British Journal of Dairy Sciences 1(2): 30-33, 2010
ISSN: 2044-2440

Submitted date: August 15, 2010 Accepted date: September 13, 2010 Published date: December 15, 2010

Enumeration and Identification of Microflora in *Roub*, A Sudanese Traditional Fermented Dairy Product

M.O.M. Abdalla and S. I.K. Hussain
Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Shambat, Postal Code 13314, Khartoum North, Sudan

Abstract: This investigation was carried out to isolate and identify the predominant microflora in *Roub* samples collected from different parts of the Sudan. A total of 30 samples were collected from El-Obied, North Kordofan State; Nyala, South Darfur State and Abu Naama, The Blue Nile State, in sterile bottles and transported in a cool box at 4ºC for analysis. The samples were subjected to microbiological examination (total viable bacteria, *Staphylococcus aureus*, *Salmonella* sp., lactic acid bacteria and yeasts and moulds). The results showed the occurrence of coliform bacteria, *S. aureus*, lactic acid bacteria, yeasts and moulds in the samples, while *Salmonella* sp. was not detected. Coliform bacteria were not detected in samples collected from Abu Naama area, and detected in 30 and 20% of samples collected from El Obied and Nyala areas respectively. *S. aureus* was detected in 40, 60 and 20% of samples from El Obied, Nyala and Abu Naama, respectively while yeasts and moulds were detected in 100, 90 and 90% of samples from El Obied, Nyala and Abu Naama areas, respectively. Llactic acid bacteria were detected in all samples under study.

Key words: Enumeration, fermentation, identification, microflora, *Roub*, Sudan

INTRODUCTION

The nature of fermented dairy products is different from one region to another depending on the local indigenous microflora. While *Leuconostoc* is responsible for traditional fermentation of milk in temperate climates, *Lactobacillus* and *Streptococcus* are responsible for fermentation in tropical and subtropical climates (Kurmann, 1994). Environmental conditions in each country affect the properties of the predominant native microflora limiting the use of some universal starters, and the rational solution is the selection of starter cultures from the native flora that could be used successfully in the dairy industry.

Roub is a traditional fermented dairy product produced in the rural areas of the Sudan from cow's milk when plenty of milk is available during the rainy season. *Roub* is made from surplus unheated milk by inoculating with starter culture from the fermentation of the previous day. After coagulation, the curd is churned early in the morning either by *Sîin* (made of tanned goat skin) or by *Bokhsa* (a gourd made from dried fruit of the plant *Lagenaria peucantha*). After the separation of the cream (locally known as *Fursa*), the remaining part is the *Roub* which is either drunk by diluting with water (called *Gobasha*) or added to powdered okra to make a soup eaten with a pudding (Abdelgadir *et al.,* 1998, 2001; Hussain, 2010).

The microbiology of *Roub* collected from the market was studied by Saeed (1981), El Mardi (1988) and Abdelgadir *et al.* (2001). The dominant lactic acid bacteria were *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus fermentum*, *Streptococcus salivarius*, *Lactococcus lactis* ssp. *lactis*, *Leuconostoc mesenteroides* ssp. *cremoris* and *Lactobacillus acidopilus*. The dominant yeasts were *Saccharomyces cereviae*, *Saccharomyces globosus*, *Saccharomyces exigus*, *Kluyveromyces bulgaricus*, *Kluyveromyces lactis* and *Candida kefir*. Abdelgadir *et al.* (2008) isolated *Streptococcus infantarius* ssp. *infantarius* as a potential human pathogen from Sudanese traditional camel milk product, *Gariss* samples collected from the market.

Many pathogenic microorganisms were isolated from traditionally fermented dairy products of different parts of the world. These organisms are *S. aureus*, *Bacillus cereus*, Klebsiella, coliforms (Beukes *et al.*, 2001; Lore *et al.*, 2005; Uzeh *et al.*, 2006). The main reason for the isolation of pathogenic organisms from traditionally fermented dairy products is the method of manufacture which involves the use of unpasteurized milk. This study aimed at isolating and identifying the predominant microflora in *Roub* from different areas in Sudan.

