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MICROBIOLOGICAL ANALYSIS OF YOGHURT PURCHASED IN KHARTOUM STATE, SUDAN

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ABSTRACT

This study was conducted to evaluate the microbiological quality of yoghurt purchased in Khartoum State during its shelf life and to compare the yoghurt produced in different factories. A total of 36 yoghurt samples were collected from December to February/2019-2020. Samples were collected from 9 groceries and 9 supermarkets with 18 samples for factory A and B in the three localities of Khartoum State. The samples were subjected to the total coliform count (TCC), tentative isolation of the *Escherichia coli* and *Salmonella* and the detection of pH and temperature values of yoghurt. By using the Most Probable Number Method (MPN) the TCC in day one of factory A was found 4.6±1.8cfu/ml and 315.2±105.2cfu/ml for factory B, whereas it was found 6.9±4cfu/ml for factory A and 1100 cfu/ml for factory B in day ten. There were significant differences in the TCC of day one and day ten between the two factories with $p \leq 0.05$. The TCC in day one for the groceries and supermarkets in the State was 129.8cfu/ml and 190cfu/ml and increased in day ten to 551cfu/ml and 555cfu/ml, respectively. Statistically there were no significant differences between groceries and supermarkets in day one and day ten with $p \leq 0.05$. The mean pH observed in these samples in day one for factory A was 5.6 and 5.2 for factory B, and decreased in day ten to 4.6, 4.4 for factory A and B, respectively. These values were above the yoghurt acceptable pH of Sudanese standard and metrology organization (SSMO) (4.5) in day one, but it decreased in day ten matching the SSMO with about 17 (48.6%) of samples. E. coli was detected in 12 (33.3%) of the samples. One sample was positive for both *Salmonella* and *Escherichia coli*. The refrigerator temperature of the groceries and supermarkets in the three localities was above the optimum storage temperature (5℃). The highest ambient temperature in the groceries and supermarkets was detected in Bahri 39.5℃ and 38℃ in groceries and supermarkets, respectively. In conclusion the overall hygienic quality of yoghurt samples was lower than the limits recommended by the SSMO. It was recommended that all producers should maintain adequate hygienic practices to produce good quality and healthy yoghurt.

KEYWORDS: Yoghurt; bacterial contamination; pH and storage temperature values.

INTRODUCTION

Yoghurt is the most common dairy products consumed around the world, and its sensory attributes, have a large effect on consumer acceptability.[1] Yoghurt is produced when milk or milk products coagulates, causing the lactic acid contained in it to coagulate, via the action of bacterial enzyme lactase provided by the bacteria *Streptococcus thermophilus, Lactobacillus bulgaricu*s breaks down the sugar compound glucose and galactose that the lactose is composed of, under anaerobic conditions.[2] Yoghurt is highly vulnerable to bacterial contamination and hence it is easily perishable.[3] There are reported cases of food infection and intoxication largely due to poor hygiene in the production, processing and storage of such foods.[4] However, some microorganisms such as *Staphylococcus aureus, Escherichia coli*, Coliform bacteria and Fungi have been well documented as yoghurt borne infection.[5] Therefore, to create public health concern, microbiological assessments are necessary for yoghurt. Microbiological methods can reduce economic losses by the early detection of inadequate processing, packaging or refrigeration.[6] Yoghurt is one of the popular dairy products in Sudan. Moreover, it is produced commercially by the specialized dairy plants. The types of micro-organisms present reflect to some extent, the standard of plant hygiene.[7] However, little work in Sudan was done to evaluate the hygienic quality in terms of its microbial loads and shelf life. Due to the health implication of poorly produced yoghurt and other dairy products, this study was done to assess the

microbiological analysis of yoghurt purchased in Khartoum state.

MATERIAL AND METHODS

Source of yoghurt samples

Thirty-six Stratified random samples of yoghurt produced by 2 different factories in Khartoum State were collected to include supermarkets and groceries. Samples were collected in day one of the production and the last day of the shelf life. Markets were classified as follows: supermarkets which relatively large food store located usually in or near a main street, usually contains high quality refrigerators placed inside the main building and groceries which are small to medium sized food stores often found within residential neighborhoods with refrigerators usually located outside the main building.

Collection and transportation of samples

The yoghurt samples were collected during the period from December to February (2019-2020) from the factory's original package at the moment the yoghurt was unloaded in the grocery stores or supermarkets in day one, and from supermarkets and groceries refrigerators at day ten, then transported immediately in an ice box until microbiological examination was carried out in the laboratory of the College of Veterinary Medicine, University of Bahri and were examined to determine the pH value of the yoghurt, presumptive isolation of *Salmonella* and *Escherichia coli* and total coliform count.

