Effects of Storage Condition and Time on the Microbiological Attributes of some Stored Yoghurts Marketed in Enugu Metropolis

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ABSTRACT

This study comparatively investigated the effects of storage condition and time on the microbiological qualities of some stored yoghurts marketed in the Enugu metropolis. The experiment was factorial (2x6x4) involving a completely randomized design (CRD). A total of 96 samples comprising four samples of yoghurt coded A, B, C and D were sourced from different producers and designated as A – Food Science and Technology lab (FST), B – Aqua Rapha yoghurt, C – Chariot yoghurt and D – A.S yoghurt samples. The samples were stored for a period of seventy days under two storage conditions – refrigerated (5^0C) and room temperatures (28 ± 2^0C). All samples were analyzed for microbiological parameters such as total viable count (cfu), coliform (cfu), fungal/yeast (cfu) . Statistical analysis was conducted using two-way ANOVA to determine the mean differences. The results showed that total viable count, coliform count, yeast /fungal counts increased with storage time but the rate of increase was significantly higher(<0.05) in room samples. The room samples contained higher total viable, yeast/fungal and coliform counts than the refrigerated counterpart at all storage time irrespective of sample. Deterioration with storage time in terms of total viable count, coliform count was found to be more in sample A and least in sample C . .

INTRODUCTION

When fermented food are not subjected to further technological transformation such as pasteurization or high pressure treatments, they can be used as vehicle for probiotics (Adams and Mitchel 2002). Probiotics are live microorganisms which when administered in adequate amount confer a high benefit to the host. Major efforts have been directed towards maintaining the highest number of live microorganisms at the time of consumption. A number of factors affect the loss of viability of probiotic organisms in yoghurt such as the acidity of products, composition of ingredients used, storage conditions, amount of culture inoculated, time of incubation, sensitivity to antimicrobial substances produced by starter bacteria and lack of nutrients in the milk. The minimum level of probiotics is crucial in determining the overall health value of that particular yoghurt. To obtain therapeutic benefits, Robinson (2002) and Kurman and Rasic (1991) suggested that the minimum level for probiotic bacteria in yoghurt is 10^5 - 10^6 viable cells per gramme of product. During milk fermentation processes, lactic acid bacteria are exposed to various environmental stress conditions such as temperature fluctuation, acid, pH, high osmotic pressure and absence of available nutrients. Champagne *et al*. (1991) reported that pH must be controlled at a range of 5.5-70 to ensure higher biomass yield and survival after freezing. Occurrence of yeast and coliform in dairy product is significant as they cause spoilage and effect some biochemical changes that may adversely affect public health (Jordano *et al*., 1991). There have been reported cases of food infection and intoxications largely due to poor hygiene in production, processing and storage of such foods (Ananias and Roland, 2017) Significant effect of total viable count in yoghurt and

lactic acid values have health implications. Milk and yoghurts are highly vulnerable to bacterial, coliform, fungal and yeast contamination and are highly perishable (Girma et., al 2014). In order to protect the public health, microbiological assessment is necessary for milk and yoghurt. Hence the objective of this work was to access the microbiological properties of some yoghurts marketed in Enugu metropolis. The data from this study will serve as baseline information for researchers working on yoghurt brands and consolidate the trust and claims on probiotic yoghurt.

MATERIALS AND METHODS

Collection of Raw Materials

Three different yoghurts (72 hours after production) were sourced from yoghurt producers within the Enugu metropolis. The samples from producers were kept at room temperature as at the time of collection before they were sent to the Lab for analysis. The fourth sample was prepared in Food Science and Technology (FST) Laboratory, Ebonyi State University, Abakaliki. The four samples were represented with A, B, C and D where sample A is FST laboratory yoghurt, sample B is Aqua Rapha yoghurt, sample C is Chariot yoghurt and sample D is A.S yoghurt sample

Sample Storage

Forty eight (48) 50 cL pet containers of twelve containers for each yoghurt sample were used. The samples were divided into two batches of twenty_four (24) samples each. One batch was refrigerated at a temperature of 5^0C while the other batch was kept in an open shelve in a room at a temperature range of 28 ± 2^0C respectively. The samples were all stored for seventy days.

