an insufficient heat treatment or is re-contaminated afterwards [3].

3.3 *Pseudomonas aeruginosa*

The storage period significantly affected *Pseudomonas spp.* count in samples of all treatments stored at 5℃ and 25℃, increasing from day 1 to the end of storage period, except for T1 samples stored in 5℃, which showed a decreasing trend in count (Table 3). Nusty off-flavour in cream or butter is often due to 2-methoxy-3-alkypyrazine produced by *Pseudomonas traetolens* which is a psychotropic strain. Rancidity of butter may result from the activity of lipases in the raw milk or the residual heat-stable microbial lipase in the finished butter. Microbial lipases remaining in the butter can hydrolyze the fat even during frozen storage [9]. During preparation of butter, care has to be taken not only to deactivate the native lipase present in the milk, but also to deactivate and prevent contamination of butter with spoilage microorganisms which may lead to butyrification during storage [7]. *Pseudomonas aeruginosa* was not detected in butter samples from Beni-Suef governorate in Egypt [5]. Suryavanshi and Ghosh [7] reported that *Pseudomonas aeruginosa* MCIM-2036 was capable of producing lipase enzyme which was showing a significant activity at 5℃, and also producing mesophilic lipases active at 25℃, leading to hydrolysis of triglycerides that caused change in the flavor of butter.

3.4 Proteolytic Bacteria

The count significantly increased during storage period in samples stored at 5℃ and 25℃. The count increased from $\log_{10}$ 2.35 cfu/g, $\log_{10}$ 2.26 cfu/g, $\log_{10}$ 2.50 cfu/g and $\log_{10}$ 2.61 cfu/g at day 1 to $\log_{10}$ 2.71 cfu/g, 3.06 cfu/g, 2.89 cfu/g and 2.96 cfu/g at the end for samples in T1, T2, T3 and T4 stored at 5℃ respectively, while for

Table 1. Effect of storage temperature (℃) and per iod (days) on total viable bacteria count ($\log_{10}$ cfu/g) of cows' milk butter

Storage temperature (℃)	Storage period (days)	Treatment[1]				CV	SE	*P*
		T1	T2	T3	T4			
5	**1**	3.47^{cB}	3.52^{dA}	2.70^{eD}	2.99^{dC}	2.35	0.003	0.02
	15	3.58^{dB}	3.62^{cA}	2.96^{dC}	2.99^{dC}	3.18	0.002	0.01
	30	3.71^{cB}	3.84^{bA}	3.49^{cC}	3.33^{cD}	5.17	0.003	0.01
	45	4.04^{bA}	3.86^{bB}	3.78^{bC}	3.86^{bB}	2.96	0.004	0.01
	60	4.29^{aB}	4.40^{aA}	3.96^{aC}	3.95^{aC}	3.24	0.002	0.01
	CV	4.09	3.20	3.56	3.41			
	SE	0.002	0.005	0.003	0.004			
	P	0.017	0.019	0.013	0.012			
25	**1**	3.47^{eB}	3.52^{dA}	2.70^{eD}	2.99^{eC}	3.29	0.001	0.01
	7	4.13^{dB}	4.52^{cA}	3.96^{dC}	3.61^{dD}	3.07	0.003	0.007
	14	5.03^{cA}	4.70^{bB}	4.72^{cB}	4.53^{cC}	2.87	0.002	0.01
	21	5.72^{bB}	5.69^{aC}	5.79^{bA}	5.72^{bB}	3.16	0.005	0.01
	28	7.24^{aC}	5.71^{aB}	7.68^{aB}	7.89^{aA}	2.84	0.002	0.01
	CV	2.97	3.14	3.46	3.57			
	SE	0.004	0.002	0.001	0.003			
	P	0.01	0.01	0.01	0.01			

Means in each row (upper case) and column (lower case) bearing similar superscripts are not significantly different (P>0.05); CV = Coefficient of variation (%); SE=Standard error of means; SL= Significance level

