Microbiology Research Journal International
19(6): 1-6, 2017; Article no.MRJI.33150
Previously known as British Microbiology Research Journal
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Microbiological Characteristics of Traditionally Fermented Milk (*Roub*) Manufactured and Sold in Khartoum State, Sudan

Mohamed Osman Mohamed Abdalla[1*] and Safinaz Adil Sir El-Khatem Osman[1]

[1]Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Shambat P.O.Box 32, Postal Code 13314, Khartoum North, Sudan.

Authors' contributions

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

Article Information

DOI: 10.9734/MRJI/2017/33150
Editor(s):
(1) Giuseppe Blaiotta, Department of Food Science, Via Università, Italy.
Reviewers:
(1) Supreena Srisaikham, Burapha University Sakaeo Campus, Thailand.
(2) Marco Antonio Sloboda Cortez, Federal Fluminense University, Brazil.
(3) Dalia El Khoury, University of Guelph, Canada.
Complete Peer review History: http://www.sciencedomain.org/review-history/18894

Original Research Article

Received 31st March 2017
Accepted 29th April 2017
Published 3rd May 2017

ABSTRACT

Aims: This study was conducted to evaluate the microbiological quality of *roub* collected from different areas in Khartoum State, Sudan during the storage period.
Methodology: Thirty *roub* samples were collected from Khartoum, Khartoum North and Omdurman areas in sterile polyethylene bags, preserved in sample containers in ice (4ºC) and transported to the laboratory for analysis. Microbiological [total viable bacteria (TVB), coliform bacteria, lactobacilli, yeasts and moulds] characteristics were determined at 1, 7, 14 and 20-day intervals.
Results: The results showed that all microorganisms tested (except coliform bacteria) were significantly (P<0.001) affected by the area from which samples were collected, with TVB being high in Omdurman (Log 6.35±1.027 cfu/gm), while lactobacilli (Log 7.75±0.775 cfu/gm) and yeasts and moulds (Log 7.49±0.685 cfu/gm) were high in Khartoum North, and coliform bacteria were high in Khartoum (Log 6.29±2.497 cfu/gm). The storage period significantly (P<0.001) affected all

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

microorganisms tested. Coliform bacteria and lactobacilli were significantly (P<0.001) affected by the storage period in samples collected from Khartoum, while only coliform bacteria were significantly (P<0.001) affected in samples collected from Khartoum North. All microorganisms were significantly (P<0.001) affected by the storage period in samples collected from Omdurman except coliform bacteria which were not detected.

Conclusion: It is concluded that there was a significant variation in microbial contamination of the product collected from different areas in Khartoum State, as well as the storage period affecting the microbial count of the product.

Keywords: Contamination; fermented milk; microbiological; lactic acid bacteria roub.

1. INTRODUCTION

The traditional fermentation takes place as a result of the activities of natural flora present in the food or from the environment. Fermentation of milk is a very ancient practice of man which has been passed down from generation to generation [1]. The introduction of fermented milk products to human civilization dates back many thousand years, and is believed to be originated in the Middle East before the Phoenician, and spread through central and Eastern Europe [2,3]. In Africa, fermented foods are still frequently prepared in small quantities using traditional methods by rural communities through spontaneous fermentation or by adding a small amount of previously fermented product as a starter [4]. The characteristics of these products are influenced by the quality and type of raw milk used, the production methods and the regional climatic conditions [5,6]. The nature of fermented products is different from one region to another depending on the local indigenous microflora, which in turn reflect the climatic conditions of the area. Thus, traditional fermented milk in regions with a cold temperature climate contained mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp*., while thermophilic bacteria, which include mostly *Lactobacillus* and *Streptococcus*, prevailed in regions with a hot, subtropical or tropical climate [7]. Lactic acid bacteria are widely distributed in nature and occur naturally as indigenous microflora in fermented foods. Some of the major fermentation processes are based on the use of lactic acid bacteria and their presence is crucial to the intrinsic properties of fermented foods [8]. The method of manufacture of fermented milk products is the main reason for the proliferation of pathogenic bacteria that may be present in the raw milk, since traditional dairy products are manufactured from raw unpasteurized milk [9].