Microbial Analysis

The method described by Madigan *et al.* (2017) was used.

Media Preparation

Exactly 5.0 grams of nutrient agar was dissolved in 1000 ml of distilled water. This was homogenized by bringing it to boil and sterilized at 121°C for 15 minutes using autoclaved. pH was adjusted between 7.2-7.6. It was allowed to cool to about 45°C and poured onto sterilized Petri dishes and allowed to solidify and left for 8 hours to confirm sterility.

Sample Treatment

One gramme of yoghurt sample was weighed and added to 10 ml of sterilized distilled water and was labelled as 10^{-1} dilution. The mixture was gently mixed by inverting the test tubes several times. Prepared serial dilutions of yoghurt samples were prepared by transferring 1 ml from the first dilution to 9 ml of sterile distilled water and mixed well. This procedure was repeated up to the 7th test tube with respective dilutions 10^{-3}, 10^{-4} 10^{-}graduate^6 10^{-7} using different sterile pipettes. Diluted sample (0.1 ml) from a particular dilution was pipetted and was spread uniformly using a sterilized L-rod. The plates were kept in an upright position for few minutes. They were incubated in an inverted position at 37°C for 24 hours.

Total Plate Count

The enumeration of the total viable count, coliform, lactic acid bacterial and fungal/yeast count was performed using International Dairy Federation (IDF standard 306). The number of colonies formed in each plate was counted for the different yoghurt samples. Plates with fewer than 30 colonies were designated as "too few to count". Plates with more than 300 colonies as "too numerous to count".

Statistical Analysis

All analysis were carried in triplicate and data reported as mean ± standard deviation (SD). Data were subjected to two –way analysis of variance (ANOVA)

RESULTS AND DISCUSSION

Total Viable Count of Stored Yoghurt Samples.

The results of the total viable count of test samples during refrigeration and room temperature storage are presented in Table 1. Results showed that as storage under refrigeration progressed, the total viable count for the different samples varied. At the end, all the refrigerated samples had total viable count higher than the levels in the original samples and their total viable count levels significantly differed ($p>0.05$). The same effect was observed in the room temperature stored samples. Statistical analysis showed that the main effect of storage condition, time and sample on total viable count were significant ($p>0.05$). Statistical analysis of the result also showed that the interactive effect of storage condition * sample was not significant whereas interactive effect of storage time*sample and storage condition*storage time on total viable count were significant.

Table1: Total Viable Count of Refrigerated and Room Stored Yoghurt Samples

Storage Time (Days)	Storage condition							
	Refrigeration (4-5°C)				Room (23-24°C)			
	A	B	C	D	A	B	C	D
0	$0.006_{f}{}^{b}\pm0.03$	$0.007_{e}{}^{a}\pm0.00$	$0.006_{e}{}^{b}\pm0.00$	$0.006_{f}{}^{b}\pm0.00$	$0.006_{f}{}^{b}\pm0.00$	$0.007_{d}{}^{a}\pm0.00$	$0.006_{f}{}^{b}\pm0.00$	$0.007_{f}{}^{a}\pm0.00$
14	$0.007_{c}{}^{f}\pm0.00$	$0.045_{d}{}^{b}\pm0.03$	$0.063_{a}{}^{a}\pm0.00$	$0.007_{e}{}^{f}\pm0.00$	$0.038_{e}{}^{d}\pm0.00$	$0.001_{e}{}^{g}\pm0.00$	$0.022_{e}{}^{e}\pm0.00$	$0.040_{e}{}^{c}\pm0.00$
28	$0.008_{d}{}^{g}\pm0.00$	$0.006_{f}{}^{h}\pm0.00$	$0.046_{d}{}^{f}\pm0.03$	$0.120_{a}{}^{e}\pm0.00$	$0.535_{b}{}^{b}\pm0.46$	$0.427_{a}{}^{c}\pm0.00$	$0.603_{b}{}^{a}\pm0.03$	$0.141_{b}{}^{d}\pm0.00$
42	$0.078_{b}{}^{e}\pm0.00$	$0.064_{c}{}^{g}\pm0.00$	$0.047_{c}{}^{h}\pm0.03$	$0.070_{c}{}^{f}\pm0.00$	$0.752_{a}{}^{a}\pm0.00$	$0.417_{b}{}^{c}\pm0.00$	$0.650_{a}{}^{b}\pm0.00$	$0.101_{c}{}^{d}\pm0.00$
56	$0.080_{a}{}^{b}\pm0.00$	$0.070_{a}{}^{e}\pm0.00$	$0.048_{b}{}^{g}\pm0.04$	$0.075_{b}{}^{d}\pm0.00$	$0.077_{c}{}^{c}\pm0.00$	$0.050_{c}{}^{f}\pm0.01$	$0.070_{c}{}^{e}\pm0.01$	$0.607_{a}{}^{a}\pm0.35$
70	$0.076_{c}{}^{a}\pm0.03$	$0.068_{b}{}^{d}\pm0.00$	$0.047_{c}{}^{f}\pm0.03$	$0.042_{d}{}^{g}\pm0.03$	$0.070_{d}{}^{c}\pm0.00$	$0.050_{c}{}^{e}\pm0.01$	$0.050_{d}{}^{e}\pm0.01$	$0.072_{d}{}^{b}\pm0.00$

