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Chemical and Microbiological Evaluation of Plain and Fruit Yoghurt in Khartoum State, Sudan

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Abstract: This study was conducted to evaluate the quality of yoghurt supplied to consumers from retail outlets in Khartoum State. Yoghurt samples (144 samples), which represent three different manufacturers (A, B and C) beside traditional producers (T) were collected. The samples included 96 plain yoghurt samples and 48 fruit yoghurt samples. All yoghurt samples were analyzed for chemical parameters (total solids, SNF, fat, protein, ash %, titratable acidity and pH) and the microbiological tests (total bacterial count, coliform count and the yeast and molds count). The means for total solids %, solids non-fat (SNF), fat, protein, ash % and pH for the plain yoghurt samples were 14.04±1.83, 10.86±1.53, 3.18±1.01, 3.44±0.58, 0.678±0.146 and 4.62±0.10, respectively. Whereas for the fruit yoghurt samples the means were 21.70±1.34, 19.70±1.27, 2.00±0.62, 3.90±0.50, 0.661±0.087 and 4.68±0.12, respectively. The means of microbiological measurements for the plain yoghurt samples were log 9.10±9.86, log 4.03±4.41 and log 4.09±4.57 for the total bacterial count, coliform count and yeast and molds count, respectively. Whereas in the fruit yoghurt samples the means were log 8.63±9.99, log 3.59±4.15 and log 3.15±3.64, respectively. Results obtained revealed significant variations ($p \leq 0.001$) between samples obtained from different manufacturers in their chemical composition. One hundred and thirty eight of collected samples (96.5%) were found satisfying the international standard for solids non-fat content, however, 73 yoghurt samples (50.7%) were found to have a lower fat content than the standard. In the microbiological parameters tested, the total bacterial count and yeast and molds count were not significantly different between different manufacturers. The coliform count of samples varied significantly ($p \leq 0.001$) between manufacturers and with a significance higher ($p \leq 0.05$) coliform count in samples collected from traditional manufacturers than that collected from modern manufacturers.

Key words: Yoghurt, plain, fruit, chemical parameters, microbiological measurements

INTRODUCTION

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 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). Lactic acid bacteria are fastidious microorganisms and their growth is often restricted in milk because of its paucity in essential nutrients, thus the success of milk fermentation relies most often upon the synergy between *S. thermophilus* and *L. bulgaricus*. Because both bacteria are able to grow alone in milk, this indirect positive interaction is called proto-cooperation (Courtin and Rul, 2004).

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Yoghurt is derived from the Turkish word Jugurt reserved for any fermented food with acidic taste (Younus *et al*., 2002). Consumption of yoghurt has been shown to induce measurable health benefits linked to the presence of live bacteria (Guarner *et al*., 2005). They added that a number of human studies have clearly demonstrated that yoghurt containing viable bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus*) improves lactose digestion and eliminates symptoms of lactose intolerance, thus, these cultures clearly fulfill the current concept of probiotics. Similarly, Sieber *et al*. (1997) reported that yoghurt is found to be well tolerated by lactose maldigesters.

Changes in the physical, chemical and microbiological structure of yoghurt determine the storage and shelf life of the product (Sofu and Ekinci, 2007). Ogasawara *et al*. (2006) reported that 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). Kozacinski *et al*. (2003) found in a study conducted in Croatia that the main reasons for microbiological non-acceptability of 655 samples out of 802 samples of milk and milk products were higher number of Enterobacteria, yeast and molds, higher number of total bacteria, *Escherichia coli* and *Staphylococcus aureus*.

The quality of yoghurt in local markets varies from producer to another. A practical approach towards the quality of yoghurt is to evaluate the different samples of yoghurt sold in local markets. Research in this field of quality evaluation of market yoghurt is the basic need to create awareness among common people about the existing situation and protect the consumers' health and rights. Therefore, this study is designed to evaluate market yoghurt for chemical and microbiological properties.

MATERIALS AND METHODS

Collection of Samples

One hundred and forty four commercially produced yoghurt samples were obtained from retail outlets in Khartoum State during the period from September to November 2005. The samples which represent three different manufacturers (A, B and C) beside traditional producers (T) included 96 plain yoghurt samples and 48 fruit yoghurt samples. All samples were transported to the laboratory (in an ice box), held at less than 4°C and analyzed for chemical and microbiological characteristics.