Roub is the major fermented dairy product of the Sudan produced from the surplus milk on household level during the rainy season as a method of milk preservation. Milk is collected and inoculated with starter from a previous batch and left overnight to curdle which is called *laben rayb* or *berkib*. Next day this product is churned either in *si'in* which is a leather container made of tanned goat or sheep skin, or *bukhsa* which is a gourd made from the dried fruit of plant with a lidded narrow mouth. Two products are made by this process: *samin* (fat-based product) and *roub* which is either drunken by itself or diluted with water to be drunken as *gubasha* [2,10]. In Sudan, this product is still being manufactured by traditional plants using unpasteurized milk in addition to unhygienic conditions during manufacture which may lead to contamination with pathogenic microorganisms that may be hazardous to consumers. Therefore, this study is conducted to evaluate the microbiological characteristics of traditionally manufactured fermented dairy product of the Sudan '*roub*' collected from different areas of Khartoum State during the storage period.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 30 *roub* samples (10 samples from each of Khartoum, Khartoum North and Omdurman) were collected in sterile polyethylene bags, preserved in sample containers in ice (4ºC) and transported to the laboratory for analysis. The analysis was carried out immediately on arrival, otherwise the samples were stored at 4ºC and analysis was started within a period of not more than 24 hr. Microbiological characteristics were determined at 1, 7, 14 and 20-day intervals.

2.2 Microbiological Examination

2.2.1 Preparation of sample dilutions

Eleven grams (11 gm) from a homogeneous *roub* sample were added to 99 m1 of sterile distilled water in a clean sterile flask, and shaken to make 10^{-1} dilution. One milliliter (1 ml) from the

previous dilution (10^{-1}) was aseptically transferred to 9 ml sterile distilled water. This procedure was repeated to make serial dilutions of 10^{-1} - 10^{-8} [11].

2.2.2 Total viable bacterial count

Plate count agar medium was used for the enumeration of total viable bacteria count, and the plates were incubated at 32±1ºC for 48±3 hr [11].

2.2.3 Lactobacilli count

MRS agar medium was used for the enumeration of lactobacilli; 1 ml quantities of each sample decimal dilution were streaked on pre-solidified MRS agar plates and incubated anaerobically at 35ºC for 48±3 hr [12].

2.2.4 Coliform bacteria count

MacConkey agar medium was used for the enumeration of coliform bacteria; 1 ml of each sample decimal dilution was transferred to MacConkey agar medium and incubated at 35ºC for 48 hr [13].

2.2.5 Yeasts and moulds count

This was determined by plating a suitable dilution of sample on potato dextrose agar medium acidified with 10% sterile tartaric acid, and the plates were incubated at 25ºC for 5 days [14].

2.3 Statistical Analysis

The statistical analysis was carried out using Statistical Analysis Systems (SAS, ver. 9). Completely randomized block design was used for the analysis of the data. General linear model (GLM) procedure was used to determine the effect of area from which samples were collected and storage period on the microbiological characteristics of *roub*. Mean separation was carried out by Duncan multiple range tests (P≤0.05).

3. RESULTS AND DISCUSSION

3.1 Microbiological Characteristics of *Roub* Collected from Khartoum, Khartoum North and Omdurman

The microbiological examination of *roub* samples from the three cities under study revealed that there was a significant variation (P<0.001) in the microbiological population, with total viable bacteria, lactobacilli and yeasts and moulds counts being high in Khartoum North (Log7.51±0.833 cfu/gm, Log 7.75±0.77 cfu/gm and Log 7.490±0.685 cfu/gm, respectively), while coliform bacteria count was high in Khartoum (Log 6.29±2.497 cfu/gm) and the latter were not detected in Omdurman (Table 1). The high TVBC might be due to contamination during processing and handling. The results of high TVBC in Khartoum North are in disagreement with Lore et al. [15] who reported that high total viable bacterial counts were observed conversely, relatively lower numbers of fungal flora and coliforms were encountered. Absence of coliform bacteria in Omdurman might be due to high acidity of *roub* samples in Omdurman (2.96±0.557% compared to 2.63±0.680% and 2.48±1.09% for Khartoum and Khartoum North, respectively) resulting in inhibition of coliform bacteria from growth. The production conditions of *roub* in Omdurman might have contributed to the decrease or absence of coliform bacteria. These findings are in line with Lore et al. [15] who reported the inhibition of *E. coli* and other coliforms by high acidity caused by the production of organic acids such as lactic acid in fermented products.