Values are means ± SD of triplicate analysis. Values with the same superscript and subscript within the same row and column are not significantly different. ($p>0.05$). Where A=FST lab yoghurt sample, B=Aqua Rapha yoghurt sample, C=Chariot yoghurt sample, D =AS yoghurt sample.

FAO/WHO (2002) defined yoghurt as "the coagulated milk product with a minimum of 10^7cfu/g of microorganisms. The results on total viable count obtained showed that storage condition and time affected the total viable count of yoghurt samples significantly. The range in total viable count of 0.11 cfu/ml-0.14 cfu/ml obtained in this work agreed with the result 0.83 cfu-0.85 cfu/ml of Ekram *et al*. (2011). The result on total viable count showed that all samples experienced an initial increase in total viable count as storage time progressed. Sample A stored at room temperature day 42 had the highest total viable count while samples B and D at day 0 had the least. Results showed that the effect of storage condition on total viable count depended on the storage time and whether the samples are refrigerated or stored at room temperature. The total viable count for refrigerated samples showed a peak on day 14, a sharp decrease on 28 days and levels off subsequently until the 70th day of storage. The samples stored at room temperature on the other hand exhibited sharp increase on total viable count until day 42 after which total viable count began to drop until day 70. At the end, the samples stored at room temperature maintained a higher total viable counts than the refrigerated samples. The observed rapid increase in total viable count in the samples stored at room temperature could be attributed to the optimum bacteria growth temperature provided by the room storage which gave rise to the rapid multiplication of microorganisms in the samples. The interactive effect of storage time * sample on total viable count showed no clear trend as different samples exhibited peaks in total viable count at different days of storage. These differences could be due to the variations in the chemical compositions (protein, carbohydrate and total solid) in the respective milk used for formulation of the different samples as the quantities of these nutrients available in the milk helps to grow and increase the total viable counts in the samples. The viability of the respective lactic acid bacteria used in culturing the milk may have also contributed to the values of the total viable count of the respective samples.

Coliform Count of yoghurt samples

The coliform count of test samples during refrigeration and room temperature storage are presented in Table 2. The initial coliform count of the samples was significantly different ($p>0.05$). Results showed that as storage under refrigeration progressed, the coliform count of the different samples varied. At the end of the 70 days storage, all the refrigerated samples had coliform count higher than the level in the original samples and their coliform count were significantly different. The same effect was observed in the room stored samples. Statistical analysis of the results showed that the main effects of storage condition and storage time

on coliform count were significant while source of sample has no significant effect on coliform count. Statistical analysis also showed that the interactive effects of storage condition * sample, sample * storage time and storage condition *storage time were significant.

