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Microbial Hazards Associated with Fermented Milk (Roub and Mish) Processing in Sudan*

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Abstract: The present study was conducted to evaluate the hygienic properties during manufacturing of fermented milk by isolation and identification of some potential pathogens and it also aimed for evaluation of some fermented dairy products (roub and mish in a modern dairy factory). It was based on collection of six batches of samples from the whole milk, skim milk, yoghurt and their mixture; which enter for the processing of roub and mish; roub and mish from a modern dairy factory in Khartoum State (Sudan). The 1st and 2nd batches were collected from the factory commercial production. The 3rd and 4th were done in the factory laboratory as an experiment following the same procedure, while in the 5th and 6th batches pasteurization of whole milk was done. Comparison and the counts of each of *E. coli*, *Staphylococcus aureus*, *Streptococcus* spp. and *Salmonella* spp. were estimated in those products. Higher counts were obtained for the factory commercial samples compared with those manufactured as experiment in the factory. Moreover pasteurization of the whole milk revealed lower counts, which could be attributed to elimination of contamination. When comparing roub and mish significant differences were obtained only for *E. coli* ($p \leq 0.05$) and *S. aureus* counts ($p \leq 0.05$). Similarly, between mixture and roub significance variations were reported for *E. coli* ($p \leq 0.05$) and *S. aureus* counts ($p \leq 0.001$). However, non-significance differences were found for the measurements between the different groups. This could be due to fermentation in roub and addition of spices (black cumin, fenugreek and garlic) and salt in mish. Hence the present study supported the previous reports, which stated the role of those spices as antimicrobial agents against some pathogenic bacteria.

Key words: Fermented milk (roub and mish), pasteurization, pathogenic bacteria, Sudan

Introduction

Fermented milks are products prepared by controlled fermentation of milk to produce acidity and flavour to desire level, fermented milks are the most common products from which other dairy products are also made (Thapa, 2000.). Fortunately, the dominant bacteria in fermented milks were progressive type, lactic acid streptococci and lactobacilli, which generally suppers the spoilage and pathogenic organism very effectively (Kosikowski, 1982). In the earlier days, fermentation was used to control the growth of harmful bacteria and some pathogens while making indigenous milk products (Thapa, 2000).

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Fermented milk products are of a considerable economic and dietary importance to the people of Sudan (El-Mardi, 1988; Abdel Gadir *et al*., 1998). The milk for roub (sour butter milk) is either boiled or used raw in Sudan (Abdel Gadir *et al*., 1998). Although yoghurt is highly nutritious and easily digestible due to the predigested nutrients by bacterial starter, it is less perishable in view of its unused lactose content which can be utilized for the growth of undesirable microorganisms responsible for spoilage (Durga *et al*., 1986). Mish is a special spicy fermented milk (black cumin; *Nigelia sativa* and fenugreek; *Trigonella foenum-graecum* and occasionally garlic; *Allium sativa* (El-Mardi, 1988).

Spices and medicinal plants have been economically valued since immemorial times (El-Hussein, 1984). Black cumin seeds are found to be acceptable without any health hazards associated with their consumption (El-Jassir, 1992.). Similarly, garlic was used since ancient times as food, spice or remedy, in the Middle Ages; it was used as antibiotic and is registered as a drug in several European countries (Grinwald, 1992). In Asia, fenugreek seeds *(Trigonella foenum graecum*) are consumed as spices and also as medicines (Patil *et al*., 1997).

Bacteria spoil foods as well as causing diseases to man and animals and because of their unseen activities; man is unaware of them (Singleton, 1992). Poor milk hygiene is mostly caused by pathogenic microorganism, which may result from infections of dairy animals or from secondary contamination by either milkers or soiled equipment (Chabo *et al*., 2000). Also water, which is essential commodity on dairy farms, could also have contributed significantly to the microbial quality of milk and to the occurrence of clinical or sub-clinical mastitis. Moreover, dairy cows are known to act as reservoirs of enteric pathogens like *Salmonella*, *E. coli*, *Compylobacter* spp. and *Listeria* spp. (Adesiyun *et al*., 1997.). However, during and after milking, the milk is subjected to organisms from various sources and with extended storage of milk products at refrigerated temperatures, psychrophilic or psychrotrophic organisms cause spoilage (Murphy *et al*., 2000).

Milk borne human infection or in toxications due to *Escherichia coli, Staphylococeus aureus, Listeria monocytogenes* and other pathogens have been well documented (Kosikowski, 1982). Similarly, various zoonotic agents can be transmitted to human through milk (Giovannini, 1998).

Contamination of raw milk by salmonella, usually takes place from external sources and the reduction in salmonella numbers due to freezing temperatures is limited, since it can survive a prolonged time in frozen foods (Asperger *et al*., 1994). Similarly, the survival of salmonella for at least 63 days in cultured buttermilk stored at 8°C (Wilson and Tanner, 1945). However, salmonella are readily destroyed during milk pasteurization (Asperger *et al*., 1994).

