MICROBIOLOGICAL QUALITY OF SET YOGHURT FROM RECONSTITUTED WHOLE AND MIXED MILK POWDER

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Abstract
The milk used for the manufacturing of set yoghurt in Khartoum State, Sudan includes recombined whole milk powder or a mixture of recombined whole and skim milk powder. This study was carried out to evaluate the microbiological quality of set yoghurt made from whole or mixed recombined milk powder during the storage period. Ten yoghurt batches (5 from each) from a modern dairy factory were examined a well milk samples from which the set yoghurt were to be made. The set yoghurt samples were collected immediately after processing. The samples were stored to determine their shelf life.

High total bacterial count were reported both in mixed (52.55×10^5) and whole milk powder (12.16×10^5) used for production of set yoghurt, which indicated poor quality of milk. Moreover even after pasteurization of milk, high numbers of bacteria was found to survive, this could be due to improper pasteurization or poor handling of raw milk materials. The microbial content revealed significantly ($P<0.05$) lower levels of total bacterial count (TBC) in mixed set yoghurt ($6.06\times10^{11}\pm1.86\times10^{12}$ cfu/ml) compared to whole set yoghurt samples ($1.23\times10^{12}\pm2.85\times10^{12}$ cfu/ml). Moreover coliform bacterial count was recorded to be highly significant($P<0.05$) in whole set yoghurt ($10.80\times10^4\pm28.00\times10^4$ cfu/ml) compared to mixed set yoghurt ($74.12\times10^3\pm30.31\times10^4$ cfu/ml). However the Streptococcus spp. count was found to be high in mixed set yoghurt ($2.74\times10^9\pm15.13\times10^9$ cfu/ml) compared to whole set yoghurt ($1.50\times10^9\pm52.87\times10^8$ cfu/ml). Similarly Lactobacillus spp. count was recorded to be high in mixed set yoghurt ($3.35\times10^9\pm16.15\times10^9$ cfu/ml) compared to whole set yoghurt ($1.08\times10^9\pm31.55\times10^8$ cfu/ml). Moreover Streptococcus spp., showed more growth compared with Lactobacillus spp during the storage period.
The results of this study concluded that quality of set yoghurt produced by this dairy factory need to be improved, as was indicated by the high count of TBC and coliform bacteria, which exist in the product up to 7 days of storage. Hence the present study recommends the application of hygienic control using HACCP systems in the dairy plants and training of the labours on the best manufacturing procedures.

Keywords: set yoghurt, processing, shelf life, recombined, whole and skim milk powder, microbial quality

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1. INTRODUCTION

Yoghurt is a popular fermented milk product consumed in many parts of the world. It's produced in different forms such as whole milk yoghurt, skim milk yoghurt, cream yoghurt, fruit yoghurt and liquid yoghurt (Balasubramanyam and Kulkarni, 1991). Yoghurt is an extremely popular fermented milk food in Europe, Asia and Africa (Thapa, 2000).
Starter cultures used in the dairy industry are mixtures of carefully selected lactic acid bacteria which are added to the milk to fulfill the desired fermentation (Mayo, 1993). The yoghurt culture is a system of two or more microbial populations with mutually complimenting metabolisms (Beshkova, 2002). Dairy starter cultures must reach high densities in milk in order to produce lactic acid at the required rates for manufacturing. Under these conditions, amino acids supply becomes limitant due to their scarce concentration in milk and to the auxotrophies shown by many starter bacteria (Mayo, 1993). On the other hands, post acidification was slightly higher in yoghurt prepared with lower amounts of inoculums and he concluded that the end

incubation temperature should be properly maintained to achieve maximum viability (Gregurek, 1999). It is also concluded that both storage temperature and yoghurt type should be taken into account when shelf life is specified on the basis of the lactic microflora content (Birollo et al., 2000).