Table2: Coliform Count of Refrigerated and Room Stored Yoghurt Samples

Storage Time (Days)	**Storage condition**							
	Refrigeration (4-5ºC)				Room (23-24ºC)			
	A	B	C	D	A	B	C	D
0	$0.001_{f}{}^{b}$±0.00	0.001_{f} b±0.01	$0.002_{d}{}^{a}$±0.00	$0.0010_{f}{}^{b}$±0.00	$0.001_{f}{}^{b}$±0.00	$0.001_{f}{}^{b}$±0.00	$0.002_{f}{}^{a}$±0.00	$0.0010_{f}{}^{b}$±0.00
14	$0.002_{e}{}^{d}$±0.00	$0.0030_{e}{}^{c}$±0.01	$0.0030_{c}{}^{c}$±0.00	$0.003_{e}{}^{c}$±0.00	$0.003_{e}{}^{c}$±0.00	$0.005_{e}{}^{a}$±0.00	$0.004_{e}{}^{b}$±0.00	$0.0054_{e}{}^{a}$±0.00
28	$0.010_{d}{}^{b}$±0.00	$0.0050_{d}{}^{e}$±0.01	$0.0030_{c}{}^{g}$±0.00	$0.0040_{d}{}^{f}$±0.01	$0.038_{d}{}^{a}$±0.00	$0.007_{d}{}^{c}$±0.00	$0.006_{d}{}^{d}$±0.00	$0.0070_{c}{}^{c}$±0.00
42	$0.040_{c}{}^{b}$±0.01	$0.007_{c}{}^{e}$±0.10	$0.0060_{b}{}^{f}$±0.01	$0.0050_{c}{}^{g}$±0.00	$0.054_{c}{}^{a}$±0.00	$0.010_{c}{}^{c}$±0.00	$0.009_{c}{}^{d}$±0.00	$0.009_{d}{}^{d}$±0.00
56	$0.050_{b}{}^{b}$±0.00	$0.009_{b}{}^{e}$±0.00	$0.007_{a}{}^{f}$±0.00	$0.0060_{b}{}^{g}$±0.00	$0.074_{b}{}^{a}$±0.00	$0.020_{b}{}^{c}$±0.00	$0.015_{b}{}^{d}$±0.00	$0.015_{b}{}^{d}$±0.00
70	$0.061_{a}{}^{b}$±0.00	$0.010_{a}{}^{f}$±0.00	$0.007_{a}{}^{h}$±0.00	$0.009_{a}{}^{g}$±0.00	$0.080_{a}{}^{a}$±0.00	$0.033_{a}{}^{d}$±0.00	$0.022_{a}{}^{e}$±0.00	$0.046_{a}{}^{c}$±0.00

Values are means ± SD of triplicate analysis. Values with the same superscript and subscript within the same row and column are not significantly different. (p>0.05). Where A=FST lab yoghurt sample, B=Aqua Rapha yoghurt sample, C=Chariot yoghurt sample, D =AS yoghurt sample.

The results on coliform count obtained in this study showed that storage conditions and time affected the coliform count of yoghurt samples and the effects were significant. The range on coliform count of 0.02 cfu/ml-0.03 cfu/ml csobtained in this work slighly agreed with the result of Ekram *et al*. (2011) that recorded coliform count of 4.99 and 5.15 log 10. Result differed with the range $74.12x10^{3}$cfu/ml-$19.0x10^{5}$cfu/ml recorded by Ashraf *et al*. (2011) in coliform count. Tamine and Robinson (1999) reported that the total coliform count decrease during the storage period due to the inhibitory effect of increased lactic acid production and that the presence of coliforms in the sample indicates post pasteurization contamination stages during processing. Sample A refrigerated and D room stored had the highest Coliform count of 0.14cfu/ml at days 42 and day 28 respectively. The study of the interactive effect of storage condition and storage time on coliform count showed the coliform count increased with storage time under the two storage conditions but the increase was more pronounced in room stored samples than refrigerated samples. Similarly, study of the interactive effect of storage condition and sample on coliform count showed that the refrigerated samples generally had lower coliform count than the corresponding room stored samples.These suggests that the room temperature storage provided optimum conditions for the proliferation of coliforms in the samples.The interactive effect of storage time and sample on coliform count studied revealed that the increase was most pronounced in sample A . The reason for the high coliform count of sample A could be due to poor hygiene during processing or poor microbial level of the water used during production although all the samples exhibited increase coliform count with increase in storage time

