 J Life Sci, 4(1): 11-18 (2012)

Evaluation of Ground Flaxseed Supplementation to Lactating Buffaloes Ration versus Control Milk Samples for milk and Stirred Yoghurt Production

Mervat I. Foda, S. M. Kholif, Sahar H. S. Mohamed and T. A. Morsy

Dairy Science Department., National Research Center, Dokki, Cairo, Egypt

KEYWORDS Buffalo Milk. Flaxseed. Fatty Acid Profile. Stirred Yoghurt. Microbiology Analysis. Sensory Evaluation

ABSTRACT Flaxseed contains high level of α-linolenic acid (omega- 3 fatty acid) which essential for humans and necessary to add to milk products to increase their health effects. To evaluate the acceptability of supplementing flaxseed to dairy products, fourteen lactating buffaloes were divided into two experimental groups. The first group used as control animals was fed ration without flaxseed (C) and the treated animals was fed rations supplemented with 433 g flaxseed/head/day (T_1). Flaxseed directly added to part of the control milk to design the third milk sample (T_2). All samples were used for stirred yoghurt production and their chemical composition, fatty acid profile, sensory evaluation and microbiological contents were evaluated. Flaxseed supplementation to ration increased fat content of donated milk. The results showed that supplementing control milk (T_2) with ground flaxseed significantly increased the total concentrations of CLA and omega-3 fatty acids.

INTRODUCTION

Traditionally, milk and milk products have formed one of the main components of human nutrition. The annual consumption of milk ranges from 180 down to less than 50 kg per capita in various societies (Haug et al. 2007). Milk fat contains low concentrations of n-3 fatty acids and high levels of saturated fatty acids, particularly $C_{16:0}$, which has hypercholesterolemic properties (Kennelly 1996). Research has shown several health benefits of n-3 fatty acids, which mammals cannot synthesize by themselves and thus need to consume in the diets to humans. These benefits include the decrease in cancer incidence, cardiovascular diseases, hypertension, arthritis and improving of visual acuity (Wright et al. 1998; Ruxton et al. 2004). Increasing the level of α-linolenic acid, n-3 fatty acid and other polyunsaturated long-chain fatty acids (LC PUFA) in addition to reducing the proportion of $C_{16:0}$ can be considered an attractive way to modify milk composition and would increase human consumption of milk and dairy products (Oba et al. 2009). Dietary recommendations suggest that the consumption of omega-3 LC PUFA should be increased and the dietary guidelines formulated by the UK Joint Health Claims Initiative (Baldwin and Rice 2004) suggest an intake of PUFA especially eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) at least 0.45 mg per person per day. These fatty acids are associated with reduced susceptibility to cardiovascular diseases (Breslow 2006).

Flaxseed (*Linum usitatissimum*) is one of the primary dietary sources of α-linolenic acid, which constitutes 180 g/kg of the total fatty acids (Mustafa et al. 2003). Flaxseed ingestion has been linked to reduce risk for cardiovascular disease and has a potential role in the management of diabetes and hypercholesterolemia (Paschos et al. 2008; Zhang et al. 2008). There is a market for flaxseed meal as animal feeding, human nutrition and poultry feed as it increases levels of omega 3 fatty acid in eggs (Rebolé et al. 2002). A significant increase in CLA and linolenic acid of milk and cheese can be achieved by flaxseed supplementation to ewes diet (Zhang et al. 2006). Also, feeding cows with whole flaxseed is as effective as rolled flaxseed at increasing absorption of linolenic acid concentration and its appearance in milk fat (Oba et al. 2009).

In Egypt, yoghurt is a very popular flavorful and healthful dairy product. Its production and consumption is growing continuously due to its therapeutic properties beside its high nutritive value (Karagul et al. 2004). The health promoting properties of live lactic acid bacteria in yoghurt include protection against gastrointestinal upsets, enhanced digestion of lactose and help the body assimilate protein, calcium and iron (Marona and Pedrigon 2004). The development of functional dairy products such as milk and yoghurt based on supplemented flaxseed is an interesting approach. So, the objectives of this work were to evaluate milk donated from buffaloes fed ground flaxseed versus control milk supplemented with ground flaxseed on the acceptability of liquid milk and stirred yoghurt and to study its effect on their chemical composition, fatty acid profile and the microbiological contents.

MATERIAL AND METHODS

Flaxseed (*Linum usitatissimum L.*) variety "Sakha 1" was purchased from Fiber Crop Research Center, Agriculture Research Center, Giza, Egypt. The flaxseeds dried at 55 ^{O}C for 48 hrs, then ground to pass a 1-mm screen with a mill. The chemical composition and fatty acids content of ground flaxseed are shown in Table 1.

