

Effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* on the quality of yogurt

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Abstract. Siddiq HAM, Hamid OIA. 2017. Effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* on the quality of yogurt. *Bioteknologi* 14: 25-31. The aim of this study was to evaluate the effect of adding different levels of culture starter and storage period to conventional yogurt culture. Eight liters of fresh cow milk were purchased from a dairy farm located at the College of Animal Production Science and Technology, Khartoum Sudan. The milk was pasteurized at 90°C for 30 minutes then cooled to 45°C. The pasteurized milk was then divided into four equal portions. Four treatments were carried out. The first treatment was the control sample, namely yogurt which was from conventional yogurt culture starter. In the second, third and fourth treatments, respectively, 50%, 75% and 100% of culture starter adjunct (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) were added. The inoculated milk was incubated in all treatments at 43 °C for three hours. After complete coagulation, the yogurt samples were cooled by refrigeration with a temperature of 4°C. Chemical composition and sensory evaluation were carried out on the yogurt samples in all treatments at intervals of 1, 5 and 10 days. The results indicated that significant variations ($P<0.05$) were found in the fat %, acidity % and protein % while total solids and the ash % showed no significant difference ($P>0.05$) due to the culture starter adjunct addition. However, the storage period had significant difference ($P<0.05$) on the fat, protein, total solids, and acidity while the ash content was not affected by storage period. The sensory characteristics of the yogurt samples were found to be not affected significantly ($P>0.05$) by the storage period, except the texture and overall acceptability. The addition of starter cultures had significant variations on the color, flavor, texture, taste, and overall acceptability. The results revealed that the treatments with 50% and 75% showed higher sensory scores than others.

Keywords: *Lactobacillus acidophilus*, *Lactobacillus plantarum*, yogurt

INTRODUCTION

Around the world, cow milk is used to produce fermented milk including yogurt. In the Indian subcontinent, buffalo milk and blends of buffalo and cow milk are used widely for Dahi (a type of fermented milk) made by the help of mixed mesophilic cultures (Aneja et al. 2002). Buffalo milk is the base for making yogurt using thermophilic cultures in several Asian countries, whereas the milk of sheep, goats, and camels are used for fermentation in several Middle Eastern countries. In modern times, yogurt is a significant dairy product worldwide. It is a semi-solid fermented product made from heat-treated standardized milk, mixed by the activity of a symbiotic blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (Clark and Plotka 2004; Ozer 2010).

In certain countries, the nomenclature “yogurt” is restricted to the product made exclusively from the two lactic cultures. Whereas in other countries, it is possible to label a product as “yogurt” if it is made with yogurt cultures and adjunct probiotic cultures. The more common cultures adjunct is *Lactobacillus acidophilus*, *Bifidobacterium*, *Lactobacillus gasseri* and *Lactobacillus rhamnosus* (Maity and Misra 2009; Chandan and Nauth 2012). Yogurt represents the most popular fermented milk product worldwide and originates from countries around the Balkan and the Eastern Mediterranean Sea (Staff 1998;

Walstra et al. 1999). Yogurt also has medical uses because of the probiotic characteristics, in helping on a variety of gastrointestinal conditions and in preventing antibiotic-associated diarrhea (Lourens-Hattingh and Viljoen 2001; Mazahreh and Ershidat 2009).

The art of making Zabadi (yogurt) came to Sudan from Egypt, most likely during the time of the Anglo-Egyptian rule (1898-1956). It was prepared by households, by the cow milk being boiled, cooled, and inoculated by back-shopping from a previous lot. It was then incubated in a warm carrier, where it sours and is then refrigerated. It was consumed with sugar as a dessert or eaten with wheat bread. Sometimes it is fed to babies and is often turned into sauce for porridge (Chandan 1999).

The objective of the study is to determine: (i) The effect of starter cultures (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) on the organoleptic properties of yogurt. (ii) The effect of storage period on the chemical and sensory evaluation of yogurt.

MATERIALS AND METHODS

This study was conducted during the period of October-November 2014 at the Department of Dairy Sciences and Technology, College of Animal Production Science and Technology, Sudan University of Science and Technology, Khartoum Sudan.