Lactic Acid Content of Samples

The lactic acid content of test samples during refrigeration and room storage are presented in Table 3. The initial lactic acid values of the samples were all significantly different. Results showed that as storage progressed under refrigeration, the lactic acid for the different samples varied. At the end of storage, most of all the refrigerated samples had lactic acid values higher than the level in the original sample and their lactic acid levels significantly differed from each other. Results showed that as storage progressed in room storage the lactic acid values varied. After 70 days storage period, all the room stored sample compaired with the refrigerated had higher lactic acid values than their initial lactic acid values and were all significantly different . Statistical results showed that the main effects of storage condition, time and sample source on lactic acid levels were significant. Statistical analysis of the result also showed that the interactive effect of storage condition*sample, storage time*sample and storage condition*storage time on lactic acid content were significant.

Table 3: Lactic Acid mmol/L of Refrigerated and Room Stored Yoghurt Samples

Storage Time (Days)	Storage condition							
	Refrigeration (4-5ºC)				Room (23-24ºC)			
	A	B	C	D	A	B	C	D
0	$0.037_{c}{}^{a}\pm0.00$	$0.003_{b}{}^{c}\pm0.00$	$0.030_{d}{}^{b}\pm0.00$	$0.030_{e}{}^{b}\pm0.00$	$0.037_{e}{}^{a}\pm0.00$	$0.003_{e}{}^{c}\pm0.00$	$0.030_{c}{}^{b}\pm0.00$	$0.030_{f}{}^{b}\pm0.00$
14	$0.036_{d}{}^{d}\pm0.00$	$0.003_{b}{}^{h}\pm0.00$	$0.030_{d}{}^{f}\pm0.00$	$0.032_{d}{}^{e}\pm0.00$	$0.069_{d}{}^{a}\pm0.00$	$0.006_{d}{}^{g}\pm0.00$	$0.06_{d}{}^{c}\pm0.00$	$0.068_{e}{}^{b}\pm0.00$
28	$0.038_{b}{}^{e}\pm0.00$	$0.003_{b}{}^{h}\pm0.00$	$0.034_{b}{}^{g}\pm0.01$	$0.037_{c}{}^{f}\pm0.00$	$0.365_{a}{}^{a}\pm0.00$	$0.151_{b}{}^{d}\pm0.00$	$0.250_{a}{}^{b}\pm0.01$	$0.198_{b}{}^{c}\pm0.01$
42	$0.039_{a}{}^{e}\pm0.00$	$0.003_{b}{}^{h}\pm0.00$	$0.031_{c}{}^{g}\pm0.00$	$0.038_{b}{}^{f}\pm0.00$	$0.300_{c}{}^{c}\pm0.01$	$0.150_{c}{}^{d}\pm0.01$	$0.250_{a}{}^{a}\pm0.01$	$0.200_{a}{}^{b}\pm0.10$
56	$0.004_{e}{}^{g}\pm0.00$	$0.003_{b}{}^{h}\pm0.00$	$0.035_{a}{}^{f}\pm0.00$	$0.038_{b}{}^{e}\pm0.00$	$0.350_{b}{}^{a}\pm0.01$	$0.170_{a}{}^{d}\pm0.01$	$0.200_{b}{}^{b}\pm0.10$	$0.180_{c}{}^{c}\pm0.01$
70	$0.036_{d}{}^{e}\pm0.00$	$0.035_{a}{}^{f}\pm0.00$	$0.030_{d}{}^{g}\pm0.00$	$0.144_{a}{}^{d}\pm0.19$	$0.350_{b}{}^{a}\pm0.01$	$0.170_{a}{}^{b}\pm0.00$	$0.030_{c}{}^{g}\pm0.01$	$0.150_{d}{}^{c}\pm0.01$

Values are means ± SD of triplicate analysis. Values with the same superscript and subscript within the same row and column are not significantly different. (p>0.05).Where A=FST lab yoghurt sample, B=Aqua Rapha yoghurt sample, C=Chariot yoghurt samples D =AS yoghurt sample.