The ranges in mean counts of *E. coli* per milliliter of bulk milk and composite milk were 8.4×10^{3} to 2.0×10^{5} and 2.1×10^{1} to 2.0×10^{2} cfu mL^{-1}, respectively and it could pose a health risk to consumers (Adesiyun *et al*., 1997). Moreover the sufficient number of *E. coli* to produce illness, is suggested to be 10^{6} cells or more (Asperger *et al*.,1994).

The present study was a contribution to the evaluation and comparison of the hygienic quality (isolation and identification of some pathogenic organisms) of fermented milk (Roub and Mish) in Sudan.

Materials and Methods

Source of Samples

The present study was conducted during the period from January to March 2002. Two batches of whole milk, skim milk and yoghurt, which used for processing of roub and mish from one of the dairy factory in Khartoum North city, were analyzed. Similarly roub and mish samples, obtained from

the same products, were also collected and analyzed during this study. On another trail a total of four experiments as an attempt to process roub and mish in the factory's laboratory were tried following the same procedures of the factory for two batch. However in the other pasteurization for whole milk was done before the processing. The same conditions for these fermented milks were nearly applied by incubating the mixtures at room temperature (30±2°C for 18±1 h) after addition of starter culture to obtain roub. Further fermentation of roub to mish with the addition of salt, fenugreek, garlic and black cumin, was done.

Collection of the Samples

Samples from each of whole milk, skim milk and yoghurt were collected after through mixing, in sterile bottles (10 mL) and transported quickly to the laboratory for microbiological examination. Each batch of sample was done on a separate week, since the collection of roub was done on the next day after collecting original samples (whole milk, skim milk and youghurt) used for its mixture. The mish was collected on the third day.

Microbiological Examination

All the samples were enumerated for the same pathogens. *Escherichia coli* count was done on MacConkey agar, *Staphylococcus aureus* count on mannitol salt agar and *Salmonella* spp. count was done using Deoxycholate Citrate Agar (DCA) (Barrow and Feltham, 1993). Similarly *Streptococcus* spp. counts on modified Edward's medium (Asperger *et al*., 1994).

Serial dilutions of the samples were prepared (10^{-1}-16^{-10}) (Richardson, 1985). The enumeration was carried out aseptically by plating the selected dilution into duplicate plates of the selected media. Purification and identification of each organism was done (Asperger *et al*., 1994).

Statistical Analysis

All data were analyzed statistically using Complete Randomized Design by SPSS program (Statistical Packages for Social Sciences). The LSD test was used to detect difference between means (Snedecor and Cochran, 1980).

Results

Incidences and Frequencies of Some Pathogens Isolated During Different Processing Procedures

The number of individuals of *E. coli*, *S. aureus*, *Streptococcus* spp. and *Salmonella* spp. isolated from processed samples were 15, 12 and 7, respectively (Table 1). Similarly the factory commercial products showed high counts of microorganisms than the samples, which were manufactured in laboratory. Moreover, *E. coli* was not isolated from the pasteurized milk samples.

Table 1: Incidences and frequencies of total pathogens isolated during different processing procedure

	No. of pathogenic bacteria isolated		
Groups	Factory	Lab. (1)	Lab. (2)
Whole milk	5	3	1
Skim milk	3	3	1
Yoghurt	4	2	1
Roub	1	2	2
Mish	2	2	2
Total	15	12	7

Lab. (1) = Processing following the same procedure of factory
Lab. (2) = Processing after pasteurized of whole milk

Table 2: Comparison of the differential counts of pathogenic bacteria isolated from milk and fermented milk products

	Log. *E. coli* (cfu mL^{-1})			Log. *S. aureus* (cfu mL^{-1})		
Milk products	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.
Whole milk	2.31±2.77	0.00	6.64	1.29±1.99	0.00	3.87
Skim milk	1.85±2.43	0.00	5.11	2.06±2.46	0.00	4.90
Yoghurt	2.11±2.65	0.00	6.52	0.48±1.19	0.00	2.90
Mixture	2.31±2.59	0.00	6.58	1.04±1.63	0.00	3.37
Roub	0.35±0.88	0.00	2.15	0.00	0.00	0.00
Mish	1.55±2.42	0.00	4.78	1.00±2.45	0.00	6.00
	Log. *Strep.* spp. (cfu mL^{-1})			Log. *Salmonella* spp. (cfu mL^{-1})		
Milk products	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.
Whole milk	1.48±2.30	0.00	4.60	1.15±1.93	0.00	4.61
Skim milk	1.75±3.50	0.00	7.00	1.84±2.35	0.00	4.90
Yoghurt	1.79±2.78	0.00	5.67	1.12±2.74	0.00	6.71
Mixture	1.76±2.74	0.00	5.46	1.48±2.19	0.00	5.66
Roub	1.49±2.43	0.00	5.64	2.36±3.67	0.00	7.30
Mish	1.52±2.44	0.00	5.48	1.11±2.71	0.00	6.63

