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Chemical Composition and Microbial Load of Set Yoghurt from Fresh and Recombined Milk Powder in Khartoum State, Sudan

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ABSTRACT

Yoghurt is usually made from recombined whole and skim milk powder, however some new dairy factories started to use fresh milk instead of powder milk The aim of the present study was to evaluate some chemical constituents and microbiological load during storage period of plain set yoghurt samples (n = 96). Random yoghurt samples (recombined milk powder; A, B and fresh milk; C, D) were collected from market in Khartoum State in order to compare the effect of the type of milk and the storage on the quality of yoghurt. Analysis of yoghurt samples, revealed significant ($p<0.01$) differences in Total Solids (TS), Solids Not Fat (SNF), fat, protein and ash contents due to variations of manufacturers and type of milk used. The titratable acidity showed non significant effect due to the type of milk used. The TS, SNF and protein contents and titratable acidity of the collected yoghurt samples were significantly ($p<0.01$) affected during the storage period, whereas fat and ash contents were not significantly affected by storage period. Microbiological analysis showed that total bacterial count, coliform count and yeast and mould counts were significantly ($p<0.05$) affected by the manufacturers and storage period. However coliform count and yeast and mould counts were not significantly affected by the type of milk used. The study concluded that the plain set yoghurt made from powder and fresh milk agrees with the Sudanese standards for yoghurt chemical composition. However all yoghurt samples revealed high microbial loads, hence, it is recommended the implementation of good hygienic conditions during processing and storage of yoghurt to obtain good quality product.

Key words: Commercial yoghurt, constituents, quality, hygiene, contaminants, shelf life, local market

INTRODUCTION

Yoghurt is a semi-solid fermented milk product consumed in most parts of the world and the changes in the physical, chemical and microbiological structure of yoghurt determine the storage and shelf life of the product (Sofu and Ekinci, 2007). Yoghurt is a very popular fermented milk product produced by lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus salivarius* sp. *thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* (Tamime and Robinson, 1999). Yoghurt has many forms including drinkable (liquid) or solid, low fat or fat free, fruity or cereal flavored and is a healthy and nutritious food (McKinley, 2005).

There has been a phenomenal increase in the production of fermented milks in developed countries and most of this increase is attributed to the healthy image associated with yoghurt

(Karagul-Yuceer *et al*., 2004; Valli and Traill, 2005). Furthermore, the World Health Organization has supported the use of yoghurt in nutritional recovery (WHO, 2000). The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhanced digestion of lactose by maldigesters, decreased risk of cancer and lower blood cholesterol. In addition it improved immune response and help the body to assimilate protein, calcium and iron (Marona and Pedrigon, 2004). Moreover Van Neil *et al*. (2002) found that therapy using *Lactobacillus* strains offered a safe and effective means of treating acute infectious diarrhea in children.

In Sudan, yoghurt in the modern dairy plants was usually made from powder milk (Attita Allah *et al*., 2010). El-Bakri and El-Zubeir (2009) found that the commercially available yoghurt in Khartoum State, Sudan has a good chemical quality when compared to international standards. However yoghurt which is made from fresh milk, which was introduced recently by some of the relatively new factories, was not evaluated. Hence aim of this study was to evaluate some selected chemical and microbiological properties of plain set yoghurt that produced from fresh and recombined milk from different factories during storage period.

MATERIALS AND METHODS

Sources and collection of yoghurt samples: This study was carried out during the period November 2008-February 2009.on plain set yoghurt produced by two dairy factories (A, B) which produce plain set yoghurt from fresh milk and two other factories (C, D) that produce plain set yoghurt from recombined milk.

Ninety six plain set yoghurt samples were purchased from local market in Khartoum North. Twenty four samples from each factory were examined from four batches. All samples were transported in an ice box, held at 4-7°C to laboratory of Department of Dairy Production, Faculty of Animal Production, University of Khartoum and analyzed for some chemical and microbiological characteristics in days 3, 6 and 9 from production day.