[1] Treatments 1, 2, 3 and 4 refer to butter manufactured in Khartoum North; Omdurman, the dairy plant and the laboratory respectively

Table 2. Effect of storage temperature (℃) and per iod (days) on coliform bacteria count (log_{10} cfu/g) of cows' milk butter

Storage Temperature (℃)	Storage period (days)	Treatment[1]				CV	SE	*P*
		T1	T2	T3	T4			
5	**1**	0.73^{cC}	0.70^{dC}	0.86^{aB}	0.97^{aA}	2.29	0.009	0.01
	15	0.74^{cC}	0.74^{cC}	0.86^{aB}	0.97^{aA}	3.05	0.007	0.02
	30	0.76^{bC}	0.87^{bB}	0.86^{aB}	0.97^{aA}	5.48	0.001	0.01
	45	0.76^{bC}	0.87^{bB}	0.86^{aB}	0.97^{aA}	2.99	0.002	0.01
	60	0.81^{aD}	0.93^{aB}	0.86^{aC}	0.97^{aA}	3.37	0.003	0.01
	CV	4.12	3.29	3.17	3.48			
	SE	0.006	0.004	0.005	0.007			
	P	0.01	0.02	0.91	1.12			
25	**1**	0.73^{eC}	0.70^{eC}	0.86^{eB}	0.97^{eA}	3.41	0.003	0.008
	7	1.25^{dC}	1.36^{dB}	1.38^{dB}	1.64^{dA}	3.17	0.002	0.005
	14	1.44^{cC}	1.61^{cB}	1.64^{cB}	2.08^{cA}	2.56	0.005	0.009
	21	1.90^{bD}	2.17^{bC}	2.08^{bB}	2.38^{bA}	3.84	0.004	0.007
	28	2.14^{aC}	2.51^{aA}	2.38^{aB}	2.41^{aB}	2.66	0.001	0.01
	CV	2.88	3.52	3.46	3.34			
	SE	0.002	0.005	0.008	0.006			
	P	0.02	0.01	0.01	0.009			

Means in each row (upper case) and column (lower case) bearing similar superscripts are not significantly different (P>0.05); CV = Coefficient of variation (%); SE =Standard error of means; SL= Significance level
[1] Treatments 1, 2, 3 and 4 refer to butter manufactured in Khartoum North, Omdurman, the dairy plant and the laboratory respectively

Table 3. Effect of storage temperature (℃) and per iod (days) on *Pseudomonas aeruginosa* count (log_{10} cfu/g) of cows' milk butter

Storage Temperature (℃)	Storage period (days)	Treatment[1]				CV	SE	*P*
		T1	T2	T3	T4			
5	**1**	3.35^{aA}	2.26^{eD}	2.50^{eC}	2.61^{eB}	2.59	0.002	0.009
	15	2.44^{eC}	2.46^{dC}	2.54^{dB}	2.66^{dA}	3.64	0.008	0.02
	30	2.54^{dC}	2.64^{cB}	2.63^{cB}	2.74^{cA}	5.43	0.001	0.01
	45	2.66^{cC}	2.83^{bA}	2.73^{bB}	2.86^{bA}	2.84	0.003	0.02
	60	2.71^{bD}	3.07^{aA}	2.89^{aC}	2.96^{aB}	3.58	0.005	0.01
	CV	4.25	3.39	3.16	3.40			
	SE	0.006	0.004	0.007	0.003			
	P	0.01	0.02	0.01	0.02			
25	**1**	3.35^{cA}	2.26^{eD}	2.50^{eC}	2.61^{cB}	3.41	0.007	0.008
	7	2.83^{eB}	2.42^{dD}	2.92^{dA}	2.61^{cC}	3.84	0.006	0.02
	14	3.23^{dA}	2.59^{cD}	2.98^{cB}	2.90^{bC}	2.31	0.004	0.01
	21	3.69^{bB}	3.42^{bD}	3.49^{bC}	3.84^{aA}	3.15	0.009	0.01
	28	3.83^{aA}	3.45^{aC}	3.55^{aB}	3.85^{aA}	2.77	0.005	0.01
	CV	2.66	3.25	3.40	3.71			
	SE	0.001	0.003	0.003	0.005			
	P	0.01	0.02	0.02	0.02			