Feeding Trial: Fourteen lactating dairy buffaloes (aged 5-6 years, weighed 561±10 kg) in early lactation were divided into two experimental groups using completely random design. The experimental period was 120 days. Control animals were fed a ration contained concentrates: berseem clover: rice straw (60: 20: 20, on DM basis), while treated animals were fed a control ration supplemented with 433 gm ground flaxseed/animal/day (about 3.33 % of the total ration). This study was conducted at the Experimental Station Farm, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, and Dairy Science Department, National Research Center, Dokki, Giza, Egypt, from May to September 2010.

Milk Preparation: Pooled milk samples, control (C) and (T_1) donated from animals fed flaxseed. Milk and stirred yoghurt were analyzed for total fat, lactose and total protein contents by near infrared analysis using a Milko-Scan (model: Foss 4000, Foss Food Technology, Hillerød, Denmark). Total solids and ash contents were determined according to the method 930.15 of (AOAC 2000). The pH values were determined using a digital pH-meter with a glass electrode, Model GC, Germany.

Fatty Acids Analysis

Milk samples were extracted and methylated according to the method 996.06 of the AOAC (1998). Tritridecanoin (Sigma, Oakville, ON, Canada) was used as the internal standard. Individual FA was identified by comparison of gas chromatography peaks with known standards (GLC-463; Nu-Chek Prep Inc.). Fatty acid methyl esters were separated on an Agilent 6890 GLC fitted with an Agilent auto sampler (model 7683, Agilent injector, Agilent Ltd., Mississauga, ON, Canada) and a flame ionization detector. Agilent Chemstation Rev. B.01.03 (204) software was used for chromatogram integration and analysis. Samples were introduced onto a 100m Supelco (Oakville, ON, Canada) SP-2560 column (part number 24056) via 1µl splitless injections. The temperature program was: level one, 45 ^{O}C held for 4min; level two, 45-150 ^{O}C at 13.0^{O}/min increments, then held for 47 min; level three, 150–215 ^{O}C at 4^{O}C / min increments, then held for 35 min. The injector temperature was set at 250 ^{O}C and the detector was set at 260^{O}C. Column head pressure was set at 30 psi. A 4 mm i.d. splitless injection liner (Agilent Ltd., Mississauga, ON, Canada) was used for all injections. Gas flow rates were: helium (carrier) 1.1 ml/min, helium (make up) 25 ml/min, compressed 350 ml/min and hydrogen 35 ml/min.

***Stirred Yoghurt Manufacture*:** Milk samples were skimmed using cream separator Lab. The control milk sample was divided into two groups; the first group was used as a control (C) while the second group was supplemented with 3.33% grounded flaxseed (T_2) to reach the same level as buffaloes ration supplementation. Milk samples (control, T_1 and T_2) were standardized to ≈ 15.0 milk solids using skim milk powder, heated to 85 ^{O}C for 20 min, cooled to 42 ^{O}C then inoculated with (1:1) *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus salivarius* subsp *thermophilus* obtained from Chr. Hansens Lab., Denmark. The inoculated samples were incubated at 42 ^{O}C until coagulation, stirred and packed in plastic cups. All samples were stored at 6 ± 1 ^{O}C for 15 days and sampled after 1, 3, 6, 9, 12 and 15 days for chemical analysis, microbiological and sensory evaluation. All experiments were carried out in triplicate.

Chemical Analysis

Microbiological Analysis: Stirred yoghurt samples (25 g) were homogenized for 1 min with 225 ml of tri-sodium citrate (2% w/v) as a sterile solution. Decimal dilution were prepared in 9 ml sterile NaCl (0.85 %). The microorganisms content of stirred yoghurt samples was determined as follows: Total bacterial counts (TBC) were enumerated on Plate Count Agar (Oxoid, Ltd), following the pour plate method, incubated at 37 ^{O}C for 48 hrs (APHA 1992). Coliform groups were detected according to FDA (2002) using Violet Red bile Agar (Difco) and the plates were incubated at 32 -35 ^{O}C for 24 hrs. Moulds and yeasts were counted on Potato Dextrose Agar media (Oxoid, Ltd) adjusted to pH 3.5 and incubated at 25 ^{O}C for 4-5 according to FDA (2002). Proteolytic bacteria were counted on supplemented skim milk (10 %) then incubated at 32 ^{O}C for 48 hrs as described by Frank et al. (1992). Lipolytic bacteria were enumerated on Tributyrim Agar (Oxoid, Ltd) incubated at 30 ^{O}C for 48 hrs according to the method described by Luck (1981). Lactic acid bacteria count: Yoghurt starter *Lactobacillus delbrueckii* supsp *bulgaricus* and *Streptococcus salivarius* supsp *thermophilus* were counted anaerobically on MRS agar medium at 37 ^{O}C for 48 hrs and aerobically on M 17 agar medium at 37 ^{O}C for 48 hrs according to the method described by Dave and Shah (1996).