Analysis of yoghurt samples

Chemical analysis: Total solids content was determined according to the modified method of to AOAC (2003). Protein content was determined by Kjeldahal method described by Bradley *et al*. (1992). Fat content was determined by Gerber method and the solids not fat were obtained by subtracting fat from total solids according to AOAC (2003). The ash content was determined by the method described by Bradley *et al*. (1992).

Microbiological analysis: All media were obtained in dehydrated forms and were prepared according to the manufacturer's instructions. Plate count agar was used to determine the total bacterial count (Houghtby *et al*., 1992). MacConkey agar was used to determine the coliform count (Christen *et al*., 1992) and Potato dextrose agar was used to determine yeast and mould counts (Frank *et al*., 1992).

Glasswares such as Petri-dishes, test tubes, pipettes, flasks and bottles were sterilized in a hot oven at 170°C for two hours, whereas distilled water and tips were sterilized by autoclaving for 15 min at 121°C (Marshall, 1992).

Pour plate technique was used for total bacterial and coliform counts and surface (spread) plate technique was done for yeast and mould counts. One milli liter from a homogenous sample was serially diluted into 9 mL ringer solution to prepare eight fold dilutions from 10^{-1} to 10^{-8}

(Houghtby *et al*., 1992). Then for total bacterial and coliform counts, one ml of each sample was transferred into a sterile duplicate plate and 15-20 mL of the selected media was added. The medium was mixed immediately and shake for 5-10 sec. For yeast and mould, one milli liter of diluted samples were spread over pre prepared dried plates. Then the cultured plates were incubated at 32°C for 48 h, 37°C for 24 h and 25°C for 5 days for the total bacterial, coliform and yeast and mould, respectively. The plates containing 25-250 cfu were enumerated for total bacterial count, whereas the plates containing 15-150 cfu were enumerated for coliform and yeast and mould count (Christen *et al*., 1992).

Statistical analysis: The data were analyzed statistically using completely randomized design. The analysis of variance (ANOVA) tests were carried out by using the general linear model procedure of the SPSS (Version 13.0). The means were separated by Duncan Multiple range test. Significant differences were determined at p = 0.05. The value of total bacteria, coliform and yeast and mould counts were transformed into log values.

RESULTS

Chemical composition: The means for Total Solids (TS), solids not fat (SNF), fat, protein and ash content and titratable acidity for recombined milk yoghurt were 14.02±0.91, 10.95±0.78, 3.06±0.41, 3.89±0.51, 0.66±0.09 and 1.31±0.19, respectively. Whereas for the fresh milk yoghurt the means were found as 15.04±0.87, 11.51±0.82, 3.53±0.14, 4.42±0.23, 0.82±0.17 and 1.33±0.15, respectively (Table 1). The chemical contents of yoghurt samples were affected significantly ($p<0.01$) due to variations of the manufacture and type of milk used, while non significant differences were found for titratable acidity due to variation of type of milk used. Similarly the storage period affected significantly ($p<0.01$) Total Solids (TS), Solids Not Fat (SNF) and protein

Table 1: Compositional content and microbiological quality of plain set yoghurt samples collected from different manufacturers