Means in each row (upper case) and column (lower case) bearing similar superscripts are not significantly different (P>0.05); CV = Coefficient of variation (%); SE =Standard error of means; SL= Significance level
[1] Treatments 1, 2, 3 and 4 refer to butter manufactured in Khartoum North, Omdurman, the dairy plant and the laboratory respectively

samples stored at 25℃ the count increased to log_{10} 3.94 cfu/g, log_{10} 4.02 cfu/g, log_{10} 3.98 cfu/g and log_{10} 3.97 cfu/g at the end of the storage period (Table 4). Suryavanshi and Ghosh [7] reported that unsalted butter held at room temperature, sometimes showed high counts of lipolytic and proteolytic bacteria but there was no correlation with defective flavor. Butter is

exposed to contamination with several types of micro-organisms from different sources under stable conditions. These contaminants found a way to grow and multiply in butter leading to undesirable changes which render the product of inferior quality and unmarketable leading to economic losses, unfit for human consumption or may cause public health hazards.

3.5 Lipolytic Bacteria

Lipolytic bacterial count followed the same trend as proteolytic bacteria count increasing significantly during storage period in samples of all treatments stored at 5℃ and 25℃ (Table 5). These results are lower than those reported by Kacem and Karam [1], Asresie et al. [2] and Idoui et al. [6]. However, no lipolytic bacteria were detected in butter of cow's milk in East Algeria [18]. The lipolytic bacteria have lipolytic activity responsible for the appearance of rancid smell in butter, and the rancidity is related to the appearance of compounds of unpleasant odours (acids, aldehyds, ketons) resulting from the hydrolysis of fat content by microbial lipases [6].

3.6 Yeasts and Moulds

The samples of all treatments showed a significant increase in yeasts and moulds count during the storage period at 5℃ and 25℃, with the increase in samples stored at 25℃ being higher. Yeasts and moulds count increased from $\log_{10}$ 2.57 cfu/g, $\log_{10}$ 2.62 cfu/g, $\log_{10}$ 2.77 cfu/g and $\log_{10}$ 2.49 cfu/g at day1 to $\log_{10}$ 3.39 cfu/g, $\log_{10}$ 2.94 cfu/g, $\log_{10}$ 3.03 cfu/g and $\log_{10}$ 3.08 cfu/g at day 60 for T1, T2,T3 and T4 samples stored at 5℃, and to $\log_{10}$ 4.84 cfu/g, $\log_{10}$ 4.89 cfu/g, $\log_{10}$ 4.94 cfu/g and $\log_{10}$ 4.90 cfu/g at day 28 for samples stored at 25℃ (Table 6). Counts of yeasts and moulds were between log 2.57 – 3.39 at 5℃ and log 2.57 – log 4.94 cfu/g at 25℃. These results were in agreement with Samet-Bali et al. [16] who reported yeasts and moulds count of $\log_{10}$ 4.80±0.00 in Turkish butter, Karagözlü and Ergonul [19] who reported yeast and mould counts of butter < $\log_{10}$ 1.00-6.62 cfu/g, Idoui et al. [6,17] and Gökce et al. [3]. Moulds and yeasts grow faster than bacteria and cause spoilage in food with low water activity. Beside spoilage, mycotoxin risk also exists, and the high amount of moulds and yeasts is as an indicator of incorrect processing and packaging [3]. *Geotrichum candidum* is responsible for yeast smell in butter and after a time it causes a disgusting taste and aroma, while Penicillium, Aspergillus, Mucor, Candida, Cladosporium, Fusarium, Rizopus, Torula and Geotrichum form spots on the surface and mouldy taste in butter, *Mucor stolonifer* causes lipolytic and proteolytic decomposition in butter and *Candida lipolitica* causes a caustic and cheese-like taste by exerting lipolytic activity in butter [3].