Microbial examination

All media were prepared according to manufacturer's instructions. Total coliform count was conducted using the Most Probable Number Method (MPN). The test is a method to estimate the concentration of total viable coliform in a sample by means of replicate liquid broth growth as described by FDA.[8] The presumptive isolation of *Escherichia coli* and *salmonella* was performed according to Sagar.[9]

pH Determination

The pH of the yoghurt samples was measured immediately with the aid of a pH meter after calibrating with the pH of distil water.

Temperature

The temperature of the refrigerators and the ambient temperature (temperature of the surrounding) were measured using probe thermometer. In groceries and supermarkets some of refrigerators were placed inside the main building and the others outside the main building. Some of the groceries' keepers used to switch off electric current from refrigerators during nights to reduce the running cost; all these factors were checked during the study.

Statistical Analysis

Statistical analysis was performed using SPSS version 24. Paired sample t-test, independent T-test, descriptive statistics, frequency, cross tabs and ANOVA were performed.

RESULTS

The mean of TCC in day one for factory A was 4.6cfu/ml and 315.2cfu/ml in factory B, with no significant difference between factory A and B in day one for TCC, p=0.006, The mean TCC in day ten of factory A and B were 6.9cfu/ml and 1100cfu/ml, respectively, and there was highly significant difference in day ten between the two factories with p=0.000. The mean pH value of factory A and B in day one and day ten were 5.6, 5.2 and 4.6, 4.4, respectively. There was significant difference of pH in day one between the factories A and B with p=0.000 and there was no significant difference between the two factories in day ten with p= 0.21(table 1).

Table 1: The pH and total coliform count in day one and day ten of the factories (A and B).

Parameter	Factory	Mean	Std. Deviation	Std. Error Mean	Significance
TCC- day 1	A	4.6111	8.02129	1.89064	
	B	315.2222	446.50825	105.24300	.006
TCC- day 10	A	6.9333	17.15247	4.04287	
	B	1100.0000	.00000	.00000	.000
pH- day1	A	5.6111	.10226	.02410	
	B	5.2889	.27630	.06512	.000
pH- day10	A	4.6706	.54517	.13222	
	B	4.4778	.32459	.07651	0.218

Significance ≤ 0.05

The mean coliform count in day one was 129.8cfu/ml in groceries and 190cfu/ml in supermarkets. There was no significant difference in the mean count between groceries and supermarket with p≤ 0.612. The mean coliform count in day ten was 551.2cfu/ml in groceries and 555.6cfu/ml in supermarkets. Statistically there was insignificant difference between the two means with p≤ 0. 982 (figure1).

The mean pH value in day one was 5.4 in groceries and supermarkets, and the mean pH in day ten was 4.6 in the groceries and 4.5 in the supermarkets. There was insignificant difference in the pH between the groceries

and supermarkets in day one and day ten with p≤ 0.454 and p≤0.721, respectively (figure1). Two (5.6%) of the samples showed pH level 4 - 4.6 in day one, while 22 (62.8%) of the samples were between 4- 4.6 pH in day ten.

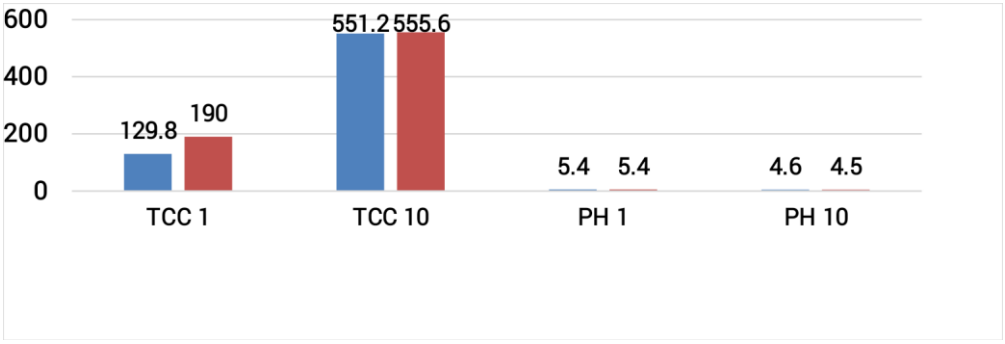

Figure 1: The total coliform count and pH of groceries and supermarkets.

Twenty-three (63.8%) of the samples were negative for presumptive isolation of *Salmonella* and *Escherichia coli* and one sample was positive for both. Also 12 (33.3%) of the samples were positive for *Escherichia coli* and negative for *Salmonella* (table 2). While, 28 (77.8%) of the samples with TCC were less than 100cfu/ml in day one, 18 (50%) of the samples were found with total coliform count less than 100cfu/ml in day ten. Paired sample t-test showed significant difference p≤0.05 between coliform count in day one and day ten. Also the difference in pH between day one and day ten was statistically significant with p≤0.05 (table 3).

Table 2: Crosstab for the isolation of *Salmonella* and *E. coli*.

		Salmonella		
		Negative	**Positive**	**Total**
E.coli	Negative	23	0	23
	Positive	12	1	13
	Total	35	1	36

Table 3: Paired sample T test.