3.2 Effect of Storage Period on Microbiological Characteristics of *Roub*

TVB, coliform bacteria and yeasts and moulds counts significantly (P<0.001) decreased towards the end of the storage period, while lactobacilli count showed fluctuating pattern decreasing at day 7 (Log 7.42±0.880 cfu/gm), followed by a slight increase to Log 7.77±1.148 cfu/gm at day 14 before decreasing at the end of storage period (Table 2). In samples from Khartoum, coliform bacteria and lactobacilli counts significantly (P<0.001) increased to a maximum at day 14 (Log 6.81±2.596 and Log 7.82±1.587 cfu/gm, respectively) before decreasing towards the end of storage period, while TVBC gradually but insignificantly increased during the storage period, and yeasts and moulds count insignificantly increased to Log 7.29±1.422 cfu/gm at day 14 followed by a decrease to Log 6.98±1.467 cfu/gm at the end (Table 3). Table 4 revealed that the storage period did not significantly (P>0.05) affect TVB, lactobacilli and yeasts and moulds counts of samples from Khartoum North, although TVBC increased at day 7 then decreased thereafter, while lactobacilli count gradually decreased, and yeasts and moulds count decreased to a minimum at day 14 followed by a slight increase

at the end. Coliform bacteria count significantly (P<0.001) decreased as the storage period progressed. Table 5 revealed that TVB, lactobacilli and yeasts and moulds counts significantly (P<0.001) decreased towards the end of the storage period of samples collected from Omdurman, while coliform bacteria were not detected. The results of TVBC are in agreement with the findings of Mathara et al. [16], Savadago et al. [7], Al-Tahiri [17], Hassan et al. [18] and Abdalla and Hussain [9], and in disagreement with Lore et al. [15] and Awad [19]. The results of coliform bacteria count are in agreement with Savadago et al. [7], Al-Tahiri [17], Lore et al. [15], Cetinkaya and Soyutemiz [20] and Abdalla and Hussain [9] who reported low coliform bacteria numbers in different fermented dairy products. However, these results are not in line with Uzeh et al. [21] who reported a mean coliform bacterium count of 4.25×10^7 cfu/ml in *wara* 'a fermented dairy product of West Africa'. Results of lactobacilli count are in accord with Mathara et al. [16], Savadago et al. [7], El-Baradei et al. [22] and Jokovic et al. [23]. Abdalla and Hussain [9] reported a mean lactic acid bacteria count of Log 7.80 cfu/gm, Log 7.90 cfu/gm and Log 7.50 cfu/gm in *roub* samples collected from El-Obeid, Nyala and Abu Naama areas of the Sudan, respectively. However, these results are not in line with Abdalgadir et al. [24] and Hassan et al. [18] who reported that the mean lactobacilli counts were Log 6.83±0.33 cfu/gm and Log 6.55±0.32 cfu/gm in *gariss* 'a fermented camel milk' from transhumance and nomadic herders of the Sudan, respectively. Results of yeasts and moulds are in line with Hassan et al. [18] who reported that yeasts count comprised mean of Log 6.99±0.13 cfu/gm and Log 7.02±0.30 cfu/gm in samples collected from transhumance and nomadic herders, respectively. However, these findings are in disagreement with Uzeh et al. [21] who reported that the mean fungal count of *wara* was 1.31×10^7 cfu/gm.

Table 1. Microbiological characteristics of *roub* samples collected from Khartoum, Khartoum north and Omdurman (mean ±SD)

Microorganisms	Area from which samples were collected			*p*
	Khartoum	Khartoum North	Omdurman	
TVBC	7.38±1.357[a]	7.51±0.833[a]	6.35±1.027[b]	<0.0001
Coliform bacteria	6.29±2.497[a]	5.39±3.026[b]	ND	<0.0001
Lactobacilli	7.41±1.276[b]	7.75±0.777[a]	7.09±1.149[c]	<0.0001
Yeasts and moulds	7.17±1.316[b]	7.49±0.685[a]	6.05±0.0.833[c]	<0.0001

Means in each raw bearing similar superscripts are not significantly different (P>0.05)
SD ≡ Standard deviation; ND≡ Not detected

Table 2. Effect of storage period on the microbiological characteristics of *roub* samples collected from areas under study (mean± SD)