Materials

Eight liters of raw cow milk were purchased from the Dairy Farm, College of Animal Production Science and Technology, Sudan University of Science and Technology, Hillat Kuku. The sterilized containers which were used for the collection of samples, culture starter of yogurt (*S. thermophilus* and *L. bulgaricus*) and culture starter adjunct of *L. acidophilus* and *L. plantarum* (1: 1) were brought from Vitane Pharma GmbH, 82515 Wolfratshausen, Germany.

Adjunct starter culture preparation

One liter of skim milk was sterilized at 85 °C for 30 min and cooled to 40-45 °C then inoculated with the culture starter adjunct at the rate of 2% and incubated at 45°C until coagulation occurred.

The starter culture:

Conventional yogurt and culture starter adjunct were added at the rate of 2% of the milk used for yogurt making.

Yogurt making process

Yogurt was prepared as described by Staff (1998), namely, eight liters of cow raw milk was heated in a water bath at 85 °C for one hour and cooled to 45°C. Then the milk was divided into four portions, the first portion was used as control only with yogurt culture starter. To the 2nd, 3rd, and 4th portions, 50%, 75% and 100% of culture starter adjunct (*L. acidophilus* and *L. plantarum*) was added to each respectively and packed into plastic cups (200 mg capacity) in triplicates for each treatment and then incubated at 43°C for 3 hrs. Samples from different treatments were stored in a refrigerator at 4 °C for 1, 5 and 10 days; chemical and sensory evaluation of the yogurt samples were analyzed for the determined period.

Chemical analysis

Fat content

The fat content was determined by Gerber method according to Bradly et al. (1992). In a clean dry Gerber tube, 10 ml of sulphuric acid (density 1.8 g/ml at 20 °C) was poured. 10.94 ml of milk sample was added with amyl alcohol (1-2 ml) to tube. This was followed by the pouring of distilled water into it. The content was thoroughly mixed until no white particles could be seen. The Gerber tube was centrifuged at 1100 revolution per minute (rpm) for 4-5 min. The fat column was then read immediately.

Protein contents

The protein content was determined by Kjeldahl method according to AOAC (1990). (1) *Digestion*: ten ml of milk were weighed and poured in the flask. Concentrated sulphuric acid (25ml) was added to the flask. The flask was heated until a clear solution was obtained. Then, the flask was removed and allowed to cool. (2) *Distillation*: the digested sample was poured in volumetric flask (100 ml) and diluted to 100ml with distilled water. Five milliliters of it was distilled using 10 ml of 40%

NaOH. The distillate was poured into a conical flask (100ml) containing 25 ml of 2% boric acid plus 3 drops of indicator (bromocresol green + phenolphthalein red). The distillation was continued until the volume in the flask was 75 ml, then the flask was removed from the distillatory. (3) *Titration*: the distillate was titrated with 0.1N HCl until the end point (red color) was obtained. The protein content was calculated by the following equation:

$$\text{Nitrogen \%} = \frac{T \times 0.1 \times 20 \times 0.014 \times 100}{w}$$

$$\text{Protein \%} = \text{N\%} \times 6.38$$

Where:

T= Titration figure

W = Weight of the original sample

0.1 N = Normality of HCL

0.014 = the atomic weight of nitrogen/100

20 = Dilution factor

Total solid (T.S.) content

The total solid content was determined according to the modified method of AOAC (1990). Three grams of sample was weighed into a dry oven flat-bottomed aluminum dish, and heated on steam bath for 10-15 min. The dish was placed in an oven at 105° C overnight, and then cooled in desiccators and weighed quickly. The weighing was repeated until the difference between the two readings was < 0.1mg. The total solid content was calculated by the following equation:

$$\text{T.S\%} = \text{W1/Wo} \times 100$$

Where:

W1 = Weight of sample after drying

Wo = Weight of sample before drying

Ash content

The ash content was determined according to AOAC (1990). Five grams of the sample was weighed and put into a suitable crucible and evaporated to dryness on steam bath. Then it was placed in a muffle furnace with temperature of 55-60° C until ashes were carbon-free (2-3 hrs.), then the crucibles were coded in a desiccator, and the weight of the ash content was calculated with the following equation:

$$\text{Ash\%} = \text{W1/W} \times 100$$

Where:

W1 = Weight of ash

W= weight of sample

Sensory evaluation

Sensory profiling of the yogurt sample was conducted using conventional profiling by untrained panelists according to Larmond (1977). Ten panelists were selected among staff and students at the College of Animal Production Science and Technology, Sudan University of

Science and Technology, Khartoum, Sudan. The panelists were given a hedonic questionnaire (Appendix NO1) to evaluate taste texture, color, and flavor and overall acceptability of coded samples of cow milk yogurt which were stored for different period (1, 5, 10 days). They were scored on a scale of 1-7 (1=not acceptable, 7= acceptable). Each attribute was evaluated in triplicate, and the values were then averaged.

Statistical analysis

Statistical analysis was carried out with SPSS (2008) version 17. General linear model was used for data analysis (Factorial design) to test the effect of culture starter adjunct addition and storage period on the quality of yogurt. Least significant difference (LSD) was used for mean separation between the treatments. Alpha level 5% was used in this study.

RESULTS AND DISCUSSION

Effect of different levels of adjunct starter culture on the chemical characteristics of yogurt

Data in Table 1 showed the effect of culture starter adjunct on the physicochemical composition of set yogurt. The results indicated that the culture starter adjunct had significant ($P < 0.01$) effect on the fat content of set yogurt (Table 1). The highest fat content ($3.56 \pm 0.06\%$) was for the control yogurt, while the lowest value ($3.28 \pm 0.06\%$) was for the one with 75% culture starter adjunct. The titratable acidity of the yogurt samples showed significant ($P < 0.05$) variations due to the different level of culture starter adjunct (Table 1). As the level of culture starter adjunct increased, the titratable acidity decreased. However, the lowest acidity ($0.94 \pm 0.02\%$) was for the yogurt with 75% culture starter adjunct.

Statistical analysis revealed that culture starter adjunct had no significant ($P > 0.05$) effect on the total solids and ash contents of the set yogurt (Table 1). The results of the study demonstrated that (Table 1) addition of the culture starter adjunct had significant effect on the crude protein content. The highest protein content ($5.52 \pm 0.05\%$) was for the yogurt with 50% each conventional and culture starter adjunct, whereas the lower one was for the yogurt with 75% adjunct starter culture.

Effect of storage period on the chemical composition of set yogurt:

Data in Table 2 shows the effect of storage period on the chemical composition of the set yogurt. The results indicated that the storage period had significant ($p < 0.05$) effect on the fat content of set yogurt (Table 2). The highest fat content ($3.62 \pm 0.05\%$) was on day 1. The lowest value ($3.28 \pm 0.05\%$) was at day 5. The titratable acidity of the yogurt samples showed significant ($p < 0.05$) variations due to the storage period (Table 2). As the storage period progressed, the titratable acidity increased. However, the highest acidity ($1.21 \pm 0.02\%$) was at day 10.

The results of the study demonstrated that (Table 2) the storage period had significant effect on total solid. The

highest total solid ($12.52 \pm 0.04\%$) was at day 5. The lowest value ($12.30 \pm 0.04\%$) was at day 10. Statistical analysis revealed that storage period had no significant ($p > 0.05$) effect on ash content of the set yogurt (Table 2). The results indicated that the storage period had significant ($p < 0.05$) effect on the crude protein content. The highest protein content ($5.97 \pm 0.05\%$) was on day 1. The lowest value ($4.96 \pm 0.05\%$) was at day 5.

Effect of storage period and levels of culture starter adjunct on chemical composition of yogurt

The results indicated that the storage period and different level of culture starter adjunct had significant ($p < 0.05$) effect on the fat content of set yogurt (Table 3). The highest fat content ($3.9 \pm 0.15\%$) was for the control yogurt on day 1. The lowest value ($2.8 \pm 0.15\%$) was for the one with 75% culture starter adjunct on day 5. The results of the study showed that (Table 4) the addition of the culture starter adjunct and the storage period had significant ($p < 0.05$) effect on the titratable acidity of the yogurt samples. The highest titratable acidity ($1.4 \pm 0.03\%$) was for the one with 50% culture starter adjunct on day 10. The lowest value ($0.56 \pm 0.05\%$) was for the one with 75% culture starter adjunct at day 1.