Each brand/type was obtained from three different points in Khartoum State (Khartoum, Khartoum North and Omdurman). Guided by date coding, only samples that were within three days of manufacture were chosen for the present study.

Microbiological Analysis

Samples were examined for total bacterial count (TBC) according to Houghtby *et al*. (1992), coliform counts and yeasts and molds count according to Harrigan and McCance (1976). Plate count agar No. 298 (Biomark Laboratories) was used for enumeration of total bacterial count. MacConkey's agar (Oxoid, CM 115) was used to determine coliform counts. Yeast and molds count were obtained using Potato dextrose agar (Hi Media Laboratories Ltd., M096) acidified with a sterile 10% tartaric acid. Plates for enumeration of TBC and coliform incubated at 32°C for 48 h and 37°C for 24 h, respectively. In case of yeast and molds count plates were incubated at 28°C for 5 days. Developed colonies were counted using manual colony counter. The plate counting 25-250 colonies were selected as described by Houghtby *et al*. (1992). The number reciprocal of the dilution factor was recorded as cfu mL^{-1}.

Chemical Analysis

The total solids content and the protein content were determined according to the modified method described by Bradley *et al*. (1992). The fat was determined by Gerber method and the ash content was determined by draft oven method described by Bradley *et al*. (1992). The pH of yoghurt samples was determined using electronic pH meter (HANNA pH 210).

Statistical Analysis

The data of the present study were analyzed statistically using randomized complete block design. ANOVA test was used to determine the significance level of the treatments, while the Least Significant Difference (LSD) was used for mean separation at $p\leq 0.05$. The analysis was carried out using SPSS for Windows 10.0 package program. The microbiological data was presented as log mL^{-1}.

RESULTS AND DISCUSSION

Statistical analysis of the results of chemical composition of yoghurt samples revealed that all parameters varied significantly ($p<0.001$) according to the manufacturer (Table 1, 3). On the other hand, there were no significant variations between plain yoghurt samples in different batches except

Table 1: Compositional content of yoghurt samples collected from different manufacturers

Variables		A	B	C	T	Total plain
TS (%)	Mean	16.210±0.970	14.040±0.530	13.800±1.950	11.850±1.570	14.040±1.830
	Range	14.510-17.77	13.330-15.16	13.490-15.46	9.140-14.63	9.140-17.77
SNF (%)	Mean	12.800±0.900	11.300±0.440	10.600±1.600	9.080±0.810	10.860±1.530
	Range	11.210-14.02	10.530-12.16	9.780-11.96	7.730-10.28	7.730-14.02
Fat (%)	Mean	3.410±0.640	2.750±0.260	3.821±0.209	2.770±1.690	3.180±1.010
	Range	2.800-5.000	2.150-3.200	3.404-4.238	0.500-4.700	0.500-5.000
Protein (%)	Mean	3.850±0.540	3.680±0.470	3.390±0.700	3.030±0.380	3.440±0.580
	Range	2.680-5.720	2.320-4.470	1.970-3.930	2.320-3.930	1.970-5.720
Ash (%)	Mean	0.743±0.061	0.744±0.188	0.701±0.168	0.551±0.129	0.678±0.146
	Range	0.567-0.810	0.483-1.035	0.520-0.822	0.276-0.725	0.276-1.035
pH	Mean	4.710±0.070	4.590±0.080	4.510±0.630	4.580±0.090	4.620±0.100
	Range	4.590-4.830	4.440-4.730	4.400-4.820	4.400-4.730	4.400-4.830
TBC (log10 mL^{-1})	Mean	8.450±8.880	8.330±8.700	8.390±8.670	8.650±10.15	9.100±9.860
	Range	5.340-10.53	0.000-8.380	6.600-8.940	7.810-10.85	0.000-10.85
Coliform log10 mL^{-1})	Mean	3.580±3.850	3.540±3.840	3.360±3.760	4.550±4.620	4.030±4.410
	Range	0.000-4.360	0.000-4.340	0.000-3.110	2.720-5.250	0.000-5.250
Yeast and moulds (log10 mL^{-1})	Mean	3.840±4.440	3.470±4.090	3.900±4.580	4.450±4.600	4.090±4.570
	Range	0.000-6.120	0.000-4.790	0.000-5.420	2.540-5.260	0.000-5.420