The microflora of yoghurt was influenced by culture type, pH, flavuor type and storage time or their interactions (Yüceer et al., 2001). Also in a study to determine shelf life of concentrated yoghurt when stored at 5, 15 and 25˚ C, the total count of psychrotrophic yeast, yeast and moulds and lactic acid bacteria were increased irrespective of storage temperature, moreover the pH of samples decreased and titratable acidity was increased (Al-kadamany et al., 2000). Heat treatment of milk, proper handling and strict hygienic measures before manufacturing of dairy products should be very well monitored (Abdalla and Ibtisam, 2006). The packaging materials are often considered a critical control point in HACCP systems of food companies, therefore the methods for the determination of the microbial contamination rate of plastic cups, especially for dairy products must reliably detect single mould, yeast and coliform (Tacker et al., 2002). The increased sugar led to an increase of fermentation time and decrease in yoghurt shelf life (Shah and Ravula, 2000). One of the important factors through food manufacturing is hygienic management. Thus, food manufactures prove their hygienic activities by taking certifications like a Hazard Analysis and Critical Control Point (HACCP) (Ogasawara et al., 2006). Quality, safety and acceptability of traditional fermented foods may be significantly improved through the use of starter cultures selected on the basis of multifunctional considerations (Holzapfel, 2002). Starter cultures are one of the most important factors affecting yoghurt quality, the qualitative and quantitative assessment of yoghurt starter bacteria is valuable because some countries have proposed standards of quality for yoghurt which include the requirement of minimum number of viable yoghurt organisms must be present at the time of sale (Schuck, 2002).

Yoghurt is one of the popular dairy products in Sudan. Moreover it is produced commercially by the specialized dairy plants. However little work in Sudan was done to evaluate the hygienic quality in terms of its microbial loads and shelf life. Hence the present study was designed in order to compare the effect of different raw milk mixture on set yoghurt properties and the development of the starter culture used for set yoghurt during processing and storage of yoghurt.

2. MATERIAL AND METHODS

Source of yoghurt samples

Ten batches of yoghurt samples (60 samples from recombined whole milk powder set yoghurt and 60 samples from mixture of recombined whole and skim milk powder set yoghurt) were collected during the period from May to December 2005.

Collection and transportation of samples

Ten samples of whole milk powder and a mixture of whole milk powder + skim milk powder (35-40 ml) were collected, before and after pasteurization of milk in sterile bottles from a dairy factory in Khartoum State. Then forty samples of set yoghurt from recombined whole milk powder set yoghurt and mixed recombined whole + skim milk powder set yoghurt were collected during incubation (200 grams cups). Moreover sixty samples from each type of set yoghurt were collected during storage and examined every second day (up to 11 days).

The samples were transported in an ice box to the laboratory of Department of Dairy Production, Faculty of Animal Production, University of Khartoum and examined to determine bacterial loads and the development of starter culture.

Microbial examination

The milk samples were collected into clean and sterile bottles, and then stored immediately at 5˚ C until examination was carried out.

The milk samples and yoghurt samples were examined for total bacterial count, coliform count, *Lactobacillus spp.* count and *Streptococcus spp.* count. The microbial counts for milk samples and yoghurt samples were carried out after 24 hours after termination of incubation.

All media were prepared according to manufacture's instructions. Plate count agar was used for determination of total bacterial counts and violet red bile agar was used for detection and enumeration of coliform bacteria (Harrigan and McCance, 1976). The MRS broth (Oxoid) was used for enumeration of Lactobacillus (Man et al., 1960) and M-17 broth (Oxoid) was used for enumeration of Streptococcus (Harrigan and McCance, 1976). Glassware such as Petri-dishes, test tubes, pipettes and flasks were sterilized in hot oven at 160˚ C for one hour. Ringer solution used in the preparation of serial dilution was sterilized by autoclaving at 121˚ C for 15 minutes. Pour plate technique was used to determine the counts of bacteria during the present study in duplicate (Harrigan and McCance, 1976).

Statistical analysis

Data of the present experiment was analyzed using a completely randomize design. The analysis of variance and the significant differences between means were determined using Duncan Multiple range test using SPSS version 10.

3. RESULTS AND DISCUSSION

High total bacterial counts were reported both in mixed ($52.55\times10^{5)}$ and whole milk powder (12.16×10^{5}) used for production of set yoghurt (Table 1). This indicated poor quality of milk (Birollo et al., 2001). Moreover even after pasteurization of milk, some bacteria are found to survive (Table 2 and Table 3). This could be due to improper pasteurization or handling of raw materials.