Sensory Evaluation

Descriptive Sensory Analysis: Fifteen panelists from the staff members of the Dairy Dept., National Research Center, Cairo, Egypt (5 female and 10 male, aged between 30 and 40 years) who has experience with milk and yoghurt descriptive vocabulary were participated. Milk samples were sensory evaluated fresh, while yoghurt were evaluated fresh and during the cold storage period for 15 days using a scheme of 30 points for taste and appearance, 20 points for odor and color, while 100 points for total acceptability. Panel members also instructed to report any defects or unpleasant flavors.

Consumer Acceptance Panel: Seventy consumers from different areas in Cairo were chosen randomly to participate in the preference test (T_1 and T_2 fresh milk only). Each consumer was asked to express his preference on a five point hedonic scale and report any unpleasant taste. Consumers were provided water at room temperature and rinse thoroughly after testing each milk sample to clean their palate.

Statistical Analysis

All data were analyzed using PROC MIXED of SAS software (version 8.1, 2000). Data were analyzed as a completely random design where treatment was the main source of variation. When a significant F-test was detected (that is, $P < 0.05$), treatment means were separated using Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Table 1 shows that ground flaxseed is rich in fat, protein and dietary fiber. These results are in agreement with those obtained by Vijaimohan et al. (2006) who reported that the chemical analysis of flaxseed average of about 30- 45% oil, includes 51-55 % α-linolenic acid, (18:3 n-3 omega-3 fatty acid), a precursor to eicosapentanoic acid, 20-25% protein, 20-28 % total dietary fiber, 4-8 % moisture and 3-4 % ash. The composition of flaxseeds can vary with genetics, growing environment, seed processing and methods of analysis. Flaxseeds contain substantial amounts (5-8%) of soluble fiber mucilaginous material and it has a high water holding capacity (Vijaimohan et al. 2006). Ground flaxseed is naturally low in saturated fatty acids (11.35%) and contains a moderate amount of monounsaturated fatty acids (21.92%) as shown in Table 1. Roughly, 66.12% of the fatty acids in flaxseed oil are polyunsaturated fatty acids. Flaxseed is rich in α- linolenic acid, omega-3 fatty acids (50.51%) it contains a low amount of linoleic acid, omega-6 fatty acids (15.61%). Due to the high α- linolenic content, flaxseed has omega-6/omega-3 fatty acid ratio of 0.309: 1. These results are in agreement with those obtained by Bahagat (2010) and El-Beltagi et al. (2007) who found that fatty acids analysis of the flaxseed of the cultivars grown in Egypt contained 46-50% linolenic acid whereas the mean value of linoleic acid, oliec acid and stearic acid were 18.20, 22.0 and 3.70% respectively. Similar results were also obtained by Nykter et al. (2006) and Choo et al. (2007).

Chemical Composition of Milk Samples

Data presented in Table 2 shows that milk produced from buffaloes fed flaxseed (T_1) contained higher fat contents compared to control sample. The same results were found by Dhiman et al. (2000) who mentioned that feeding fat through seeds maintains or increases milk fat content. Total solids content in milk from buffaloes fed flaxseed was slightly increased com-

Table 1: The chemical composition and fatty acids content of ground flaxseed

Items	*%*
Moisture content	4.68
Ash	4.10
Total protein	23.76
Total carbohydrates	18.84
Crude fiber	10.09
Total fat	43.21
Fatty acids (as % of total fatty of acids)	
C16:0 (Palmtic acid)	6.738
C16:1n-7 (Palmitoleic acid)	0.100
C18:0 (Stearic acid)	4.616
C18:1n-9 (Oleic acid)	21.819
C18:2 n-6 (Linoleic acid)	15.608
C18:3 n-3 (α- Linoleic acid)	50.509
Saturated fatty acids (SFA)	11.354
Unsaturated fatty acids (USFA)	88.036
Mono-unsaturated fatty acids (MUFA)	21.919
Poly-unsaturated fatty acids (PUFA)	66.117
Total n-6	15.608
Total n-3	50.509
PUFA n-6/ n-3	0.309:1
SFA / PUFA	0.129

pared to the control sample which may be due to the higher fat content. However, the pH values did not changed in both milk samples.