The results on lactic acid obtained in this study showed that storage conditions and time affected the lactic acid content of yoghurt samples significantly. The range in lactic acid 0.003-0.365 mmol/L obtained in this work is lower compared with the result of Mohammed and El Zubeir (2007) that recorded $7.21x10^{1}$-7.5x10 mmol/L in lactic acid. The result in the lactic acid of this work is also comparable with the range of 0.823-0.770 mmol./L recorded by Mortazavian *et al*. (2011) in lactic acid. National Yoghurt Association defined active culture as "final product that contains live lactic acid bacteria in amount $>10^{8}$cells/g at the end of manufacture" (Mazahreh and Ershidat, 2009). Viability of yoghurt and probiotic bacteria assessed in yoghurt made from different commercial starter cultures were dependent on the species and strains of associative yoghurt organisms. The storage temperature of yoghurt had effects on the viability of *bifido* bacteria but not on *l.acidophilus*. The results obtained in this study showed that all samples experienced an increase in lactic acid as storage time progressed. Sample A had the highest lactic acid content of 0.35mmol/L and at day 70 while sample B had the least lactic acid content of 0.003mmol/L at day 0.

The interactive effect of storage condition and time on lactic acid showed that though lactic acid increased in the test yoghurts with time, the increase was more elaborate in the room stored samples than in refrigerated sample. The interactive effect of storage condition and sample on lactic acid also showed that the refrigerated samples contained lower lactic acid values than their corresponding room counterparts. This observation could be attributed to the fact that refrigeration help to inhibit the growth of lactic acid producing bacteria during storage while room temperature provide optimum condition for microbial growth. The interactive effect of storage time and sample on lactic acid content showed that all the sample increased in their lactic acid content during storage especially on the 14th and 28th after which they showed a decline. Sample A however showed the highest rise in lactic acid content. The reason for the high lactic acid content of sample A could be due to its high total solid content. It could also be due to the nature and activity of the lactic acid bacteria used in production of the sample.

Fungal/Yeast Count of Stored Yoghurt Samples

The fungal/yeast count of test samples during refrigeration and room storage is presented in Table 4. Results showed that as storage under refrigeration progressed the fungal/yeast counts varied. At the end of the 70 days storage, most of the refrigerated samples had fungal/yeast count higher than the level in the original sample and their fungal/yeast counts were significantly different. The same effect was observed under room storage. Statistical analysis showed that the main effect of storage conditions, time and sample on fungal/yeast count were significant. Statistical analysis of the result also showed that the interactive effect of storage condition* sample, storage time*sample and storage condition * time on fungal/yeast content were significant.

Table 4: Fungal/Yeast count cfu/mlof Refrigerated and Room Stored Yoghurt Samples

Storage Time (Days)	Storage condition							
	Refrigeration (4-5ºC)				Room (23-24ºC)			
	A	B	C	D	A	B	C	D
0	$0.012_{e}{}^{b}\pm0.00$	$0.001_{d}{}^{c}\pm0.00$	$0.001_{e}{}^{c}\pm0.00$	$0.026_{b}{}^{a}\pm0.00$	$0.012_{e}{}^{b}\pm0.00$	$0.001_{f}{}^{c}\pm0.00$	$0.001_{f}{}^{c}\pm0.00$	$0.026_{e}{}^{a}\pm0.0$
14	$0.016_{d}{}^{d}\pm0.00$	$0.003_{c}{}^{f}\pm0.00$	$0.002_{d}{}^{e}\pm0.00$	$0.029_{a}{}^{b}\pm0.00$	$0.021_{a}{}^{c}\pm0.00$	$0.002_{f}{}^{e}\pm0.00$	$0.002_{e}{}^{e}\pm0.00$	$0.036_{d}{}^{a}\pm0.0$
28	$0.012_{e}{}^{c}\pm0.00$	$0.004_{b}{}^{e}\pm0.00$	$0.003_{c}{}^{f}\pm0.00$	$0.019_{c}{}^{b}\pm0.00$	$0.104_{b}{}^{a}\pm0.00$	$0.003_{f}{}^{f}\pm0.00$	$0.007_{d}{}^{g}\pm0.00$	$0.011_{f}{}^{d}\pm0.0$
42	$0.019_{b}{}^{e}\pm0.00$	$0.003_{c}{}^{g}\pm0.00$	$0.004_{b}{}^{f}\pm0.00$	$0.019_{c}{}^{e}\pm0.00$	$0.104_{b}{}^{b}\pm0.00$	$0.048_{c}{}^{d}\pm0.00$	$0.062_{c}{}^{c}\pm0.00$	$0.112_{a}{}^{a}\pm0.0$
56	$0.018_{c}{}^{e}\pm0.00$	$0.003_{c}{}^{g}\pm0.00$	$0.005_{a}{}^{f}\pm0.00$	$0.019_{c}{}^{d}\pm0.00$	$0.102_{c}{}^{a}\pm0.00$	$0.049_{b}{}^{c}\pm0.00$	$0.067_{a}{}^{b}\pm0.00$	$0.067_{b}{}^{b}\pm0.0$
70	$0.020_{a}{}^{d}\pm0.00$	$0.030_{a}{}^{e}\pm0.00$	$0.004_{b}{}^{g}\pm0.00$	$0.018_{d}{}^{f}\pm0.00$	$0.105_{a}{}^{a}\pm0.01$	$0.050_{a}{}^{c}\pm0.01$	$0.065_{b}{}^{b}\pm0.00$	$0.065_{c}{}^{b}\pm0.0$