The results revealed that the storage period and different level of culture starter adjunct had significant ($p < 0.05$) effect on the total solid of yogurt samples (Table 5). The highest total solids content ($12.9 \pm 0.05\%$) was for the one with 100% culture starter adjunct at day 5, the lowest value ($12.2 \pm 0.20\%$) was for the one with 50% culture starter adjunct at day 10. The storage period and different level of culture starter adjunct had significant ($p < 0.05$) effect on the ash of yogurt (Table 6). The highest ash content ($0.81 \pm 0.02\%$) was for the one with 75% culture starter adjunct on day 5, the lowest value ($0.73 \pm 0.15\%$) was for the control at day 5.

The storage period and different level of culture starter adjunct had significant ($p < 0.05$) effect on the crude protein content of set yogurt (Table 7). The highest protein content ($6.0 \pm 0.08\%$) was for the one with 50% culture starter adjunct on 10 days, the lowest value ($4.0 \pm 0.04\%$) was for the one with 75% culture starter adjunct at day 5.

Effect of culture starter adjunct on the sensory characteristics of yogurt

The addition of culture starter adjunct had significant ($p < 0.01$) effect on the color of yogurt (Table 8), the highest value (8.07 ± 0.34) was for the control yogurt while the lowest one (6.80 ± 0.34) was for the one with 100% culture starter adjunct. The results showed that culture starter adjunct had significant ($p < 0.05$) effect on the flavor of yogurt (Table 8). The highest scores (7.20 ± 0.28) was for the one with 75% culture starter adjunct. However, the lowest value (5.60 ± 0.28) was for the one with 100% culture starter adjunct. The texture and taste of the yogurt samples were found to be affected significantly ($p < 0.05$) by the culture starter adjunct addition. The highest texture value (7.33 ± 0.32) was for the one with 50% culture starter adjunct, and the lowest value ($5.07 \pm 0.22\%$) was for the one with 100% culture starter adjunct. The highest taste scores

(6.87 ± 0.41) was for the control yogurt and the one with 50% culture starter adjunct, the lowest value (4.87 ± 0.41) was for the yogurt with 100% culture starter adjunct.

Overall acceptability of yogurt samples (Table 8) was significantly ($p < 0.05$) affected by the culture starter adjunct addition, the highest score (7.53 ± 0.25) was the one with control yogurt. The lowest value (5.93 ± 0.25) was for the one with 100% culture starter adjunct.

Effect of storage period on the sensory characteristics of yogurt

The data in Table 9 showed that no significant ($P < 0.05$) variations were observed in the color, flavor, and taste of the yogurt samples. However, significant differences ($P < 0.05$) were in the texture and overall acceptability of the yogurt samples. The color of the yogurt samples did not change during the storage period, the highest color scores (7.95 ± 1.43) were at day 5 and the lowest value was at day 1.

Table 1. Effect of different level of starter culture on chemical characteristics of set yogurt.

Treatments	Chemical composition (%)				
	Fat	Acidity	T.S	Ash	Protein
A	$3.56 \pm 0.06a$	$1.20 \pm 0.02b$	12.38 ± 0.05	0.75 ± 0.02	$5.33 \pm 0.5b$
B	$3.51 \pm 0.06b$	$1.23 \pm 0.02a$	12.40 ± 0.05	0.80 ± 0.02	$5.52 \pm 0.05a$
C	$3.28 \pm 0.06d$	$0.94 \pm 0.02d$	12.35 ± 0.05	0.80 ± 0.02	$4.48 \pm 0.05d$
D	$3.43 \pm 0.06c$	$0.99 \pm 0.02c$	12.51 ± 0.05	0.80 ± 0.02	$5.03 \pm 0.06c$
Sig	*	**	NS	NS	**

Note: Means with different superscript in the same column are significantly ($p < 0.05$) different. A = control yogurt with conventional culture starter, B = yogurt with 50% conventional culture starter and 50% culture starter adjunct, C = yogurt with 75% culture starter adjunct and 25% conventional culture starter, D = yogurt with 100% culture starter adjunct.