Microorganisms	Storage period (days)				*p*
	1	7	14	20	
TVBC	7.22±0.995[a]	7.33±0.898[a]	7.05±1.281[b]	6.71±1.493[c]	<0.0001
Coliform bacteria	4.18±3.559[a]	4.13±3.452[a]	3.87±3.735[b]	3.40±3.576[c]	<0.0001
Lactobacilli	7.59±0.904[ab]	7.42±0.880[b]	7.77±1.148[a]	6.87±1.294[c]	<0.0001
Yeasts and moulds	7.10±1.050[a]	6.93±1.059[ab]	6.92±1.145[b]	6.66±1.332[c]	<0.0001

Means in each raw bearing similar superscripts are not significantly different (P>0.05)
SD ≡ standard deviation

Table 3. Effect of storage period on microbiological assessment of *roub* samples collected from Khartoum (Mean ±SD)

Microorganisms	Storage period (days)				*p*
	1	7	14	20	
TVBC	7.19±1.258[a]	7.34±1.195[a]	7.48±1.434[a]	7.49±1.542[a]	0.6021
Coliforms bacteria	6.11±2.442[b]	6.12±2.304[b]	6.81±2.596[a]	6.13±2.652[b]	<0.0002
Lactobacilli	7.55±1.027[a]	7.03±0.849[b]	7.82±1.587[a]	7.23±1.402[b]	<0.0004
Yeasts and moulds	7.20±1.203[a]	7.22±1.179[a]	7.29±1.422[a]	6.98±1.467[a]	0.5923

Means in each raw bearing similar superscripts are not significantly different (P>0.05)
SD ≡ standard deviation

Table 4. Effect of storage period on the microbiological characteristics of *roub* samples collected from Khartoum north (Mean ±SD)

Microorganisms	Storage period (days)				*p*
	1	7	14	20	
TVBC	7.48±0.957[a]	7.67±0.656[a]	7.50±0.839[a]	7.38±0.855[a]	NS
Coliform bacteria	6.44±2.389[a]	6.26±2.201[a]	4.79±3.270[b]	4.07±3.467[c]	<0.0008
Lactobacilli	7.96±0.717[a]	7.82±0.825[a]	7.59±0.722[a]	7.60±0.804[a]	0.2350
Yeasts and moulds	7.55±0.601[a]	7.51±0.755[a]	7.35±0.686[a]	7.54±0.694[a]	0.0902

Means in each raw bearing similar superscripts are not significantly different (P>0.05)
SD ≡ standard deviation

Table 5. Effect of storage period on the microbiological characteristics of *roub* samples collected from Omdurman (mean ±SD)

Microorganisms	Storage period (days)				*p*
	1	7	14	20	
TVBC	6.98±0.624[a]	6.98±0.598[a]	6.17±1.026[b]	5.25±0.643[c]	<0.0005
Coliform bacteria	ND	ND	ND	ND	-
Lactobacilli	7.28±0.827[b]	7.14±0.798[b]	7.88±0.965[a]	5.79±0.783[c]	<0.0009
Yeasts and moulds	6.55±1.017[a]	6.07±0.518[b]	6.11±0.708[b]	5.47±0.646[c]	<0.0003

Means in each raw bearing similar superscripts are not significantly different (P>0.05)
SD ≡ standard deviation; ND≡ not detected

4. CONCLUSION

Microbiological characteristics of the product showed that samples from Khartoum North were highly contaminated compared to samples from Omdurman area which were the least contaminated, and this might be attributed to processing conditions in each area. Sample of Omdurman area were of better quality from microbiological point of view probably due to the fact that either the raw milk used for the manufacture of the product was of good quality or the processing conditions were of good standards. During the storage period, all microorganisms showed a decrease in the count probably due to increasing acidity of the product.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. The technology of traditional milk products in developing countries. FAO Animal Production and Health Paper 85. Food and Agriculture Organization, Rome, Italy; 1990.
2. Mohammed Salih AM, El Sanousi SM, ElZubeir IEM. A review on the Sudanese traditional dairy products and technology. International Journal of Dairy Science. 2011;6(4):227-245.
3. Heita L. Antimicrobial activity profile of traditional fermented milk starter cultures from North-Eastern Namibia. M.Sc. Thesis, University of Namibia, Namibia; 2014.
4. Oywole OB. Lactic fermented foods in Africa and their benefits. Food Control. 1997;8(5-6):289-297.
5. Mensah P. Fermentation - the key to food safety assurance in Africa? Food Control. 1997;8:271-278.
6. Wouetrs JTM, Ayad EHE, Hugenholtz J, Smit G. Microbes from raw milk and fermented dairy products. International Dairy Journal. 2002;12(2-3):91-109.
7. Savadogo A, Ouattara CAT, Savadogo PW, Ouattara AS, Barro N, Traore AS. Microorganisms involved in fulani traditional fermented milk in Burkina Faso. Pakistan Journal of Nutrition. 2004;3(2): 134-139.
8. Harun-ur-Rashid MD, Togo K, Miyamoto T. Identification and characterization of dominant lactic acid bacteria isolated from traditional fermented milk Dahi in Bangladesh. World Journal of Microbiology and Biotechnology. 2007;23:125–133.
9. Abdalla MOM, Hussain KIS. Enumeration and identification of microflora in Roub, A Sudanese traditional fermented dairy