Table 1: Bacteriological contents of recombined whole milk powder and recombined mixed milk powder used for producing yoghurt

Treatment		Mean ± S. d	Min.	Max.	Mean sq.	Sig.
TBC	Mixed	$52.55\times10^{5}\pm 14.6\times10^{6}$	0	50.5×10^{6}	1.55×10^{14}	0.239 NS
	Whole	$12.16\times10^{5}\pm 19.75\times10^{5}$	105	52×10^{5}	1.08×10^{14}	
Coliform count	Mixed	$19.07\times10^{4}\pm 39.29\times10^{4}$	0	11×10^{5}	1.19×10^{11}	0.246 NS
	Whole	$81.50\times10^{3}\pm13.14\times10^{4}$	0	36.5×10^{4}	8.58×10^{10}	
Streptococcus spp. count	Mixed	$65.72\times10^{3}\pm 12.94\times10^{4}$	0	42×10^{4}	16.06×10^{9}	0.339 NS
	Whole	$10.58\times10^{4}\pm 13.07\times10^{4}$	35	36×10^{4}	16.93×10^{9}	
Lactobacillus spp. count	Mixed	$86.9\times10^{3}\pm 16.42\times10^{4}$	0	49×10^{4}	17.16×10^{8}	0.803 NS
	Whole	$10^{5}\pm 16.48\times10^{4}$	0	48.5×10^{4}	27.08×10^{9}	

In this and the following tables:
TBC= total bacterial count
*: $P \leq 0.005$
**: $P \leq 0.01$
***: $P \leq 0.001$
NS: $P \geq 0.05$

Table 2: Microbial content of recombined whole milk powder and recombined mixed milk powder used for yoghurt before and after pasteurization

Treatment		Mean ± S.d	Minimum	Maximum
Total bacterial counts	After	$13.93\times10^{4}\pm 42.45\times10^{4}$	0	15×10^{5}
	Before	$62.32\times10^{5}\pm 14.34\times10^{6}$	0	50.5×10^{6}
Coliform counts	After	21.00± 37.58	0	150
	Before	$27.22\times10^{4}\pm 37.27\times10^{4}$	0	11×10^{5}
Streptococcus spp. count	After	$10.21\times10^{2}\pm 23.99\times10^{2}$	0	10^{4}
	Before	$17.05\times10^{4}\pm 13.99\times 10^{4}$	10^{4}	42×10^{4}
Lactobacillus spp. count	After	$400\pm 11.25\times10^{2}$	0	5×10^{3}
	Before	$18.55\times10^{4}\pm 18.98\times 10^{4}$	0	49×10^{4}

Table 3: Variation of microbial content of recombined whole milk powder and recombined mixed milk powder using Duncan test

Treatment	TBC	Coliform count	*Streptococcus spp.* count	*Lactobacillus spp.* count
After ×Mixed	27.55×10^{4a}	35^{a}	440^{a}	$3\times10^{2\,a}$
After ×Whole	$31.77\times10^{2\,a}$	7^{a}	16.03×10^{2a}	501.50×10^{2a}
Before ×Mixed	$10.03\times10^{6\,b}$	38.15×10^{4b}	$13.10\times10^{4\,b}$	$17.35\times10^{4\,b}$
Before ×Whole	$24.30\times10^{5\,ab}$	16.30×10^{4ab}	$21.00\times10^{4\,b}$	$19.95\times10^{4\,b}$

Means with the same column being similar subscript letter are not significantly affected (P≥ 0.05).

Table 4: Bacteriological content of set yoghurt during the storage period (11 days)

Treatment		Mean ± S. d	Minimum	Maximum
TBC	Mixed	$6.06\times10^{11}\pm1.86\times10^{12}$	0	9.50×10^{12}
	Whole	$1.23\times10^{12}\pm2.85\times10^{12}$	0	1.38×10^{13}
Coliform counts	Mixed	$74.12\times10^{3}\pm30.31\times10^{4}$	0	19×10^{5}
	Whole	$10.80\times10^{4}\pm28\times10^{4}$	0	13×10^{5}
Streptococcus counts	Mixed	$2.74\times10^{9}\pm15.13\times10^{9}$	0	1.16×10^{11}
	Whole	$1.50\times10^{9}\pm52.87\times10^{8}$	12.50×10^{6}	3.65×10^{10}
Lactobacillus counts	Mixed	$3.35\times10^{9}\pm16.15\times10^{9}$	0	1.25×10^{11}
	Whole	$1.08\times10^{9}\pm31.55\times10^{8}$	35×10^{5}	1.80×10^{10}