MATERIALS AND METHODS

Collection of samples: This study was conducted at the Department of Dairy Production, Faculty of Animal Production, University of Khartoum during the period January to July 2007. A total of 30 traditionally made

Corresponding Author: M.O.M. Abdalla, Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Shambat, Postal Code 13314, Khartoum North, Sudan

Table 1: Microbiological profile (Mean±SE) of "*Roub*" samples from three different areas in Sudan

	Area of study			
Organism	El-Obeid	Nyala	Abu Naama	SL
Total viable bacteria count	8.14±0.40[a]	7.56±0.40[b]	8.07±0.40[b]	*
Coliform bacteria	5.60±2.62[a]	5.70±2.62[a]	ND	NS
Staphylococcus aureus	6.15±3.15[a]	6.18±3.15[a]	6.00±3.15[a]	NS
Salmonella sp.	ND	ND	ND	-
Lactic acid bacteria	7.80±0.57[a]	7.09±0.57[b]	7.51±0.57[ab]	*
Yeasts and moulds	5.53±1.41[a]	4.64±1.41[a]	5.50±1.41[a]	NS

Means within each row bearing the same superscripts are not significantly different (p>0.05)
*: p<0.05, NS = Not Significant, SL = Significance Level, SE = Standard error, ND = Not detected

Table 2: Microbiological profile (Log_{10} cfu/ml) of "*Roub*" and percent positive samples (% +ve) from El-Obeid area

Sample No.	TVBC	*Salmonella* sp.	Coliform bacteria	*S. aureus*	Yeasts and moulds	Lactic acid bacteria
1	7.53	ND	ND	ND	4.00	7.18
2	8.29	ND	6.0	6.00	5.83	8.06
3	8.19	ND	6.0	ND	5.97	7.48
4	7.64	ND	6.3	ND	5.20	7.04
5	7.89	ND	ND	ND	5.08	7.66
6	7.76	ND	ND	6.60	4.70	7.26
7	8.24	ND	ND	6.00	5.65	7.92
8	8.34	ND	ND	6.90	5.90	7.98
9	8.47	ND	ND	ND	5.15	8.29
10	8.19	ND	ND	ND	4.85	7.40
Mean±SE	8.14±0.40	ND	5.60±2.62	6.15±3.15	5.53±1.41	7.80±0.57
% +ve	100	0	30	40	100	100

TVBC = Total viable bacteria count, ND = Not detected, % +ve = Pecent positive samples

Roub samples were obtained from three regions in Sudan; El-Obied, North Kordofan State; Nyala, South Darfur State and Abu Naama, The Blue Nile State. The samples were collected in sterile bottles, transported to the laboratory in a cool box at 4ºC before analysis. Samples were analyzed within 48 h of collection.

Microbial enumeration and isolation: Eleven grams of *Roub* samples were transferred aseptically into a sterile bottle containing 99 mL sterile peptone water and mixed thoroughly. Serial dilutions (10^{-1} -10^{-8}) were made for each sample and 1 mL of the appropriate dilution was transferred into a sterile Petri dish and pour plated using the appropriate culture media (for yeasts and moulds, 0.1 mL was transferred into solidified medium and spread plated). Plate count agar (Scharlau Chemie S.A., Barcelona, Spain) was used for the enumeration of total viable bacteria, and the plates were incubated at 32ºC for 48 h (Houghtby *et al*., 1992). MRS agar medium (Scharlau Chemie S.A.) was used for enumeration of total lactic acid bacteria and incubated at 35ºC for 48 h. Mannitol salt agar medium (Scharlau Chemie S.A.) was used for the enumeration of *S. aureus* and the plates were incubated at 37ºC for 48 h. Salmonella Shigella agar medium (Scharlau Chemie S.A.) was used for detection of *Salmonella* sp. and incubated at 35ºC for 48 h. McConkey agar medium (Scharlau Chemie S.A.) was used for the numeration of coliform bacteria and the plates were incubated at 32ºC for 24 h. Potato dextrose agar medium (Scharlau Chemie S.A.) was used for the enumeration of yeasts and moulds and plates were incubated at 30ºC for 5 days. Biochemical tests were carried out according to Barrow and Feltham (1993).