Values are means ± SD of triplicate analysis. Values with the same superscript and subscript within the same row and column are not significantly different. (p>0.05). Where A=FST lab yoghurt sample, B=Aqua Rapha yoghurt sample, C=Chariot yoghurt sample, D =AS yoghurt sample.

The results on fungal/yeast count showed that storage conditions and time affected the fungal/yeast count of yoghurt samples and the effects were significant. The range of fungal/yeast analysis of 0.00 cfu/ml-0.11cfu/ml obtained in this work is lower, when compared to the result of Suriyarachichi and Fleet (1981). However, the result of this work is also lower than the range 0.465 cfu/ml-0.627 cfu/ml obtained by Ekram and El Zubeir (2011). The decrease in fungal/ yeast count of samples could be due to level of hygiene observed during processing and storage condition. It might also be due to the microbial quality of water. El Bakri and El Zubeir (2009) reported that the increase of yeast/fungal count in fermented dairy products might be due to insufficient hygiene practices during processing. Montagna *et al*. (1998) reported that fungi in commercial yoghurt generally correspond to poor cleaning practices and the use of unhygienic techniques or inadequate storage conditions. They added that fungal contamination could occur during transportation and sales. They also suggested that the tolerable limit of mould and yeast in yoghurt should be equal to or less than 50 cfu/ml yeast in milk produced. Yeast in milk produced abundant gas, limited acidity and appreciable units of ethanol (Dave, 1998). Contamination of yoghurt with the yeast resulted in gassy alcoholic fermentation and a fruity odour with eventual spoilage of the product. The spoilage of yoghurt by yeast is recognized by the development of yeasty off flavours, loss of texture quality due to gas production and swelling and eventual blowingoff of the product container. International Dairy Federation(1990) shows that yoghurt should not contain more than one yeast cell/g of a product if produced under good GMP and if the product is correctly stored under refrigeration (5^{0}C). The result of this study showed that some samples experienced a change in fungal/yeast count as storage time progressed. Sample A and C had the highest and least fungal/yeast count during storage.

The results showed that the interactive effect of storage condition * time on fungal/yeast count showed the samples experienced increase in fungal /yeast count as the storage time increased but the magnitude of increase depended on whether the samples are refrigerated or room stored. The room samples maintained higher fungal/yeast count throughout storage period unlike the refrigerated samples that had lower fungal/yeast count as storage time progressed. The interactive effect of storage condition *sample on fungal/yeast count studied also showed that the room stored samples generally had significantly higher fugal/yeast count than the corresponding refrigerated sample. The interactive effects of storage time * sample on fungal/yeast count studied show no clear trend as different samples exhibited different positive and negatives peaks at different times during storage which is attributable to different level of hygiene deployed during their manufacture and handling .

CONCLUSION

The study establishes that room storage of yoghurt leads to rapid deterioration and proliferation of the inherent microorganism in the yoghurt which result in the decrease in chemical and sensory qualities of the product with storage time. Thus extensive storage of yogurt at ambient conditions may render the product unsafe for consumption

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