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Preservation of Raw Milk of Khartoum State (Sudan) by the Lactoperoxidase System*

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Abstract: This study was carried out at Khartoum State to evaluate the hygiene and the quality of milk after preservation with Lactoperoxidase Enzyme System (LPS). The milk was collected directly from a traditional farm, the collection and examination of samples was done during April- June 2005. The result indicated that non significant differences were found for total bacterial counts, coliform counts and acidity in the treated milk samples. Moreover this study showed that total bacterial counts and the acidity were not affected by temperature (room and refrigerator) after treatment with Lactoperoxidase System (LPS). Also the treatment of raw milk with the LPS revealed non significant differences in acidity due to variation of storage temperature. However the result indicated that there were significant differences in total bacterial and coliform counts ($p<0.001$) of the raw milk stored at both room and refrigerator temperatures. The raw milk samples revealed the presence of five organisms and they were identified as *Escherichia coli*, *Citrobacter freundii*, *Yersinia enterocolitica*, *Staphylococcus aureus* and *Enterococcus feacalis*. However the treated milk (with lactoperoxidase system) revealed the presence of *E. feacalis*, which take time before it was disappeared (9 days for refrigerated treated milk). Thus the present study encourages the uses of lactoperoxidase enzyme system for preservation of milk where cooling facilities are not available to ensure its safety. However the addition of the enzymes should be done by trained authorized persons.

Key words: Lactoperoxidase system, bacteria, bovine milk, storage, shelf life, Sudan

Introduction

Milk is magnificent medium for growth of microorganisms and therefore risk for quick microbial deterioration of quality is present from time of milking to the time of use in the milk processing plant (IDF, 1994). Measurement of bacterial numbers in milk is of interest because of their direct role in milk spoilage and because they are indicator of poor hygienic production (Harding, 1999).

A growing list of ingredients are being used and investigated for use in fluid milk processing to inhibit microbial activity and many of these components have exhibited successful protection against the proliferation of lactic acid bacteria and molds (Yuan, 2001). He also added that protein and peptide such as lactoperoxidase, lactoferrin, bacteriocins, lysosome and xanthine oxidation, occurring naturally

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in milk and have antimicrobial properties. FAO/ WHO strongly discourages the preservation of milk by chemical means, except the application of H_2O_2 at native LPS and in the case of H_2O_2, it must be completely destroyed before consumption (Ozer *et al*., 2003).

Lactoproxidase system is based on the activation of the lactoproxidase, thiocyanate and hydrogen peroxide system (naturally present in milk in varying concentrations) to inactivate several vital metabolic bacterial enzymes, consequently blocking their metabolism and ability to multiply (Gaya *et al*., 1991). Lactoperoxidase system (LPS) is active against both Gram positive and Gram negative microbes to varying extents (Marks *et al*., 2001). Activation of lactoperoxidase system was investigated as potential alternative method to sustain the safety of milk by inhibiting certain microorganisms with known pathogenic potential like *Staphylococcus aureus* and *Escherichia coli* (Kangumba *et al*., 1997).

Recently FAO has conducted large-scale field trials in Pakistan, Philippines and Cuba, which have shown promising results, however, the application of this method has to follow specific guidelines as spelt out by Codex Alimentarius and IDF (Lambert, 1993). The application of the lactoperoxidase system of milk preservation at collection centers aimed at raising regional awareness to safe, cheap and effective alternative milk preservation method (Codex Alimentarius Commission, 2004).

Most of the consumers in Sudan use raw milk that supplied to them from far away without cooling as the marketing system of milk are weak and on a transitional stage. This milk is contaminated by various organisms (Yagoub *et al*., 2005), which necessitate the introduction of safe methods of preservation suitable for the local conditions. Hence the present study was carried out as a trial to evaluate the preservation of milk with lactoperoxidase enzyme system, a newly enzyme kits that have been recently approved and introduced to the developing countries by the FAO.