Parameters		A	B	Recombined milk yoghurt	C	D	Fresh milk yoghurt
Total solid (%)	Mean±SD	14.72±0.56	13.31±0.56	14.02±0. 91	15.79±0.51	14.29±0.35	15.04±0.87
	Range	13.62 –15.70	12.32-14.63	12.32-15.70	15.16-16.73	13.56-15.00	13.56-16.73
Solids not fat (%)	Mean±SD	11.32±0.579	10.57±0.59	10.95±0.78	12.22±0.46	10.81±0.38	11.51±0.82
	Range	10.12-12.14	9.23-12.36	9.23-12.40	11.56-13.39	10.06-11.7	10.06-13.39
Fat (%)	Mean±SD	3.402±0.11	2.72±0.31	3.06±0.41	3.57±0.17	3.48±0.09	3.53±0.14
	Range	3.20-3.60	2.10-3.10	2.10-3.60	3.30-3.90	3.20-3.60	3.20-3.90
Protein (%)	Mean±SD	3.92±0.41	3.87±0.47	3.89±0.51	4.51±0.24	4.33±0.18	4.42±0.23
	Range	2.87-4.47	3.39-4.47	2.87-4.47	3.75-4.82	3.93-4.64	3.75-4.82
Ash (%)	Mean±SD	0.66±0.06	0.66±0.11	0.66±0.09	0.89±0.21	0.75±0.07	0.82±0.17
	Range	0.44-0.82	0.37-.086	0.37-0.86	0.550-1.095	0.62-1.13	0.550-1.13
Titratable acidity	Mean±SD	1.23±0.19	1.39±0.16	1.31±0.19	1.30±0.08	1.35±0.19	1.33±0.15
(%)	Range	1.00-1.90	1.18-1.90	1.00-1.90	1.12-1.46	1.09-1.93	1.09-1.930
TBC (log10 cfu g^{-1})	Mean±SD	8.30±0.20	8.35±0.16	8.32±0.18	8.40±0.19	8.47±0.12	8.43±0.16
	Range	7.70-8.56	7.95-8.60	7.70-8.60	7.95-8.66	8.19-8.73	7.95-8.73
Coliform count	Mean±SD	5.3±1.21	4.69±1.6	4.99±1.50	4.86±1.34	5.43±0.89	5.15±1.17
(log10 cfu g^{-1})	Range	0.00-6.18	0.00-5.93	0.00-6.18	0.00-6.11	0.00-6.00	0.00-6.11
Yeast and mould	Mean±SD	6.30±0.46	5.26±2.04	5.78±1.56	5.49±1.92	5.80±1.80	5.65±1.6
count (log10cfu g^{-1})	Range	5.00-6.90	0.00-6.56	0.00-6.90	0.00-6.56	0.00-6.92	0.00-6.92

Khartoum State, (A-B-C-D-) =Manufactures, (A+B) = Plain set yoghurt from recombined milk, (C+D) =Plain set yoghurt from fresh milk, TBC: Total bacterial count

Table 2: Variations of chemical and microbiological measurement of plain set yoghurt samples between manufactures

	Manufactures			
Parameters	A	B	C	D
Total solids (%)	14.72^{b}	13.31^{d}	15.79^{a}	14.29^{c}
Solid not fat (%)	11.32^{b}	10.57^{d}	12.22^{a}	10.81^{c}
Fat (%)	3.40^{c}	2.72^{d}	3.57^{a}	3.48^{b}
Protein (%)	3.92^{c}	3.87^{c}	4.51^{a}	4.33^{b}
Ash (%)	0.66^{c}	0.66^{c}	0.89^{a}	0.75^{b}
Titratable acidity (%)	1.23^{c}	1.39^{a}	1.30^{b}	1.35^{ab}
TBC (log10 cfu g^{-1})	8.30^{c}	8.35^{bc}	8.40^{b}	8.47^{a}
Coliform count(log 10cfu g^{-1})	5.30^{ab}	4.68^{c}	4.86^{bc}	5.43^{a}
Yeast and moulds count (log 10 cfu g^{-}1)	6.30^{a}	5.26^{b}	5.49^{b}	5.80^{ab}

Means in the same row followed by the same letter (s) do not differ significantly at (p>0.05), TBC: Total bacterial count

Table 3: The effect of milk types on the chemical and microbiological parameters of plain set yoghurt samples

Parameters	Recombined milk yoghurt	Fresh milk yoghurt
Total solids (%)	14.02^{b}	15.04^{a}
Solids not fat (%)	10.95^{b}	11.51^{a}
Fat (%)	3.06^{b}	3.53^{a}
Protein (%)	3.89^{b}	4.42^{a}
Ash (%)	0.66^{b}	0.82^{a}
Titratable acidity (%)	1.31^{a}	1.33^{a}
TBC(log10 cfu g^{-1})	8.32^{b}	8.43^{a}
Coliform count (log 10 cfu g^{-1})	4.99^{a}	5.15^{a}
Yeast and mould count (log 10 cfu g^{-1})	5.78^{a}	5.65^{a}