The mean total bacteria count (TBC) was $1.23\times10^{12}\pm 2.85\times10^{12}$ cfu/ml for recombined whole milk set yoghurt was higher than the mean of TBC for recombined mixed milk set yoghurt ($6.06\times10^{11}\pm 1.86\times10^{12}$ cfu/ml) as shown in Table 4. This may be due to the high fat content in whole milk set yoghurt. The fat content affects activity and persistence of lactic acid bacteria (LAB) (Foster et al. 1958). They added that the growth of LAB is stimulated by content of free fatty acid. Also the results showed the behavior of TBC for recombined mixed milk set yoghurt and recombined whole milk set yoghurt (Figure 1). These results were higher than the result reported for yoghurt (Zabadi) samples purchased from different groceries in Khartoum and found that the mean of TBC was $1.29\times10^{9}\pm 3.79\times10^{8}$cfu/ml and this may be due to the different of sanitary conditions during processing (Sulieman, 1982). The fluctuations reported in total bacterial count may be due to the development of acidity and/or toxic products (Al-kadamany et al., 2002). However this disagrees with the report

which stated that cooling of yoghurt below 20˚C decreases the activity of LAB (Robinson, 1981). These results are higher than international standards for yoghurt which require a minimum of total viable microorganisms of 10^7cfu/ml in the finished product (CODEX STAN 243, SASO 1998). This variation may be due to the variation in storage conditions.

High coliform count were found in both mixed and whole milk powder (19.07×10^4 and 81.50×10^3 cfu/ml, respectively) as shown in Table 1. This might suggest faecal contamination. The higher count of coliform bacteria might be due to poor sanitary conditions. It may also be due to the unrefrigerater transportation and poor microbiological quality of water (Adesiyun et al. 1997). This might be due to improper pasteurization as was reflected by its survival in the milk after its pasteurization (Table 2). This supported the previous report that Enterobacteiaceae do not survive pasteurization but contamination can be due to poor post pasteurization control (Manie et al, 1999). Moreover significant differences ($P<0.05$) were reported for both TBC and coliform counts between whole and mixed milk powder (Table 3).

Coliform bacterial count from recombined whole milk set yoghurt was higher ($10.80\times10^4\pm 28.00\times10^4$cfu/ml) than the count of coliform bacterial count from recombined mixed milk set yoghurt ($74.12\times10^3\pm 30.31\times10^4$cfu/ml) as shown in Table 4 and Figure 2. This might be due to the development and increase of acidity during storage, which affects activity and persistence of bacteria (Foster, M.E. et al. 1958). Generally the coliform bacterial count decreased during storage period and this is due to the LAB (Al-kadamany et al., 2002). However higher values (log 2.11±2.65 cfu/ml) of *E. coli* for yoghurt have been reported (Abdalla and Ibtisam, 2006). The high counts of coliform bacteria might suggest unhygienic processing of yoghurt (Birollo et al., 2000) particularly after heat treatment of yoghurt (Abd El-Ghani et al., 1998). Since it was stated that *E. coli* should be less than 10^2 (SASO, 1998). Moreover the developed acidity in yoghurt should restrict the growth of these organisms as fermentation controlling the growth of harmful microorganisms (Banwart, 1981).

Figure 3 and 4 show the count of the *Lac. spp* and *Strep. spp*. and generally the count of these organisms in recombined mixed milk set yoghurt was higher than in recombined whole milk set yoghurt till the fifth day of storage for *Lactobacillus spp* and till the seventh day for *Streptococcus spp.*. It was also noted that the count of LAB increased for recombined whole milk set yoghurt for *Lactobacillus spp.* and increased more for *Streptococcus spp.*, which might be due to the decrease in the acidity in recombined mixed milk set yoghurt and high fat content in recombined whole milk set yoghurt. *Lactobacillus spp.* count ($1.08\times10^9\pm 31.55\times10^8$cfu/ml) for recombined whole milk set yoghurt was lower than the mean count of *Lactobacillus spp*. for recombined mixed milk set yoghurt ($3.35\times10^9\pm 16.15\times10^9$ cfu/ml) as shown in Table 4.

Such a difference might be due to the availability of lactose in recombined mixed milk set yoghurt which was more than in recombined whole milk set yoghurt.

The mean count of *Lactobacillus spp*. for yoghurt was $1.29\times10^9\pm5.4\times10^8$ cfu/ml (Sulieman, 1982). However the values reported by Codex stated that lactobacilli in yoghurt should be in the range of 10^7(CODEX STAN 243, 2003).