Variables		AF	BF	Total fruit	Grand total
TS (%)	Mean	21.230±1.170	22.160±1.360	21.700±1.340	16.590±3.990
	Range	19.360-24.33	19.450-24.63	19.360-24.63	9.140-24.63
SNF (%)	Mean	19.280±1.060	20.110±1.340	19.700±1.270	13.800±4.420
	Range	17.660-22.18	17.750-22.73	17.660-22.73	7.730-22.73
Fat (%)	Mean	1.950±0.790	2.050±0.410	2.000±0.620	2.320±1.490
	Range	0.800-3.900	1.200-3.000	0.800-3.900	0.500-5.000
Protein (%)	Mean	4.180±0.520	3.620±0.270	3.900±0.500	3.590±0.600
	Range	3.570-5.000	3.190-4.110	3.190-5.000	1.970-5.720
Ash (%)	Mean	0.650±0.077	0.673±0.097	0.661±0.087	0.670±0.129
	Range	0.500-0.766	0.386-0.785	0.386-0.785	0.277-1.035
pH	Mean	4.630±0.090	4.730±0.130	4.68±0.120	4.640±0.110
	Range	4.480-4.800	4.480-4.950	4.480-4.950	4.400-4.950
TBC (log10 mL^{-1})	Mean	8.220±8.470	8.840±9.120	8.630±9.990	8.100±9.770
	Range	5.530-9.030	0.000-9.750	0.000-9.750	0.000-10.85
Coliform (log10 mL^{-1})	Mean	3.500±4.120	3.670±4.190	3.590±4.150	3.930±4.350
	Range	0.000-4.820	0.000-4.810	0.000-4.820	0.000-5.250
Yeast and moulds (log10 mL^{-1})	Mean	3.630±3.660	3.380±3.790	3.150±3.640	3.940±4.490
	Range	0.000-3.200	0.000-4.470	0.000-4.470	0.000-5.420

In this and the following tables: A, B, C and T = Plain yoghurt samples, AF and BF = Fruit yoghurt samples

Table 2: Comparison between plain and fruit yoghurt samples collected from different manufacturers using one way ANOVA analysis

	Plain yoghurt			Fruit yoghurt		
	Batch	Manufacturer	Batch×Manufacturer	Batch	Manufacturer	Batch×Manufacturer
Measurement	F	F	F	F	F	F
TS (%)	0.848^{NS}	73.027***	0.791^{NS}	4.691***	10.630**	0.828^{NS}
SNF (%)	2.464*	155.746***	1.488^{NS}	5.019***	9.480**	1.338^{NS}
Fat (%)	0.411^{NS}	6.286***	0.347^{NS}	11.032***	1.298^{NS}	5.464***
Protein (%)	3.861***	22.032***	1.813*	1.438^{NS}	21.747***	0.681^{NS}
Ash (%)	5.105***	25.352***	3.878***	1.597^{NS}	0.956^{NS}	0.870^{NS}
pH	0.597^{NS}	13.952***	1.198^{NS}	8.036***	23.494***	3.490**
TBC (cfu mL^{-1})	0.939^{NS}	2.105^{NS}	1.004^{NS}	0.987^{NS}	2.546^{NS}	0.898^{NS}
Coliform (cfu mL^{-1})	1.291^{NS}	14.294***	0.932^{NS}	1.642^{NS}	0.124^{NS}	0.198^{NS}
Yeast and moulds (cfu mL^{-1})	0.638^{NS}	2.117^{NS}	1.048^{NS}	1.889^{NS}	3.181^{NS}	1.793^{NS}

NS = Not significant (p>0.05), **Significance (p<0.01), ***Significance (p<0.001)

Table 3: Differences between manufacturers in the chemical and microbiological measurements of plain yoghurt samples

	Manufacturer				
Measurement	A	B	C	T	Grand mean
TS (%)	16.209±0.972^{c}	14.043±0.527^{b}	14.061±1.947^{b}	11.851±1.565^{a}	14.041±1.829
SNF (%)	12.802±0.902^{d}	11.297±0.444^{c}	10.240±1.599^{b}	9.084±0.809^{a}	10.856±1.533
Fat (%)	3.406±0.640^{b}	2.746±0.260^{a}	3.820±0.720^{b}	2.767±1.690^{a}	3.185±1.010
Protein (%)	3.848±0.540^{b}	3.684±0.468^{b}	3.201±0.701^{a}	3.029±0.378^{a}	3.441±0.581
Ash (%)	0.743±0.061^{c}	0.744±0.188^{c}	0.673±0.168^{b}	0.551±0.129^{a}	0.678±0.146
pH	4.712±0.070^{b}	4.590±0.080^{a}	4.601±0.630^{a}	4.576±0.090^{a}	4.620±0.100
TBC (log 10 mL^{-1})	8.460±8.870^{a}	8.320±8.700^{a}	8.280±8.690^{a}	9.640±10.15^{a}	9.080±9.860
Coliform (log 10 mL^{-1})	3.570±3.850^{a}	2.530±3.840^{a}	2.200±3.760^{a}	10.540±4.620^{b}	4.030±4.410
Yeast and molds (log 10 mL^{-1})	3.840±4.440^{ab}	2.460±4.100^{a}	4.040±4.580^{ab}	4.430±4.600^{b}	4.080±4.570