Materials and Methods

Sources of Samples

This study was carried out using bovine milk from a traditional farm in Khartoum State, Sudan during the period of April to June 2005.

Collection and Transportation of Milk Samples

Milk samples were collected directly from farm in Jabal Awlyaa, Khartoum, Sudan. The samples were kept in plastic containers with capacity of 5 L. The samples were transported after one hour of milking to the Microbiological laboratory (School of Biotechnology, El Neelian University). Part of the samples (treated) was preserved with lactoperoxidase system, which contains thiocyanate and hydrogen peroxide. The LPS was obtained from Ministry of Animal Resources, Sudan. The kits were offered to them by FAO for field trials of the LPS in Sudan. Then both treated (by the addition of lactoperoxidase) and non treated milk samples (control; with out addition of the lactoperoxidase) were distributed into 100 sterile bottles. The bottles were grouped into 4 groups that included: control milk samples that kept at room temperature (CR), control milk samples that persevered at refrigerator temperature (CC), treated milk samples that kept at room temperature (TR) and treated milk samples that preserved at refrigerator temperature (TC).

Measuring of Milk Acidity

The acidity of the milk samples was determined; at zero time and then each hour up to nine hours then daily up to 12 days; according to the method described in the AOAC (1990). Clot on boiling test of the milk samples were done daily according to Foley *et al*. (1974).

Microbiological Examination of Milk Samples

Plate count agar was used for determining total bacterial counts, MacConkey agar was chosen as a differential selective medium for coliform bacterial counts and mannitol salt agar was a differential and selective plate medium used to isolate *Staphylococcus aureus*.

Serial dilutions from the milk samples were prepared aseptically according to the methods described by Richardson (1985). The incubation was done at 32°C for 24- 48 and 37°C for 24 h for the total bacterial counts and coliform counts, respectively (Richardson, 1985) and 4-7°C for 2-10 days for the psychotropic counts (Ballou *et al*., 1995).

The developed colonies were counted using manual colony counter and the number of the total bacterial count was calculated as colony forming unit/mL (Christen *et al*., 1992). Identification of the isolated bacteria was done according to Barrow and Feltham (1993).

Results and Discussion

Table 1 shows that the treated milk samples did not show any significant differences in total bacterial counts, when kept at room temperature ($4.22\times10^{9}\pm4.4\times10^{9}$cfu mL^{-1}) or refrigerator ($2.68\times0^{9}\pm4.2\times10^{9}$ cfu mL^{-1}). Also the present study revealed that acidity of treated milk was not significantly different due to storage temperature, when adding the lactoperoxidase enzymes. This might indicated that LPS could be a method of milk preservation when cooling facilities are not available which supported Lambert (1993) and Kangumba *et al*. (1997). The suppression of bacteria by LPS (Table 1) indicated the antimicrobial effect of LPS, which supported McLay *et al*. (2002), Florisa *et al*. (2003) and Dufour *et al*. (2004).