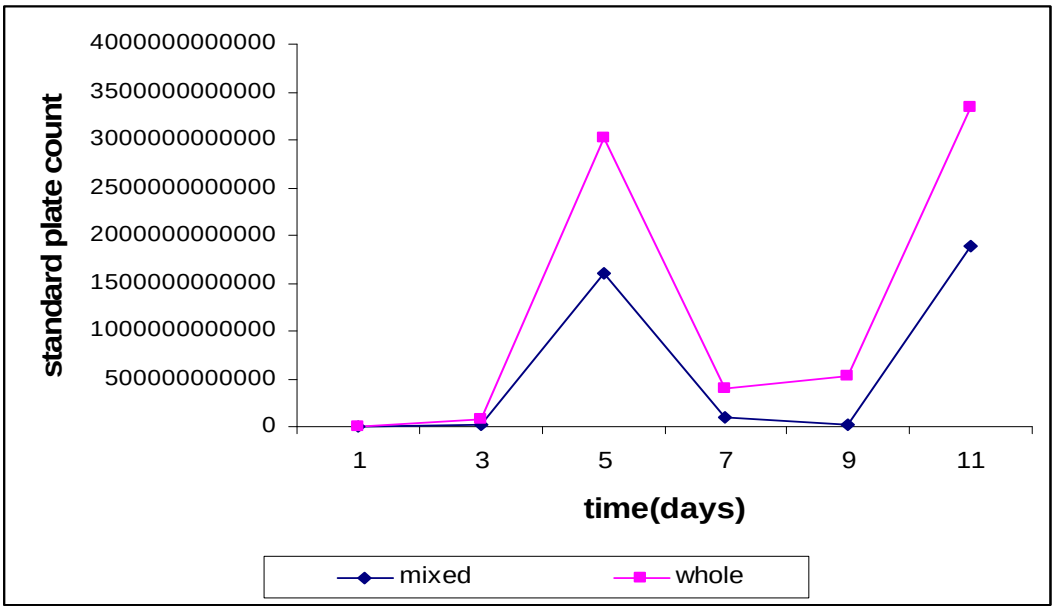

Fig. 1: Total bacterial counts of set yoghurt during storage period

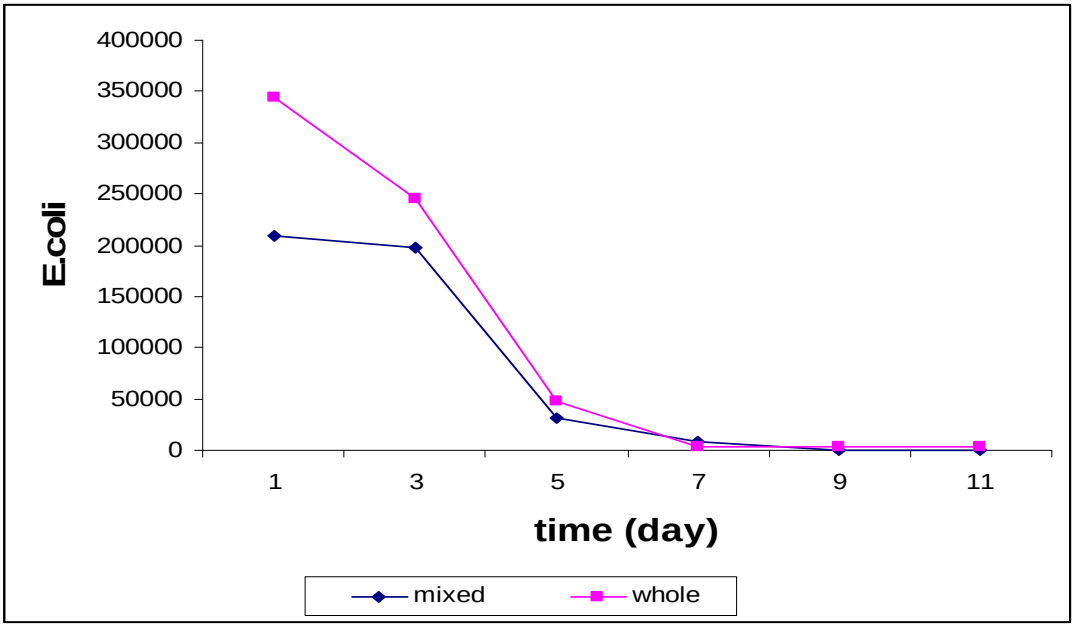

Fig. 2: Coliform count of set yoghurt during storage period

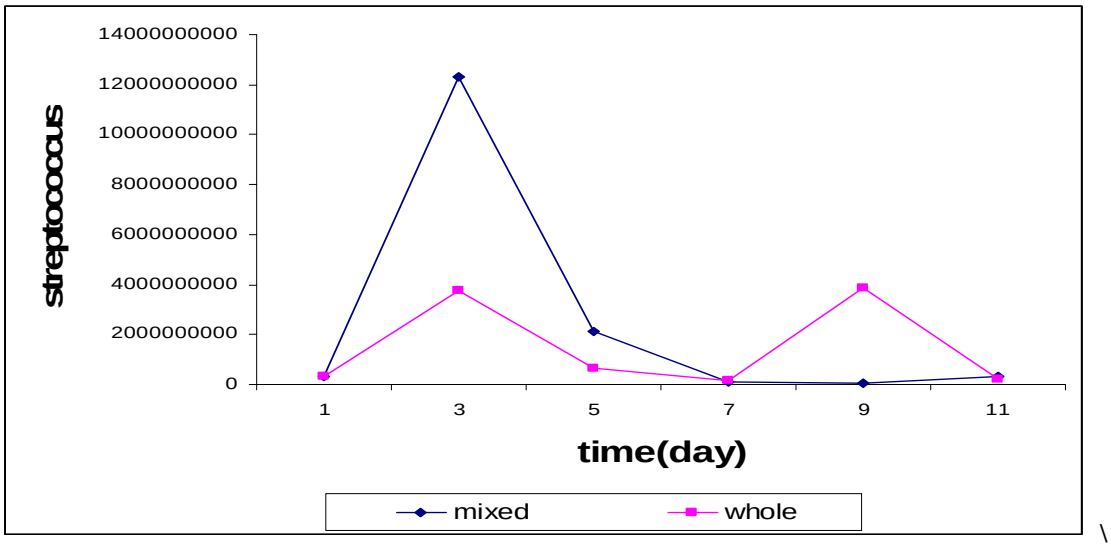

Fig. 3: *Streptococcus* count of set yoghurt during storage period

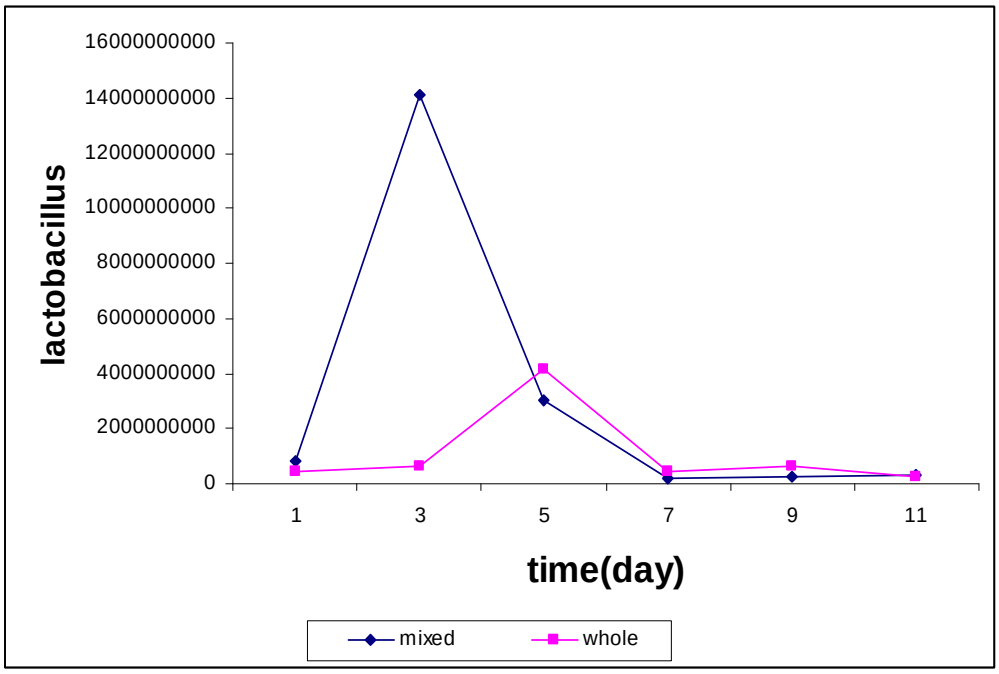

Fig. 4: *Lactobacillus* count of set yoghurt during storage period

4. CONCLUSIONS

The results of this study concluded that quality of set yoghurt produced by the selected dairy factory need to be improved, as was indicated by the high count of TBC and coliform bacterial count, which exist in the product up to 7 days of storage. The international, regional and Sudan Standards stated lower values than it was found during the present study. Hence the present study recommends the application of hygienic control using HACCP systems in the dairy plants and training of the labours on the best manufacturing procedures. Coordination between dairy sector, research institutes and the Sudanese Standards and Metrology Corporation for establishment of the standards and legislation in dairy industry are urgently needed. Further studies and research are needed to evaluate and improve the hygienic quality of dairy products in the country.

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