Table 2. Effect of the storage period on the chemical characteristics of set yogurt.

Storage/days	Chemical composition (%)				
	Fat	Acidity	T.S	Ash	C.P
Day 1	$3.62 \pm 0.05a$	$0.97 \pm 0.02b$	$12.40 \pm 0.04b$	0.79 ± 0.02	$5.97 \pm 0.05a$
Day 5	$3.28 \pm 0.05c$	$1.09 \pm 0.02c$	$12.52 \pm 0.04a$	0.79 ± 0.02	$4.84 \pm 0.05c$
Day 10	$3.43 \pm 0.05b$	$1.21 \pm 0.02a$	$12.30 \pm 0.04b$	0.79 ± 0.02	$4.96 \pm 0.05b$
Sig	**	**	**	NS	**

Note: Means with different superscript in the same column are significantly ($p < 0.05$) different.

Table 3. Effect of the storage period and different level of culture starter adjunct on the fat content (%) of yogurt.

Treatment	Storage period			Sig
	day1	Day5	Day 10	
A	3.9 ± 0.15	3.5 ± 0.10	3.3 ± 0.00	
B	3.7 ± 0.17	3.3 ± 0.06	3.6 ± 0.30	
C	3.3 ± 0.26	2.8 ± 0.15	3.7 ± 0.30	
D	3.6 ± 0.00	3.5 ± 0.11	3.1 ± 0.7	**
Sig	**			

Note: A = control yogurt with conventional culture starter, B = yogurt with 50% conventional culture starter and 50% culture starter adjunct, C = yogurt with 75% culture starter adjunct and 25% conventional culture starter, D = yogurt with 100% culture starter adjunct.

Table 4. Effect of storage period and different levels of culture starter adjunct on the acidity (%) of yogurt.

Treatment	Storage period			Sig
	Day 1	Day 5	Day 10	
A	1.1 ± 0.05	1.2 ± 0.05	1.3 ± 0.01	
B	1.1 ± 0.00	1.1 ± 0.01	1.4 ± 0.03	
C	0.56 ± 0.05	1.1 ± 0.02	1.1 ± 0.18	
D	1.1 ± 0.04	0.86 ± 0.01	1.0 ± 0.01	**
Sig	**			

Note: A = control yogurt with conventional culture starter, B = yogurt with 50% conventional culture starter and 50% culture starter adjunct, C = yogurt with 75% culture starter adjunct and 25% conventional culture starter, D = yogurt with 100% culture starter adjunct.

Table 5. Effect of storage period and different level of culture starter adjunct on total solid of yogurt.

Treatments	Storage period			Sig
	day 1	day 5	day 10	
A	12.4± 0.05	12.4± 0.05	12.3± 0.21	
B	12.5±0.25	12.6±0.15	12.2±0.20	
C	12.5±0.08	12.3±0.05	12.4±0.05	
D	12.4±0.15	12.9±0.05	12.3±0.03	**
Sig	**			

Note: A = control yogurt with conventional culture starter, B = yogurt with 50% conventional culture starter and 50% culture starter adjunct, C = yogurt with 75% culture starter adjunct and 25% conventional culture starter, D = yogurt with 100% culture starter adjunct.

Table 6. Effect of storage period and different level of culture starter adjunct on ash content (%) of set yogurt.

Treatment	Storage period			Sig
	Day 1	Day 5	Day 10	
A	0.75± 0.05	0.73± 0.15	0.77± 0.05	
B	0.80±0.00	0.80±0.00	0.80±0.00	
C	0.80±0.00	0.81±0.02	0.80±0.00	
D	0.80±0.00	0.80±0.02	0.80±0.00	**
Sig	**			

Note: A = control yogurt with conventional culture starter, B = yogurt with 50% conventional culture starter and 50% culture starter adjunct, C = yogurt with 75% culture starter adjunct and 25% conventional culture starter, D = yogurt with 100% culture starter adjunct.

Table 7. Effect of the storage period and different level of culture starter adjunct on protein contents of set yogurt.