The acidity of raw milk initially was 0.209% and it was observed to show approximately constant levels until it increased to 0.265% after 9 days of storage (Table 2), which was due to the action of lactoperoxidase system. This supported the previous reports of McLay *et al*. (2002), Florisa *et al*. (2003) and Dufour *et al*. (2004). Similarly non significant variations were found for the coliform counts in the treated milk samples that kept at room temperature ($2.32\times10^{9}\pm2.9\times10^{9}$cfu mL^{-1}) and refrigerator ($1.40\times10^{9}\pm 2.4\times10^{9}$cfu mL^{-1}). This result might be due to powerful effect of the antimicrobial effect of the lactoperoxidase system, which supported Gaya *et al*. (1991), Florisa *et al*. (2003) and Garcia -Graells *et al*. (2003). On the other hand highly significant variations ($p<0.001$) were observed for total bacterial counts ($1.17\times10^{10}\pm2.0\times10$ cfu mL^{-} and $1.83\times10^{9}\pm2.9\times10^{9}$cfu mL^{-1}) and coliform counts ($4.23\times10^{9}\pm1.7\times10^{9}$cfu mL^{-1} and $1.29\times10^{9}\pm1.9\times10^{9}$cfu mL^{-1}) for the non treated (control) milk samples kept at room and refrigerator temperature, respectively (Table 1). This could be due to the effect of cooling (IDF, 1994). This might indicate the usefulness of applying the use of LPS for preservation of milk especially where cooling facilities are not available as was stated before by Lambert (1993). The higher counts of both total and coliform bacteria obtained during this result might be due to the effect of bacterial growth in high temperature as reported by Yuan (2001). However Durfour *et al*. (2004) reported that two hours exposure of combined LPS resulted in decrease of numbers of viable bacterial attached to the stainless steel. Similarly the present study revealed non significant differences for acidity, when comparing the milk samples that were kept at refrigerator and room temperature, which indicated that this milk contain higher load of bacteria.

Table 2 shows the effect of LPS on the keeping quality on milk during storage, as the total bacterial count at the beginning of the storage period was 9.63×10^{9} cfu mL^{-1} and it decreased to 3.0×10^{6}cfu mL^{-1} after 10 days of storage. Also coliform count at the beginning of the storage revealed 4.05×109 cfu mL^{-1} and it decreased to 1.0×10^{6} cfu mL^{-1}. This might be due to the killing and

Table 1: Comparison of the microbial loads and acidity of the control raw milk and lactoperoxidase treated milk samples

		Lactoperoxidase treated milk samples			
Measurements	Storage conditions	Means±SD	Minimum	Maximum	Level of Sig.
Total bacterial counts (cfu mL^{-1})	Room	$4.22\times10^{9}\pm4.4\times10^{9}$	4.0×10^{8}	1.20×10^{10}	NS
	Refrigerator	$2.68\times10^{9}\pm4.2\times10^{9}$	2.0×10^{6}	1.50×10^{10}	
	Total	$3.17\times10^{9}\pm4.0\times10^{9}$	2.0×10^{6}	1.50×10^{10}	
Coliform bacterial counts (cfu mL^{-1})	Room	$2.32\times10^{9}\pm2.9\times10^{9}$	2.0×10^{6}	5.00×10^{9}	NS
	Refrigerator	$1.40\times10^{9}\pm2.4\times10^{9}$	0	6.00×10^{9}	
	Total	$1.70\times10^{9}\pm2.9\times10^{9}$	0	6.00×10^{9}	
Acidity (%)	Room	0.2272±0.0146	0.20	0.27	NS
	Refrigerator	0.2258±0.0153	0.20	0.27	
	Total	0.2263±0.0150	0.20	0.27	
		Control raw milk samples			
Measurements	Storage conditions	Means±SD	Minimum	Maximum	Level of Sig
Total bacterial counts (cfu mL^{-})	Room	$1.17\times10^{10}\pm2.0\times109$	9.00×10^{9}	1.50×10^{10}	0.001***
	Refrigerator	$1.83\times10^{9}\pm2.9\times10^{9}$	2.0×10^{6}	7.00×10^{9}	
	Total	$3.17\times10^{9}\pm4.0\times10^{9}$	2.0×10^{6}	1.50×10^{10}	
Coliform bacterial counts (cfu mL^{-1})	Room	$4.23\times10^{9}\pm1.7\times10^{9}$	4.0×10^{6}	6.00×10^{9}	0.002***
	Refrigerator	$1.70\times10^{9}\pm2.9\times10^{9}$	0	6.00×10^{9}	
	Total	$1.29\times10^{9}\pm1.9\times10^{9}$	0	5.00×10^{9}	
Acidity (%)	Room	0.2267±0.0139	0.20	0.26	NS
	Refrigerator	0.2262±0.0155	0.20	0.27	
	Total	0.2263±0.0150	0.20	0.27	