Microbial Properties of Samples

The means log counts of *Escherichia coli*, *Staphylococcus aureus, Streptococcus* spp. and *Salmonella* spp. in whole milk were 2.31±2.77, 1.29±1.99, 1.48±3.30 and 1.15±1.93 cfu mL^{-1} (Table 2). Similarly, skim milk samples revealed means log counts of 1.85±2.43, 2.06±2.46, 1.75±3.50 and 1.84±2.35 cfu mL^{-1} for *E. coli*, *Staphylococcus aureus*, *Streptococcus* spp. and *Salmonella* spp., respectively (Table 2). In yoghurt samples (Table 2) the mean log count of *E. coli* was 2.11±2.65, for *S. aureus* was 0.48±1.19 cfu mL^{-1}, for *Streptococcus* spp. was 1.79±2.78 and for *Samonella* spp. was 1.12±2.74 cfu mL^{-1}. The mixture samples revealed mean log count for *E. coli* of 2.31±2.59, *S. aureus* of 1.04±1.63 cfu mL^{-1}, *Streptococcus* spp. of 1.76±2.74 and *Salmonella* spp. of 1.48±2.19 cfu mL^{-1} (Table 2). In Roub samples *E. coli* revealed a mean log counts of 0.35±0.88 cfu mL^{-1}, for *Streptococcus* spp. a mean log counts of 1.49±2.43 cfu mL^{-1} and *Salmonella* spp. a mean log counts of 2.36±3.67, however *S. aureus* was not detected. Mish samples revealed mean log for *E. coli* counts of 1.55±2.42, *S. aureus* mean log count of 1.00±2.45, *Streptococcus* spp. mean log count of 1.52±2.44 cfu mL^{-1} and *Salmonella* spp. mean log counts of 1.11±2.71 cfu mL^{-1} (Table 2).

Comparison Between Mixture, Roub and Mish

Comparing mixture and roub there were significant differences observed between *E. coli* ($p \leq 0.05$) and *S. aureus* ($p \leq 0.001$). However, non significant variations were found between *Salmonella* spp. counts and *Streptococcus* spp. counts. Also comparison of mixture and mish revealed non significant variations for all measures. Comparing roub and mish revealed non significant differences for all measures except for *E. coli* ($p \leq 0.01$) and *S. aureus* ($p \leq 0.05$).

Discussion

The presence of some pathogens in the dairy products (*E. coli*, *S. aureus*, *Streptococcus* spp. and *Salmonella* spp.) during different processing procedures indicated the lower standards of hygiene in the selected dairy factory. This could be due to the traditional method of distribution of milk to consumers, retailers and factories, which were transported into large plastic containers that still practiced in Sudan. In addition, those containers are opened frequently that milk is subjected to contamination (Murphy and Boor, 2000). It might also be due to unrefrigerated transportation of milk

from dairy farms, through collection centers, to the major processing plant may have been responsible for the high counts of *E. coli* and *S. aureus* in milk (Asperger *et al*., 1994).

The higher incidences of *Salmonella* spp. in the samples collected from factory compared to that made by the student supported the previous report, which stated that the human handler could also play a role in contributing to health risk by contaminating milk (Giovannini, 1998). Moreover, *E. coli* was not isolated from the pasteurized milk samples. This could be due to that generally, the Enterobacteriaceae do not survive pasteurization but contamination can be due to poor post pasteurization control (Manie *et al*., 1999).

The mean log count of *Escherichia coli*, *Staphylococcus aureus, Streptococcus* spp. and *Salmonella* spp. in whole milk demonstrated that raw milk could be a source of pathogenic bacteria (Giovannini, 1998). The presence of pathogenic organisms in samples could be due to unhygienic conditions on practice during production or may be due to inadequate cleaning of utensils (Jay, 1986). Similarly, inadequately sanitized milk utensils are the most common sources of coliform in raw milk (Murphy and Boor, 2000).

Mish samples revealed increase in the means log counts for *E. coli*, *S. aureus* and *Streptococcus* spp., while counts of *Salmonella* spp. were decrease compared to roub samples (Table 2). This might be due to the fermentation effect as one of the potential health benefits of lactic acid bacteria is the protection against enteric infections (Perdigon *et al*., 1995). Moreover, the present results for *S. aureus* (Table 2) were lower than that obtained for roub and mish (Manal, 2001). The results reported during this investigation showed a remarkable effect of the spices on the growth of *Staphylococcus aureus* (Table 2), which were supported the findings that the volatile oil in the black cumin inhibited the growth of *Staphylococcus aureus* (Hanfey and Hatem, 1991). Moreover, the bacterial count of *Staphylococcus aureus* decrease from log 7.45 to log 7.34 cfu mL^{-1} in mish samples (Manal, 2001). Hence it is suggested that detail studies should be conducted on the fate of pathogenic bacteria due to fermentation and spices that traditionally added as a preservative.

The present study concluded that heat treatment of milk, proper handling and strict hygienic measures before manufacturing of dairy products should be very well monitored. Fermentation and addition of traditional spices proved to have a very significant effect of pathogenic organisms. Further studies are needed to estimate the different concentrations of spices in order to inhibit the growth of some pathogenic bacteria such as *E. coli, S. aureus, Salmonella* spp. and other food-borne pathogens.

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