Table 4. Effect of storage temperature (℃) and per iod (days) on proteolytic bacteria count ($\log_{10}$ cfu/g) of cows' milk butter

Storage Temperature (℃)	Storage period (days)	Treatment[1]				CV	SE	*P*
		T1	T2	T3	T4			
5	**1**	2.35^{eC}	2.26^{eD}	2.50^{dB}	2.61^{dA}	2.84	0.004	0.01
	15	2.44^{dC}	2.46^{dC}	2.49^{dB}	2.62^{dA}	3.43	0.001	0.02
	30	2.54^{cB}	2.64^{cA}	2.63^{cA}	2.65^{cA}	5.47	0.005	0.02
	45	2.66^{bD}	2.83^{bA}	2.73^{bC}	2.86^{bA}	2.76	0.007	0.01
	60	2.71^{aD}	3.06^{aA}	2.89^{aC}	2.96^{aB}	3.55	0.005	0.01
	CV	4.17	3.99	3.81	3.53			
	SE	0.001	0.004	0.006	0.002			
	P	0.03	0.02	0.02	0.02			
25	**1**	2.35^{eC}	2.26^{eD}	2.50^{eB}	2.61^{cA}	3.45	0.008	0.02
	7	2.86^{dB}	2.80^{dC}	2.91^{dA}	2.61^{cD}	3.14	0.001	0.01
	14	3.20^{cC}	3.27^{cB}	3.11^{cD}	3.31^{bA}	2.64	0.003	0.02
	21	3.88^{bB}	3.73^{bC}	3.92^{bA}	3.95^{aA}	3.47	0.004	0.01
	28	3.94^{aC}	4.02^{aA}	3.98^{aB}	3.97^{aB}	2.68	0.001	0.01
	CV	2.51	3.94	3.23	3.09			
	SE	0.005	0.008	0.009	0.004			
	P	0.02	0.02	0.02	0.02			

Means in each row (upper case) and column (lower case) bearing similar superscripts are not significantly different (P>0.05); CV = Coefficient of variation (%); SE =Standard error of means

SL= Significance level; [1] Treatments 1, 2, 3 and 4 refer to butter manufactured in Khartoum North, Omdurman, the dairy plant and the laboratory respectively

Table 5. Effect of storage temperature (℃) and per iod (days) on lipolytic bacteria count (log_{10} cfu/g) of cows' milk butter

Storage Temperature (℃)	Storage period (days)	Treatment[1]				CV	SE	*P*
		T1	T2	T3	T4			
5	**1**	1.33^{dA}	1.01^{dB}	0.86^{eD}	0.97^{dC}	2.58	0.004	0.01
	15	1.34^{dC}	1.02^{dD}	1.53^{dB}	1.60^{cA}	3.46	0.002	0.02
	30	1.40^{cC}	1.28^{cD}	1.57cB	1.63^{bA}	5.11	0.005	0.02
	45	1.47^{bC}	1.47^{bC}	1.61^{bB}	1.66^{aA}	2.84	0.004	0.04
	60	1.54^{aD}	1.61^{aC}	1.65^{aB}	1.68^{aA}	3.70	0.007	0.01
	CV	4.17	3.35	3.46	3.41			
	SE	0.006	0.004	0.005	0.003			
	P	0.01	0.02	0.02	0.01			
25	**1**	1.33^{eA}	1.01^{eB}	0.86^{eD}	0.97^{eC}	3.46	0.005	0.02
	7	1.42^{dD}	1.45^{dC}	1.58^{dB}	1.63^{dA}	3.88	0.003	0.02
	14	1.50^{cB}	1.50^{cB}	1.65^{cA}	1.67^{cA}	2.13	0.004	0.02
	21	1.56^{bB}	1.53^{bC}	1.69^{bA}	1.71^{bA}	3.44	0.006	0.02
	28	1.60^{aB}	1.57^{aC}	1.73^{aA}	1.75^{aA}	2.73	0.003	0.02
	CV	2.11	3.61	3.26	3.70			
	SE	0.003	0.005	0.007	0.005			
	P	0.008	0.01	0.02	0.02			