product. British Journal of Dairy Sciences. 2010;1(2):30-33.
10. Abdelgadir W, Nielsen DS, Hamad S, Jakobsen M. A traditional Sudanese fermented camel's milk product, Gariss, as a habitat of Streptococcus infantarius subsp. infantarius. International Journal of Food Microbiology. 2008;127(3):215-219.
11. Houghtby AG, Maturin LJ, Koenig KE. Microbiological count methods. In: Standard Methods of the Examination of Dairy Products. 16th edition. Marshal RT, (edt.). Washington, D.C.: American Public Health Association, USA. 1992;213-246.
12. Frank FJ, Christen LG, Bullerman LB. Tests for groups of microorganisms. In: Standard Methods for the Examination of Dairy Products. 16th edition. Marshal RT, (edt.). Washington, D.C.: American Public Health Association, USA. 1992;271-286.
13. Christen LG, Davidson PM, McAllister JS, Roth LA. Coliform and other indicator bacteria. In: Standard Methods of the Examination of Dairy Products. 16th edition. Marshal RT, (edt.). Washington, D.C.: American Public Health Association, USA. 1992;247-269.
14. Harrigan WF, McCane ME. Laboratory methods in food and dairy microbiology. London, UK; 1976. Academic Press.
15. Lore AT, Mbugua KS, Wangoh J. Enumeration and identification of microflora in suusac, a Kenyan traditional fermented camel milk product. Lebensm. - Wiss. u.-Technology. 2005;38:125-130.
16. Matharaa JM, Schillingera U, Kutimab PM, Mbuguac SK, Holzapfel WH. Isolation, identification and characterisation of the dominant microorganisms of kule naoto: The Maasai traditional fermented milk in Kenya. International Journal of Food Microbiology. 2004;64(3):269-278.
17. Al-Tahiri R. A comparison on microbial conditions between traditional dairy products sold in Karak and same products produced by modern dairies. Pakistan Journal of Nutrition. 2005;4(5):345-348.
18. Hassan RA, ElZubeir IME, Babiker SA. Chemical and microbial measurements of fermented camel milk "Gariss" from transhumance and nomadic herds in Sudan. Australian Journal of Basic and Applied Science. 2008;2(4):800-804.
19. Awad MI. The effect of management practices on the hygienic quality of raw milk produced by some dairy farms in Khartoum State. M.Sc. Thesis, Faculty of Animal Production, University of Khartoum, Sudan; 2006.
20. Cetinkaya F, Soyutemiz EG. Microbiological and chemical changes throughout the manufacture and ripening of Kashar: A traditional Turkish cheese. Turkish Journal of Veterinary and Animal Science. 2006;30:397-404.
21. Uzeh ER, Ohenhen ER, Rojugbokan KA. Microbiological and nutritional qualities of dairy products: Nono and Wara. Nature and Science. 2006;4(3):37-40.
22. El-Baradei G, Delacroix-Buchet A, Ogier CJ. Bacterial biodiversity of traditional zabady fermented milk. International Journal of Food Microbiology. 2008;121: 295-301.
23. Jokovic N, Nikolic M, Begovic J, Jovocic B, Savic D, ToPisirovic L. A survey of the lactic acid bacteria isolated from Serbian artisanal dairy product Kajmak. International Journal of Food Microbiology. 2008;127:305-311.
24. Abdelgadir WS, Ahmed TK, Dirar HA. The traditional fermented milk products of the Sudan. International Journal of Food Microbiology. 1998;44:1-13.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18894