		T	**Df**	**Sig. (2-tailed)**
Pair 1	tcc1 - tcc10	-4.682	35	.000
Pair 2	ph1 - ph10	11.476	34	.000

The mean refrigerator temperature in Khartoum groceries was 10.7℃ and the highest refrigerator temperature in Bahri groceries was 21℃. While the mean refrigerator temperature in Omdurman supermarkets was 18℃ and the lowest refrigerator temperature in Bahri supermarkets was 12℃ and there were no significant differences in refrigerators temperature between groceries and supermarkets in the three localities of Khartoum State with p≤0.05 (Table 4).

Table 4: The refrigerator temperature of groceries and supermarkets.

Refrigerator temp	**N**	**Mean**	**Std. Error**	**Minimum**	**Maximum**	**Sig.**
Khartoum groceries	3	10.7333	2.89904	5.20	15.00	.398
Bahri groceries	3	21.4667	4.96700	13.00	30.20	
Omdurman groceries	3	15.0333	5.48037	6.10	25.00	
Khartoum Supermarkets	3	15.2667	3.97548	9.80	23.00	
Bahri Supermarkets	3	12.0333	1.24141	9.90	14.20	
Omdurman Supermarkets	3	18.6667	2.33333	14.00	21.00	
Total	18	15.5333	1.57316	5.20	30.20	

The mean of ambient temperature in Bahri, Khartoum and Omdurman groceries were 39, 37, and 38℃, respectively. But in the supermarkets the mean ambient temperature in Bahri, Khartoum and Omdurman were 38, 35, and 31℃, respectively. There were no significant differences of ambient temperature between the groceries and supermarkets in the three localities with p≤0.05 (table 5).

Table 5: The ambient temperature of groceries and supermarkets.

Ambient temp	N	Mean	Std. Error	Minimum	Maximum	Sig
Khartoum groceries	3	37.6667	1.85592	34.00	40.00	.076
Omdurman groceries	3	38.3333	1.66667	35.00	40.00	
Bahri groceries	3	39.0000	.57735	38.00	40.00	
Khartoum supermarkets	3	35.0000	2.88675	30.00	40.00	
Omdurman supermarkets	3	31.3333	1.33333	30.00	34.00	
Bahri supermarkets	3	38.3333	1.66667	35.00	40.00	
Total	18	36.6111	.89712	30.00	40.00	

About 26 (72.2%) of the groceries and supermarkets kept their refrigerator inside the main building and 10 (27.8%) outside the main building. About 10 (27.8%) of groceries and supermarkets keepers used to switch off electric current from refrigerators during nights.

DISCUSSION

In this study Factory B showed significantly higher counts compared to factory A in day one which were not complying with SSMO standard (<100cfu/ml). These higher counts may be attributed to the differences in the hygienic practices and sanitation measures during manufacturing of yogurt between the two factories. The presence of coliform bacteria indicates unhygienic practices during handling of the product.[10]

The present study showed that the highest value of pH in day one was obtained from factory A (5.6). This result was higher than the values (4.61) observed by Ranathunga.[11] and also higher than (4.5) that stipulated by SSMO, while the pH-values decreased progressively in factory B (4.4) which may be due to excessive sugar fermentation and presence of lactic acid.[12,13]

The present study revealed mean TCC counts in supermarkets and groceries in day one and in day ten with differences that were statistically insignificant and this may be to the same improper storage condition practiced in both types of markets.

This study showed that 33% percent of yoghurt samples were positive for presumptive isolation of *E. coli* which indicates poor hygienic practices during processing. This result also didn't comply with SSMO limits (zero count for *Escherichia coli* and *salmonella*). The result also contradicts the scientific facts that *E. coli* is an indicator of food and water contamination from fecal sources and its mere presence in a food renders the food unfit for human consumption.[14]

In the present study one sample showed presence of *salmonella* and that agreed with Szczawiński et al.[15] who stated that the numbers of *salmonella* in yoghurt decreased linearly with storage time in the samples incubated at all temperatures.

Finally, the refrigerator temperature of the stored yoghurt in both groceries and supermarket was found above the standard reported by FAO.[16] The product should be stored at refrigeration temperature equals to (5℃) to slow down the physical, chemical and microbiological degradation.

Also the highest ambient temperature of groceries and supermarkets in this study were found in Bahri locality (39℃ and 38℃, respectively). This was not in line with that reported by Ozcooler.[17] who showed that the highest temperature point at which most refrigerators will work well is 37℃.

CONCLUSION AND RECOMMENDATIONS

The high level of TCC indicated that excessive contamination occurs during manufacturing or post contamination in processing and packaging of the product. The high ambient temperature, switching off electric current from refrigerators during nights and placing refrigerator outside the main building contributed to the high count of coliform.

The overall picture of yoghurt microbiology analysis indicates the need for emphasis on safety and quality control within processing factories by application of HACCP system.

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