Treatments	Storage period			Sig
	Day 1	Day 5	Day 10	
A	5.6± 0.07	4.9± 0.49	5.5± 0.04	
B	5.1±0.07	5.4±0.07	6.0±0.08	**
C	5.2±0.07	4.0±0.04	4.2±0.07	
D	5.9±0.07	5.0±0.07	4.1±0.05	
Sig		**		

Note: A = control yogurt with conventional culture starter, B = yogurt with 50% conventional culture starter and 50% culture starter adjunct, C = yogurt with 75% culture starter adjunct and 25% conventional culture starter, D = yogurt with 100% culture starter adjunct.

Table 8. Effect of the different level of culture starter adjunct on sensory evaluation of yogurt.

Treatment	Sensory attributes				
	Color	Flavor	Texture	Taste	Overall
A	8.07± 0.34a	7.00± 0.28 a	7.6± 0.32a	6.87± 0.41a	7.53± 0.25a
B	8.00±0.34 a	7.07±0.28 a	7.33±0.32a	6.87±0.41a	7.47±0.25a
C	7.53±0.34 a	7.20±0.28 a	6.27±0.32b	6.40±0.41 a	7.4±0.25 a
D	6.80±0.34 b	5.60±0.28 b	5.07±0.22	4.87±0.41b	5.93±0.25b
Sig	*	**	**	**	**

Note: Means with different superscript in the same Column are significantly ($p < 0.05$) different. A = control yogurt with conventional culture, B = yogurt with 50% conventional starter and 50% culture starter adjunct C = yogurt with 75% culture starter adjunct and 25% conventional starter D = yogurt with 100% culture starter adjunct.

Table 9. Effect of storage period on the sensory characteristics of set yogurt.

Storage period/day	Sensory attributes				
	Color	Flavor	Texture	Taste	Overall
Day 1	7.95±1.43	6.35±1.45	6.20±1.91 b	6.30±2.19	6.90±1.4 2 b
Day 5	7.30±2.10	6.75±1.98	6.10±2.02 cb	5.75 ±2.42	6.75±1.58 cb
Day 10	7.55±2.02	7.05 ±1.39	7.40 ±1.93 a	6.70±2.42	7.60 ±1.58 a
Sig	NS	NS	*	NS	*

Note: Means with different superscript in the same column are significantly ($p < 0.05$) different.

The flavor of the yogurt samples improved slightly during the storage period. As the storage period progressed, the flavor scores increased, and the highest scores (7.05 ± 1.39) were at day 10. Storage period affected the texture of the yogurt samples significantly ($P < 0.05$). The texture scores of the yogurt samples increased with the advancement in storage. Therefore, the highest texture scores (7.40 ± 1.93) were recorded on day 10.

Slight improvement in the taste of the yogurt samples were noticed with the longer storage periods. At day 10, the taste scored 6.70 ± 2.42 , which was the maximum. The results indicated that the storage period affected the overall acceptability significantly ($P < 0.05$). The lowest overall acceptability scored 6.75 ± 1.58 which were on day 5 while the highest ones (7.60 ± 1.58) were at day 10.

Discussion

The addition of culture starter adjunct to the yogurt improved its quality. The results showed (Table 1) that the chemical composition of the yogurt samples significantly ($P < 0.05$) affected by the addition of culture starter adjunct (*L. acidophilus* and *L. plantarum*). The decrease in the fat content of the yogurt samples with culture starter adjunct could be due to their lipolytic activities. These findings are in accordance with the results of Mutlu and Guler (2005) who observed that the fat content of bio-yogurt ranged from 3.1 to 4.5% during storage.

The titratable acidity of the yogurt samples with culture starter adjunct showed higher values. This might be due to the high potentiality of culture starter bacteria to convert lactose to lactic acid which increases the acidity of yogurt samples, or probably due to its lower buffering capacity and higher content of non-protein nitrogen. These results agree with those reported by Abrahamsen et al. (1991); Salvador and Fiszman (2004).

The protein content of the yogurt samples with 50% culture starter adjunct was higher in comparison to the other treatments (Table 1). The high protein content could be due to preservative effect of culture starter adjunction the protein content. Our findings are in line with the results of Hassan and Amjad (2010) and Janhoj et al. (2006), who showed that the protein contents of low-fat stirred yogurt ranged from 3.4-5.6%.