$^{a-d}$Values in the same row with the same alphabet do not differ significantly (p>0.05), A, B, C and T are plain yoghurt samples

in case of SNF (p<0.05), protein (p<0.001) and ash% (p<0.001). In contrast fruit yoghurt samples varied significantly (p<0.001) in all chemical composition between batches except in protein and ash % as shown in Table 2. Moreover, yoghurt samples taken from different locations in Khartoum State did not vary significantly in all chemical and microbiological parameters studied.

Table 1-3 showed the different microbiological parameters studied in the plain and fruit yoghurt samples. In plain yoghurt samples, the manufacturer from which the samples were obtained appeared to have significant effect (p<0.001) only in the coliform count, but the total bacterial count and yeast and molds count did not vary significantly (p>0.05) according to the manufacturer. On the other hand, no significant effect (p>0.05) on the results was noticed because of the batch or location of sampling. For the fruit yoghurt samples no significant variations between different manufacturers, batches or locations were observed.

In plain yoghurt samples the mean total solids content was 14.04±1.83%, which varied significantly according to the manufacturer (Table 3). Results obtained were in alliance with the results of Musaiger *et al*. (1998) for yoghurt samples collected from local market in Bahrain and that reported by Younus *et al*. (2002) for samples available in local markets in Pakistan, but higher than that found by El-Zubeir *et al*. (2005), who reported an average total solids content of 9.3±2.52% for yoghurt samples in Sudan. Also, lower total solids content were reported for yoghurt by Karagozlu *et al*. (2005) who found that yoghurt samples collected from local market in Turkey had average total solids content of 15.89%. The fruit type yoghurt samples had higher total solids content (Table 1) than that of plain yoghurt which could easily be justified by the incorporation of fruit concentrates added.

Table 2 demonstrates the higher variations in total solids content in fruit yoghurt samples because of the batches rather than the variation caused by manufacturer and this could be attributed to the variation of type of fruit between batches since sampling was done regardless of the type of fruit.

Results of chemical analysis of fruit yoghurt (Table 1) gives total solids mean of 21.7±1.34%. This difference probably exists because fruit yoghurt samples contain fruit concentrations rather than flavours added in flavoured yoghurt and the later have lower total solids than the former. Additionally the total solids content in yoghurt samples taken from traditional manufacturers represented in sample (T) was significantly lower than plant-made yoghurt samples obtained for manufacturers (A, B and C) as shown in (Table 2, 3). This could be due to the enrichment of yoghurt with milk powder the matter that causes this elevation of total solids content probably.

Yoghurt samples collected in this study recorded solids non-fat content of 10.86±1.53% for plain yoghurt samples and 19.7±1.27% for the fruit yoghurt samples (Table 1, 3). Plain yoghurt solids non-fat content results were similar to that reported by Musaiger *et al*. (1998), but lower than what reported by Karagozlu *et al*. (2005).

Fat content results obtained for plain yoghurt were higher than that reported by El Zubeir *et al*. (2005) and lower than results reported by Musaiger *et al*. (1998) and Younus *et al*. (2002). The fat percentages in plain yoghurt samples appeared to be higher (3.18±1.01%) than that recorded in fruit yoghurt samples (2.0±0.62%) (Table 1, 3), which could be attributed to the addition of fruit which have a lower fat percentage causing a noticeable decrease in the fat content of the yoghurt samples. Fat content results in fruit yoghurt samples were similar to that obtained by Rodas *et al*. (2001).

In total protein content the fruit yoghurt samples showed a higher content of 3.9±0.5% compared to 3.44±0.58% in plain yoghurt samples (Table 1). Which were higher than that found by Rodas *et al*. (2001) in fruit yoghurt (2.95±0.46). Moreover, these results were lower than that reported by El Zubeir *et al*. (2005).