*** = p< 0.001, NS = Non Significant, SD = Standard Deviation, Sig. = Significant

Table 2: Effect of lactoperoxidase enzymes system on the keeping quality of raw milk

Storage periods (h)	Total bacterial counts	Coliform counts	Acidity (%)
0	9.63×10^{9c}	4.05×10^{9b}	0.2088^{a}
4	6.87×10^{9bc}	4.33×10^{9b}	0.2233^{abc}
9	5.25×10^{9b}	4.00×10^{9b}	0.2200^{ab}
24	33.0×10^{6a}	18.0×10^{6a}	0.2275^{abcd}
48	17.0×10^{6a}	11.0×10^{6a}	0.2325^{bcde}
72	11.0×10^{6a}	5.0×10^{6a}	0.2475^{e}
96	13.0×10^{6a}	5.0×10^{6a}	0.2350^{bcde}
120	13.0×10^{6a}	5.0×10^{6a}	0.2300^{bcde}
144	11.0×10^{6a}	4.0×10^{6a}	0.2350^{bcde}
168	11.0×10^{6a}	4.0×10^{6a}	0.2400^{cde}
192	9.0×10^{6a}	2.0×10^{6a}	0.2450^{de}
216	5.0×10^{6a}	1.0×10^{6a}	0.2650^{f}
240	3.0×10^{6a}	0.00^{a}	-

Different superscript letter(s) on the same column are significantly different (p<0.05)

inhibition effect of lactoperoxidase system on the growth of organisms as reported previously (Gaya *et al*., 1991; Florisa *et al*., 2003; Garcia *et al*., 2003; Durfour *et al*., 2004; Elliot *et al*., 2004).

Isolation of Bacteria from Raw Milk

The microorganisms, which were isolated from raw milk include *E. coli*, *Citrobacter freundii*, *Yersinia enterocolitica*, *Enterococcus feacalis* and *Staphylococcus aureus*. This was supported Yagoub *et al*. (2005) who reported that the most predominant bacteria in raw milk samples investigated in Khartoum North were *Staphylococcus aureus* (30%), *Citrobacter* spp. (21.43%), *Shigella* spp. (20%), *E. coli* (14.28/%), *Enterobacter* spp. (12.86%) and *Salmonella* spp. (1.43%). However the treated milk sample revealed the presence of *Enterococcus feacalis*. This result explain the reduction

of the total number of bacteria that was found be reduced in case of lactoperoxidase treated milk (Table 1). This indicated that the presence of *E. coli*, *Citrobacter freundii*, *Yersinia enterocolitica* and *Staphylococcus aureus* in milk were inhibited by the addition of LPS, which agreed with Kangumba *et al.* (1997) and Elliot *et al.* (2004). However the present study observing a degree of resistance of *Enterococcus feacalis* to lactoperoxidase, since the inhibition occurred after prolong exposure to treatment (9 days for refrigerated treated milk). This might be because LPS is proved to have greatest activates against Gram negative bacteria (McLay *et al.*, 2002). Hence the lactoproxidase system can be used to preserve raw milk in countries where refrigeration facilities are uncommon (Lambert, 1993). Moreover FAO in collaboration with SIDA (Sweden) is planning a carry out worldwide testing in about 80 counties worldwide, following the successful field testing in several countries including Cuba, Pakistan and Philippine (Codex Alimentarius Commission, 2004).

The present study recommended the uses of LPS; as it proves to be an efficient procedure for preservation of milk; in Sudan especially at the rural areas in order to utilize the large quantities of milk that was unavailable to the consumers. This will also help in providing enough milk for the dairy factories that have just been started in and around the big towns. It is also recommended that fund should be raised from national and international bodies to facilitate the improvement of dairy sector in Sudan. Further research on antimicrobial effect on the different milk borne diseases and spoilage organisms is needed. Also field investigations are highly required to examine the LPS under the different conditions of Sudan.

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