Means in the same row followed by the same letter(s) do not differ significantly at (p>0.05). TBC: Total bacterial count

Table 4: The effect of storage period (days) on some chemical and microbiological properties of plain set yoghurt samples From Khartoum State

	Storage period (days)		
Parameters	3	6	9
Total solids (%)	14.37^{b}	14.66^{a}	14.56^{a}
Solid not fat (%)	11.07^{b}	11.37^{a}	11.26^{a}
Fat (%)	3.29^{a}	3.29^{a}	3.30^{a}
Protein (%)	4.31^{a}	4.16^{b}	4.00^{c}
Ash (%)	0.75^{a}	0.76^{a}	0.72^{a}
Titratable acidity	1.24^{c}	1.31^{b}	1.40^{a}
TBC (log 10 cfu g^{-1})	8.27^{b}	8.43^{a}	8.44^{a}
Coliform count (log 10 cfu g^{-1})	5.60^{a}	5.25^{a}	4.36^{b}
Yeast and mould count (log 10 cfu g^{-1})	5.06^{b}	5.90^{a}	6.17^{a}

^{abc}Means in the same row followed by the same letter(s) do not differ significantly at (p>0.05), TBC: Total bacteria count

contents and titratable acidity. However the storage period were not significantly (p>0.01) affected fat and ash contents of yoghurt.

Table 2 shows that yoghurt samples from manufacture (C) had the highest means for TS (15.79%), SNF (12.22%), fat (3.57%), protein (4.51%) and ash (0.89%) compared to other manufactures. Similarly the maximum and the minimum levels for those measurements were found in the yoghurt samples produced by manufacturer (C). Whereas the lowest means were

Table 5: The chemical composition and microbiological quality of plain set yoghurt within each type as affected by the storage period (days)

Storage period (days)		Total solids (%)	Solids not fat (%)	Fat (%)	Protein (%)	Ash (%)	Titratable acidity (%)	TBC (log10 cfu g^{-1})	Coliform count (log 10 cfu g^{-1})	Yeast and mould count (log 10 cfu g^{-1})
Recombined	3	13.69^{b}	10.60^{b}	3.09^{a}	4.04^{a}	0.65^{a}	1.24^{b}	8.25^{b}	5.65^{a}	5.47^{a}
milk yoghurt	6	14.21^{a}	11.16^{a}	3.05^{a}	3.90^{ab}	0.66^{a}	1.30^{ab}	8.38^{a}	5.22^{a}	5.79^{a}
	9	14.15^{a}	11.10^{a}	3.05^{a}	3.74^{b}	0.66^{a}	1.39^{a}	8.33^{a}	4.10^{b}	6.07^{a}
Fresh milk	3	15.04^{a}	11.53^{a}	3.52^{a}	4.58^{a}	0.84^{a}	1.24^{c}	8.29^{c}	5.55^{a}	4.65^{b}
yoghurt	6	15.12^{a}	11.59^{a}	3.53^{a}	4.42^{b}	0.85^{a}	1.33^{b}	8.47^{b}	5.27^{a}	6.01^{a}
	9	14.96^{a}	11.43^{a}	3.53^{a}	4.26^{c}	0.77^{a}	1.41^{a}	8.54^{a}	4.62^{b}	6.27a

Means in the same Column followed by the same letter(s) do not differ significantly at ($p>0.05$). TBC: Total bacteria count

reported for manufacture (B). Titratable acidity (1.39%) was higher in yoghurt samples from manufacture (B) and the lower value (1.23%) was found in yoghurt samples from manufacture (A). Moreover the yoghurt samples manufactured from fresh milk showed the highest mean for all chemical contents compared with that produced from powder milk (Table 3).