Variations in ash content were significant ($p \leq 0.001$) in plain yoghurt samples depending on both batches and manufacturer but not the location. On the other hand, the fruit yoghurt samples didn't show significant variations according to the batch, manufacturer or location of samples. Ash content in plain yoghurt samples were lower than that reported by El Zubeir *et al*. (2005), who reported that ash content of yoghurt had an average of 0.81±0.29%.

The pH value of all yoghurt samples collected averaged 4.64±0.11 and for the plain yoghurt samples the average was 4.62±0.10 (Table 1, 3) which was lower than that reported by El Zubeir *et al*. (2005), but higher than values reported by Karagozlu *et al*. (2005) and that reported by Younus *et al*. (2002). In fruit yoghurt samples the average was 4.68±0.12 which was higher than what obtained by Rodas *et al*. (2001) and Musaiger *et al*. (1998). These results were reasonably justified and suitable for yoghurt marketed in tropical areas because of the expected effect of bad storage conditions mainly high temperatures encountering in such zones on the acidity of yoghurt. Additionally the kind of uses of yoghurt in Sudan-particularly in Khartoum-requires yoghurt with such mild acidity as the consumer desires. Also a factor which could not be excluded or mis-evaluated is the in-consistency of power supply that subjects the product to a high temperatures shortening its shelf life and increasing the acidic flavour.

Microbiological analysis for yoghurt samples collected (Table 1, 3) revealed no significant variations between different manufacturers (Table 2) except that encountered in coliform count for the plain yoghurt samples. The average log total bacterial count for all yoghurt samples collected was 8.10±9.77 mL^{-1}. These results were higher than what reported by Al-Tahiri (2005) and Younus *et al*. (2002).

The international standards for yoghurt require minimum total viable microorganisms of 10^7 cfu mL^{-1} in the finished product. This standard was met in 138 out of 144 (95.8%) plain and fruit yoghurt samples collected. No significant differences between manufacturers or type of yoghurt were observed. The coliform count was the only microbiological parameter having a significant variation ($p \leq 0.001$) according to the manufacturer. The mean log total coliform count for all yoghurt samples collected was 3.93±4.35 mL^{-1} (Table 1, 3), which was higher than that reported by Younus *et al*.

(2002). Moreover, 43.75% of samples had coliform counts lower than 10^2 which is the maximum determined in most of the international standards (Kucukoner and Tarakci, 2003). The non complying samples might indicate the low level of hygiene during processing of yoghurt (Birollo *et al*., 2001). This illustrates the differences in those practices between different manufacturers, yet most of them appear to be microbiologically not acceptable when compared to the international standards.

When comparing samples collected from different manufacturers, the samples from the traditional producers could easily be noticed to have higher coliform counts (Table 1, 3). This could be obviously attributed to the lower hygienic standards used in the production facilities, the matter which is confirmed by the findings of Dardashti *et al*. (2001), who found that the rate of contamination in traditional processing with coliform was higher than in industrial processing.

The present data showed that 68.75% of samples collected had yeast and molds count lower than 10^3 cfu mL^{-1} determined in the international standards. The plain yoghurt samples showed a higher mean for log yeast and molds count (4.09 ± 4.57 mL^{-1}) as shown in Table 1 and 3, compared with the yeast and molds count in case of fruit yoghurt samples ($1.4\times10^3\pm4.4\times10^3$ cfu mL^{-1}). This could be attributed to the fact that the traditional yoghurt samples, which were included in the plain yoghurt samples-, had significantly ($p\leq 0.05$) higher mean yeast and molds count (Table 1, 3), the matter that raises the mean for the plain yoghurt samples. Hence, the present study suggested that the producers must give more attention to the hygienic practices in their production facilities to improve the microbial quality standards. Licenses given for small dairy producers must be issued after the assurance of a minimum level of good manufacturing practices.

The present study concluded that commercially available yoghurt has a good chemical quality when compared to the international standards. However, the microbiological quality was lower than that required by the international standards in most of yoghurt samples. Moreover plant-made yoghurt samples available in the local market appeared to be more consistent, unlike traditionally produced yoghurt which varied in compositional quality parameters tested, especially fat content. Hence it is recommended that governmental legislative bodies must have efficient role in monitoring the quality of dairy products available for consumers.

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