Statistical analyses: The samples were analyzed for total viable bacteria count, *S. aureus* count, coliform bacteria count, lactic acid bacteria count and yeasts and moulds count using Statistical Analysis Systems (SAS, ver. 16). Means were separated using Duncan multiple range test with $p \leq 0.05$.

RESULTS

Total viable bacteria count: Results showed that total viable bacteria count was significantly (p<0.05) affected by the area from which samples were collected, with the highest count being in samples collected from El-Obeid area and the lowest count in samples collected from Nyala area (Table 1). Total viable bacteria count ranged from Log_{10} 7.53 to Log_{10} 8.47 cfu/mL in El-Obeid, Log_{10} 7.34-7.69 cfu/mL in Nyala and Log_{10} 7.24-8.69 cfu/mL in Abu Naama area. All samples tested were positive (Table 2, 3 and 4).

Coliform bacteria count: Mean coliform bacteria count was not significantly affected by the area, and no coliform bacteria were detected in samples from Abu Naama area (Table 1). Coliform bacteria were detected in 30% of samples from El-Obeid and 20% of samples from Nyala area, and the total coliform count ranged between Log_{10} 6.00 - Log_{10} 6.30 cfu/mL in El-Obeid and Nyala areas (Table 2, 3 and 4).

***S. aureus* count:** *S. aureus* count did not show any significant difference in the three areas under study, although the highest count was in samples from Nyala area (Table 1). The organism was detected in 40% of samples collected from El-Obeid area, 60% in Nyala area

Table 3: Microbiological profile (Log_{10} cfu/ml) of "*Roub*" and percent positive samples (% +ve) from Nyala area

Sample No.	TVBC	*Salmonella* sp.	Coliform bacteria	*S. aureus*	Yeasts and moulds	Lactic acid bacteria
1	7.34	ND	ND	ND	4.60	6.78
2	7.43	ND	ND	ND	4.78	6.90
3	7.46	ND	ND	6.30	4.30	7.08
4	7.54	ND	ND	ND	4.78	6.95
5	7.52	ND	ND	6.30	4.60	7.00
6	7.54	ND	ND	6.00	4.30	7.20
7	7.69	ND	ND	6.30	4.90	7.28
8	7.65	ND	ND	ND	4.90	7.15
9	7.72	ND	6.00	6.60	ND	7.26
10	7.53	ND	6.30	6.60	4.60	7.08
Mean±SE	7.56±0.40	ND	5.70±2.62	6.18±3.15	4.64±1.41	7.09±0.57
% +ve	100	0	20	60	90	100

TVBC = Total viable bacteria count, ND = Not detected, % +ve = Pecent positive samples

Table 4: Microbiological profile (Log_{10} cfu/ml) of "*Roub*" and percent positive samples (% +ve) from Abu Naama area

Sample No.	TVBC	*Salmonella* sp.	Coliform bacteria	*S. aureus*	Yeasts and moulds	Lactic acid bacteria
1	7.37	ND	ND	ND	4.00	6.60
2	7.46	ND	ND	ND	3.70	5.70
3	7.87	ND	ND	ND	5.40	7.40
4	7.37	ND	ND	ND	4.60	6.60
5	7.33	ND	ND	ND	ND	6.65
6	8.48	ND	ND	6.00	5.81	7.81
7	7.24	ND	ND	ND	4.30	6.30
8	8.00	ND	ND	ND	5.51	7.51
9	8.06	ND	ND	ND	5.40	7.40
10	8.69	ND	ND	6.00	6.21	8.21
Mean±SE	8.07±0.40	ND	ND	6.00±0.00	5.50±1.41	7.51±0.57
% +ve	100	0	0	20	90	100

TVBC =Total viable bacteria count, ND = Not detected, % +ve = Pecent positive samples

and 20% in Abu Naama area. The count ranged between Log_{10} 6.00 - Log_{10} 6.90 cfu/mL and Log_{10} 6.00 - Log_{10} 6.60 cfu/mL in El-Obeid and Nyala areas, respectively (Table 2, 3 and 4).