*Means in each row (upper case) and column (lower case) bearing similar superscripts are not significantly different (P>0.05); ** = P<0.01; * =P<0.05; CV = Coefficient of variation (%)*
SE =Standard error of means; SL= Significance level; [1] Treatments 1, 2, 3 and 4 refer to butter manufactured in Khartoum North,; Omdurman, the dairy plant and the laboratory respectively

Table 6. Effect of storage temperature (℃) and per iod (days) on yeasts and moulds count (log_{10} cfu/g) of cows' milk butter

Storage Temperature (℃)	Storage period (days)	Treatment[1]				CV	SE	*P*
		T1	T2	T3	T4			
5	**1**	2.57^{eC}	2.62^{eB}	2.77^{eA}	2.49^{eD}	2.51	0.007	0.02
	15	2.68^{dD}	2.74^{dC}	2.86^{dA}	2.80^{dB}	3.13	0.009	0.01
	30	2.87^{cB}	2.83^{cC}	2.93^{cA}	2.91^{cA}	5.58	0.007	0.009
	45	3.08^{bA}	2.87^{bC}	2.96^{bB}	2.97^{bB}	2.41	0.005	0.02
	60	3.39^{aA}	2.94^{aC}	3.03^{aB}	3.08^{aB}	3.28	0.003	0.01
	CV	4.19	3.55	3.31	3.94			
	SE	0.003	0.004	0.007	0.002			
	P	0.018	0.018	0.018	0.018			
25	**1**	2.57^{eC}	2.62^{eB}	2.77^{eA}	2.49^{eD}	3.41	0.003	0.02
	7	3.51^{dD}	3.78^{dA}	3.60^{dC}	3.69^{dB}	3.16	0.001	0.01
	14	3.76^{cD}	4.12^{cA}	3.94^{cB}	3.90^{cC}	2.57	0.004	0.02
	21	4.33^{bC}	4.59^{bA}	4.37^{bB}	4.39^{bB}	3.36	0.007	0.02
	28	4.83^{aC}	4.89^{aB}	4.94^{aA}	4.90^{aB}	2.47	0.003	0.02
	CV	2.52	3.39	3.41	3.55			
	SE	0.009	0.007	0.005	0.004			
	P	0.02	0.01	0.01	0.007			

Means in each row (upper case) and column (lower case) bearing similar superscripts are not significantly different (P>0.05)
CV = Coefficient of variation; SE =Standard error of means; SL = Significance level; [1] Treatments 1, 2, 3 and 4 refer to butter manufactured in Khartoum North, Omdurman, the dairy plant and the laboratory respectively

4. CONCLUSION

This study revealed that during the storage period of butter at 5℃ and 25℃, there was a significant variation in TVB, coliform bacteria, *Pseudomonas aeruginosa,* proteolytic bacteria, lipolytic bacteria and yeasts and moulds counts between treatments with increasing counts as the storage period progressed. Butter making in Sudan is still traditional on farmer's level and batch processing in dairy plants. The highest microbial count in butter reflects the microbial quality of raw milk and cream and the technique of manufacture in Sudan. The shelf life of

traditional butter is adversely affected by the temperature becoming short under room temperature. The problem of butter making in Sudan should be identified, and the traditional methods should be improved by training the small-scale farmers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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