The total solids was found to increase significantly ($p<0.05$) from day 3 (14.37%) to day 6 (14.66%) then a non significant decrease was reported at day 9 (14.56%). The SNF also showed significant ($p<0.05$) increase from day 3 (11.07%) to day 6 (11.37%) then a non significant decrease (11.26%) was found at day 9 as shown in Table 4. The protein content was decreased significantly ($p<0.05$) from day 3 (4.31%) to day 6 (4.16%) and day 9 (4.00%). Also the titratable acidity revealed significant ($p<0.05$) increase from day 3 (1.24%) to day 6 (1.31%) and day 9 (1.40%), However the levels of fat (3.29, 3.29 and 3.30%, respectively, during 3, 6 and day 9) and ash (0.75, 0.76 and 0.72%, respectively) of plain yoghurt samples revealed non significant ($p>0.05$) changes due to the storage period (Table 4).

Non significant variations in both total solids and solids not fat were reported for fresh milk yoghurt (15.04, 15.12 and 14.96% and 11.53, 11.59 and 11.43%, respectively) during the storage period (day 3, 6 and 9, respectively). The values of recombined powder milk yoghurt (13.69, 14.21 and 14.15% and 10.60, 11.16 and 11.10%, respectively). The values for TS and SNF obtained during day 3 were significantly ($p<0.05$) lower than those found during day 6 and day 9 of storage (Table 5). Non significant variations were also found for both types of yoghurt during the storage for fat content (3.09, 3.05 and 3.05%, respectively and 3.52, 3.53 and 3.53%, respectively) and the ash content (0.65, 0.66 and 0.66%, respectively and 0.84, 0.85 and 0.77%, respectively). However the ash values were higher for fresh milk yoghurt. Significant ($p<0.05$) reduction was also reported for protein content of fresh milk yoghurt (4.58, 4.42 and 4.26%, respectively). However the protein values reported on day 6 (3.90%) showed significant ($p<0.05$) higher values than that found in day 9 (3.74%) and lower than that reported at day 3 (4.04%). Similar trend were also found for the level of acidity in both fresh (1.24, 1.30 and 1.39%, respectively) and recombined milk yoghurt (1.24, 1.33 and 1.41%, respectively) as shown in Table 5.

Microbiological loads: The mean of microbiological parameters for yoghurt made from powder milk were 8.32±0.18, 4.99±1.50 and 5.78±1.56 for log total bacterial count, coliform count and yeast and mould counts, respectively. Whereas for the fresh milk yoghurt, the means were 8.43±0.16, 5.15±1.17 and 5.65±1.86, respectively (Table 1). According to manufactures, all samples showed significant ($p<0.05$) changes in all microbiological parameters studied. Table 2 shows that yoghurt

samples from manufacture (D) had the highest mean for total bacterial and coliform counts compared to other manufactures. Whereas the manufacture (A) had the highest mean for yeast and mould count and lowest mean for total bacterial count. Yoghurt samples from manufacture (B) had the lowest mean for coliform and yeast and mould counts.

Table 3 shows that fresh milk yoghurt revealed the higher means counts for total bacterial count and coliform count and lower mean for yeast and mould count compared with that produced from powder milk. However significant ($p<0.01$) changes were found only in the total bacterial count due to the type of milk used.

Total bacterial count, coliform count and yeast and mould counts, were affected significantly ($p<0.01$) during the storage period. The total bacterial count and yeast and mould counts increased significantly ($p<0.05$) from day 3 to 6 and then gradually with non significant effect in day 9 of storage period (Table 4). The coliform count was found to decrease significantly ($p<0.05$) from day 3 to 6 and then without any significant change at day 9. The coliform count and yeast and mould counts followed the same trend in yoghurt produced from both recombined and fresh milk (Table 5). Total bacterial count increased significantly ($p<0.05$) from day 3 to 6 in both types of yoghurt and then gradually without any significance difference in day 9 of storage period in fresh milk yoghurt whereas in recombined milk yoghurt, the total bacterial count were slightly decreased at day 9 (Table 5).