The total solid and the ash content of the yogurt samples were not affected by the culture starter adjunct addition (Table 1). This result is not in accordance with those of Hassan and Amjad (2010), who reported the total solid of yogurt with different level of starter increased up to $15.60\% \pm 0.56\%$. The insignificant increase in ash contents was because of the loss of CO₂ and water during the mixing of yogurt samples. Moreover, the ash content of yogurt samples in this study was lower than those of Akin and Guler (2005) who reported the ash value of probiotic yogurt as 0.95%. This could be due to the action of *L. plantarum*.

The storage period affected the chemical composition of the yogurt significantly ($P > 0.05$) except the ash content (Table 2). The fat content was highest at day 1 then it decreased at day 5. This could be due to the lipolytic activities of the culture starter adjunct. These results were

in accordance with those of Salji et al. (1984). The results (Table 2) showed that acidity tends to increase within the 10 days-storage periods. The acidity of the yogurt samples increased significantly as the storage period progressed. This could be due to the breakdown of lactose into lactic acid by the culture starter adjunct; this may also be due to the lower buffering capacity of *Lactobacillus acidophilus* and higher content of non-protein nitrogen and vitamins which are needed for fast growing microorganisms. These findings are in line with those reported by of Nighswonger et al. 1996 and Salvador and Fiszman 2004.

The total solid of the yogurt samples increased till day 5 (Table 2), then decreased at day 10. This is probably due to the loss of moisture and the proteolytic activities of the culture starter adjunct during storage. These results were in accordance with the findings of Hassan and Amjad (2010) who reported that total solid increased up to $15.60\% \pm 0.56$ and with those of Abubakar et al. (2005).

The ash content was not affected significantly by storage period (Table 2). These results are not in agreement with those of Akin and Guler (2005), who reported that the ash value is 0.95%. The storage period affected the protein content (Table 2) of the yogurt samples significantly ($P < 0.05$). The decrease in protein could be due to the proteolytic action of the cultures. Similar findings were reported by Janhoj et al. (2006) who showed that the protein contents of low fat stirred yogurt ranged from 3.4-5.6%.

The interaction between the storage period and the addition of culture starter adjunct significantly affected the chemical composition of the yogurt samples in all treatments (Tables 3, 4, 5, 6 and 7). The lowest fat was for the yogurt sample with 75 % culture starter adjunct at day 5. However, the highest fat % was for the control yogurt samples. This could be due to lipolytic nature of the culture starter adjunct that tended to decrease the fat in the yogurt samples.

The mean sensory scores of the organoleptic evaluation and acceptability for the different yogurt samples are shown in Table 8. The statistical analysis revealed that there were significant differences ($p < 0.05$) among the yogurt samples in the sensory attributes due to the use of adjunct cultures.

Culture starter adjunct affected the color of the yogurt samples negatively. As the level of the culture starter adjunct increased, the color scores deteriorated. This could be due to the high lipolytic and proteolytic activities of the culture starter adjunct. These results agree with those of Nuser (2001). The flavor, texture and taste followed the same trend.

The best flavor and texture scores were for the control and yogurt with 50% culture starter adjunct, the improvement in flavor was probably because of high lactic acid contents which controlled the growth of desirable organisms producing flavor compound which was as the result of sugar and protein and fat degradation.

The storage period was not found to affect the sensory characteristics of the yogurt samples (Table 9) significantly ($P > 0.05$) except the texture and over all acceptability. Our results were not in line with those of Nuser (2001). The

improved in texture till day 5 was likely because of proteolytic agents on the protein breakdown, and it resulted in changing the structure of protein matrix and the texture to become soft and compact. While the deterioration in texture, thereafter, at day 10 might be attributed to further hydrolysis of protein at later stages which leads to very fine and mealy structure. The color of the yogurt in this study was not affected by the storage period.