***Salmonella* sp.:** The organism was not detected in all samples collected from the three areas under study.

Lactic acid bacteria count: Mean lactic acid bacteria count was significantly ($p<0.05$) higher in El-Obeid area, while the lowest mean count was in Nyala area (Table 1). Lactic acid bacteria were detected in all samples, and the count ranged between Log_{10} 7.04 and Log_{10} 8.29 cfu/mL in E-Obeid, Log_{10} 6.78 - Log_{10} 7.28 cfu/mL in Nyala and Log_{10} 5.70 - Log_{10} 7.81 cfu/mL in Abu Naama area (Table 2, 3 and 4).

Yeasts and moulds count: There was non-significant variation in the count of yeasts and moulds in the areas under study; however, the highest count was in samples from El-Obeid area (Table 1). Yeasts and moulds were detected in 100, 90 and 90% of samples collected from El-Obeid, Nyala and Abu Naama areas respectively, with the range being Log_{10} 4.00 - Log_{10} 5.97 cfu/mL , Log_{10} 4.30 - Log_{10} 4.90 cfu/mL and Log_{10} 3.70 - Log_{10} 6.21 cfu/mL, while yeasts and moulds were not detected in one sample from each of Nyala and Abu Naama (Table 2, 3 and 4).

DISCUSSION

Due to absence of heat treatment of milk prior to fermentation in addition to utilizing natural fermentation, it is expected that total bacterial count is high in all areas sampled.

The results of microbiological examination indicate that this product is highly contaminated with microorganisms of public health concern. The high number of total bacterial count, *S. aureus* and coliform bacteria indicates unhygienic conditions during production of milk and further processing into *Roub* without heat treatment (Hussain, 2010; Uzeh *et al*., 2006). Similar results of total bacteria count were reported for different traditional dairy products (Beukes *et al*., 2001; Mathara *et al*., 2004; Savadogo *et al*., 2004; Lore *et al*., 2005; Al-Tahiri, 2005; Hassan *et al*., 2008). The detection of coliform bacteria and *S. aureus* in high number is a public health concern since it indicates faecal contamination during production or processing of this product. These organisms were isolated by different researchers in other fermented dairy products (Savadogo *et al*., 2004; Al-Tahiri, 2005, Lore *et al*., 2005; Uzeh *et al*., 2006).

The results of lactic acid bacteria count show that fermentation is mainly carried out by lactic acid bacteria in uncontrolled conditions of fermentation. Similar results were reported by Abdelgadir *et al*. (2001),

Beukes *et al*. (2001), Mathara *et al*. (2004), Savadogo *et al*. (2004), El-Baradei *et al*. (2008), Hassan *et al*. (2008) and Jokovic *et al*. (2008).

Salmonella sp. was not detected in all samples tested. This result was in disagreement with the results reported by Abdalla and El Zubeir (2006) in mish and roub. *Roub* samples were highly contaminated with yeasts and moulds. This might be possibly due to poor processing conditions and/or uncontrolled fermentation which lead to contamination with yeasts and moulds, and this is obvious by alcoholic fermentation resulting in alcohol production in addition to lactic acid. Abdelgadir *et al.* (2001), Ali *et al.* (2002), Mathara *et al*. (2004), Savadogo *et al*. (2004), Al-Tahiri (2005), Lore *et al*. (2005) and Uzeh *et al*. (2006) detected yeasts and moulds in different traditional fermented dairy products.

CONCLUSION

In conclusion, the high number of coliform bacteria and *S. aureus* indicate the unhygienic conditions during processing and subsequent handling of the product, while high number of lactic acid bacteria indicate that the natural microflora of milk are responsible for producing lactic acid, in addition to yeasts which might produce alcohol during fermentation converting the product into slightly alcoholic.

ACKNOWLEDGMENT

The authors acknowledge the assistance of the staff of the Microbiology laboratory at the Department of Dairy Production.

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