DISCUSSION

The results obtained from chemical analysis of both type of yoghurt revealed that mean of total solids for powder milk yoghurt was lower than that obtained from fresh milk yoghurt. The higher value in fresh milk yoghurt might be because of enrichment of fresh milk with 15% milk powder (Attita Allah *et al*., 2010). Variations of some compositional contents of yoghurt were reported previously by Aly *et al*. (2004), El-Zubeir *et al*. (2005), Karagozlu *et al*. (2005) and Haj *et al*. (2007). Solids Non Fat (SNF), protein and fat content of all samples were in line with El-Bakri and El-Zubeir (2009). The result agrees with Sudanese Standards (2007), which stated that the minimum SNF content should be 8.20%. The average ash content of all samples of plain set yoghurt supported by Attita Allah *et al*. (2010). Titratable acidity revealed higher values than that reported by Haj *et al*. (2007) and lower than the results obtained by El-Zubeir *et al*. (2005). This might be due to the presence of some bacteria as shown in the present results. The differences in chemical composition between manufactures might be due to the variation of milk type and the conditions of processing that used to produce the yoghurt (Rasic and Kurmann, 1978). Moreover, Tamime and Robinson (2000) reported that the quality of yoghurt depending on the type of milk used.

The total bacterial count (TBC) of all yoghurt samples collected revealed result higher than that reported by Haj *et al*. (2007) and lower than that reported by El-Bakri and El-Zubeir (2009) Abdalla and El-Zubeir (2006) reported that higher counts were obtained for the factory commercial samples compared with those manufactured as experimental trial in the factory. Moreover pasteurization of the whole milk revealed lower counts, which could be attributed to elimination of contaminants.

The coliform count revealed results higher than that obtained by El-Bakri and El-Zubeir (2009) who found that the means level of coliform in plain and fruit yoghurt samples were log 4.03±4.41 and 3.59±4.15, respectively. Also they added that only 43.75% of samples had coliform count lower than 10^2 which is the maximum determined in most of the international standards. Tamime and

Robinson (1999) reported that the total coliform decrease during storage period due to the inhibitory effect of increase acid production. Also they added that the presence of coliforms in the samples is indicative of post pasteurization contamination at one or more stages during processing.

Yeast and mould counts revealed results higher than result obtained by El-Bakri and El-Zubeir (2009) and similar to the finding of Aly *et al.* (2004). The increase of yeast and moulds in fermented dairy products might be due to insufficient hygiene practices during processing (El-Bakri and El-Zubeir, 2009). However, Corbo *et al.* (2001) reported that most of lipolytic enzymes that are produced by the milk yeasts microbiota are frequently used for conferring different flavours of cheeses during cheese production. Montagna *et al.* (1998) cited that the fungi, in commercial yoghurt generally correspond to poor cleaning practices and the use of unhygienic techniques or inadequate storage conditions. Also they added that the fungal contamination might to occur during transformation processes and/or packaging, storage, transport and sale. Economic and sensorial losses of dairy products due to spoilage by yeast have been increasing in Turkey because of poor hygienic conditions during processing and to shorten the anticipated shelf life of products (Kavas *et al.*, 2006). Li and Li (1998) found that 67.33% of yoghurt samples collected from factories, supermarkets and retailers are contaminated by mould and yeast. They suggested that the tolerable limit of mould and yeast in yoghurt should be equal to or less than 50 cfu mL^{-1}.

The present study showed that plain set yoghurt made from powder and fresh milk present on local market in Khartoum State although showed high significant variations in chemical composition, it agreed with the Sudanese standards. However high coliform and yeast and mould counts were detected indicated high contamination and poor hygienic processing conditions. Nahar *et al.* (2007) suggested that in order to prepare good quality Dahi it is impertinent to follow strict hygienic conditions. It is concluded that sanitary standards should be established in Sudan so as to control milk production and marketing as was stated previously by Mohamed and El-Zubeir (2007).

The storage period also showed significant changes on both chemical and microbiological parameters. Hence it is recommended that monitoring procedures should be implemented on yoghurt available for the consumers to ensure that dairy product meets the standards. More research is needed about the type of milk used to produce yoghurt and its association with human health.

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