Based on the results of the study, the following conclusions were drawn: (i) The quality of yogurt was relatively improved with the addition of *L. acidophilus* and *L. plantarum*. (ii) The addition of different level of culture starter adjunct improved sensory evaluation (overall acceptability). (iii) Significant variations in the sensory characteristics and chemical composition of the yogurt with probiotics were found. (iv) The storage period has no significant effect on the sensory characteristics of yogurt.

REFERENCES

- Abrahamsen RK, Ryszard G. 1991. Fermentation of goat's milk with yogurt starter bacteria. A review. *J Cult Dairy Prod* 8: 20-26.
- Abubakar MM, Adegbola TA, Oyawoye E. 2005. Determination of physiochemical, microbial, and organoleptic properties of yogurt. *J Texture Stud* 36: 333.
- Akin MS, Guler MB. 2005. Effect of different incubation temperatures on chemical composition and sensory characteristics of bio-yogurt. *J Food Sci* 17: 67-74.
- Aneja RP, Mathur BN, Chandan RC, Banerjee AK. 2002. Technology of Indian milk products. Dairy India yearbook, New Delhi, India.
- AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists. 15th ed. Washington, DC, USA.
- Chandan RC, Nauth KR. 2012. Yogurt. In: Hui YH, Chandan RC (eds.). Handbook of Animal-based Fermented Food and Beverage Technology. 2nd ed. CRC Press, Boca Raton, FL.
- Chandan RC. 1999. Enhancing market value of milk by adding cultures. *J Dairy Sci* 82: 2245-2256.
- Clark S, Plotka VC. 2004. Yogurt and sour cream: operational procedures and processing equipment. In: Hui YH, Meunier-Goddik L, Hansen AS, Josephsen J, Nip W-K, Stanfield PS, Toldra F (eds.). Handbook of Food and Beverage Fermentation Technology. Marcel Dekker, New York, NY.
- Guler A, Mutlu B. 2005. The effects of different incubation temperatures on the acetaldehyde and viable counts of bioyogurt. *Intl J Dairy Technol* 58: 174-179.
- Hassan A, Amjad I. 2010. Nutritional evaluation of yogurt prepared by different starter cultures and their physiochemical analysis during storage. *Afr J Biotechnol* 9 (20): 2913-2917.
- Janhoj B, Charlotte B, Michael. 2006. Sensory and rheological characterization of low fat stirred yogurt. *J Texture Stud* 37: 276-299.
- Lourens-Hattingh A, Viljoen B. 2001. Review: Yogurt as probiotic carrier food. *Intl Dairy J* 11: 1-17.
- Maity TK, Misra AK. 2009. Probiotics and human health: synoptic review. *Afr J Food Agric Nutr Dev* 9: 1778-1796.
- Mazahreh, AS, Ershidat, MO. 2009. The benefits of Lactic acid bacteria in yogurt on the gastrointestinal function and health. *Pakistan J Nutr* 8 (9): 1404-1410.
- Nighswonger BD, Branshear MM, Gilliland SE. 1996. Viability of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* in fermented milk products during refrigerated storage. *J Dairy Sci* 79: 212-219.
- Nuser, SNM. 2001. The Effect of Cooking and Vacuum Packaging on the Quality of White Soft Cheese. [Thesis]. University of Khartoum, Sudan.
- Ozer, B. 2010. Probiotics dairy beverages: Microbiology and technology. In: Yildiz, F (ed). Development and Manufacture of Yogurt and Other Functional Dairy Products. CRC Press, Boca Raton, FL.
- Salji JP, Sawaya WN, Saadi SR, Safi, WM. 1984. The effect of heat treatment on quality and shelf life of plain liquid yogurt. *J Cult Dairy Prod* 19: 10-14.
- Salvador A, Fiszman SM. 2004. Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage. *J Dairy Sci* 87 (12): 4033-4041.
- SPSS. 2008. Statistical package for the social sciences (Advanced Models-base system in version 14). SPSS statistical 17.0.1
- Staff MC. 1998. Cultured Milk and Fresh Cheeses. In: Early R (ed.). The Technology of Dairy Products. 2nd ed., Blackie Academic and Professional, London.
- Walstra P, Geurts TJ, Noomen A, Jellems AN, Boekel MAJS. 1999. Dairy technology principles of milk properties and processes. Marcel Dekker, Inc. New York.