Table 2: The chemical composition of different milk samples

Items	*(C)*	*(T_1)*	*± SE*
Total solids	16.78	17.26	0.061
Total fat	6.57 [b]	7.45 [a]	0.067
Total protein	4.18	4.20	0.024
Lactose	4.75	4.78	0.065
Ash	0.782	0.829	0.009
pH	6.56	6.57	0.026

(C) milk donated from buffaloes fed ration without flaxseed and (T_1) milk donated from buffaloes fed ration supplemented with grounded flaxseed.
Each value represents an average of three samples,
[a, b] means with different superscripts are significantly (P<0.05) different.

Sensory Evaluation of Milk Samples

The results presented in Table 3 indicated that milk sample donated from buffaloes fed rations supplemented with flaxseed (T_1) got higher scores (P<0.05) with all the descriptive vocabulary compared with the control after adding ground flaxseed (T_2). These results could be due to the characteristics of flaxseed as tiny, smooth and flat ranged in color from light to reddish brown. It is also described as having a crunchy and chewy texture as well as a nutty flavor which gave milk samples unusual color, odor and taste (McKinley Health Center 2006). The results of consumer overall acceptance showed also lower scores with the direct addition of flaxseed to skimmed milk.

Table 3: Descriptive and hedonic evaluation of different milk samples

Items	*(T_1)*	*(T_2)*	*± SE*
Taste (30 points)	25.2 [a]	20.13 [b]	0.736
Appearance (30 points)	26.4 [a]	16.33 [b]	1.024
Odor (20 points)	16.67 [a]	13.07 [b]	0.540
Color (20 points)	17.53 [a]	13.13 [b]	0.525
Total acceptability	87.0 [a]	62.67 [b]	2.429
Overall acceptance	3.55 [a]	2.51 [b]	0.043

(T_1) milk donated from buffaloes fed ration supplemented with flaxseed, (T_2) control milk supplemented with flaxseed, [a, b] means with different superscripts are significantly (P<0.05) different.

Fatty Acid Composition of Different Milk Samples

Table 4 shows that milk from lactating buffaloes fed ground flaxseed (T_1) contained slightly lower amounts of short-chain and medium-chain fatty acids (C6- C10) while C16.0 was slightly higher than both control milk and the milk supplemented with ground flaxseed (T_2). Kholif et al. (2011) found that flaxseed supplementation to a buffaloes ration can be used as a nutritional strategy to reduce concentrations of saturated fatty acids and increase long-chain and polyunsaturated fatty acids in milk compared with control samples and a significant increase in CLA and linolenic acid of milk can also be achieved by flaxseed supplementation to buffaloes ration as 34g/kg DM. Mihaiu et al. (2010) reported that buffalo and cow milks contains high levels of short chain fatty acids C10 - C14, and of steric acid and a small amount of oleic acid, which stands for 33.5% at a cow and 24% at buffalo. Feeding flaxseed supplementation improved composition and nutritional properties, fatty acid profile and the CLA content of milk from cows produced during summer months (Caroprese et al. 2010). It could be seen from Table 3 that supplementation of control milk with ground flaxseed significantly increased the total concentrations of CLA and omega-3 fatty acids. These results could be due to the high amount of ∝- linolenic acid (50.51%) and omega-3 fatty acids (46.51%) in the ground flaxseed as shown in Table 1. Adding flaxseed to control milk (T_2) decreased trans form of oleic acid and increased Cis form significantly compared with other treatments. Feeding flaxseed (T_1) decreased the concentrations of saturated fatty acids and increased the concentrations of unsaturated fatty acids followed by (T_2) compared with the control. The n-6/n-3 ratio of milk supplemented with flaxseed was the lowest (0.82), followed by ration supplemented with flaxseed (4.86) then the control samples (27.0), this could be due to the low ratio of ground flaxseed 0.321:1 as shown in Table 1. Proportions of CLA, omega 3 fatty acids and unsaturated fatty acids are a good indicator for healthy milk and yoghurt for consumers.

Table 4: Fatty acids composition of different milk samples

Fatty acids (g/100g FA)	*(C)*	*(T_1)*	*(T_2)*	*± SE*
C6	1.1	0.63	1.87	0.031
C8	0.87	0.81	1.7	0.070
C10	1.71	1.68	3.26	0.096
C12	2.18	2.31	3.33	0.416
C14.0	11.27	11.59	13.41	0.008
C14.1	0.67	0.67	0.66	0.230
C15.0	-	-	0.23	
C16.0	33.67	34.2	31.29	0.311
C16.1	0.45^{c}	2.3^{a}	1.39^{b}	0.036
C17.0	-	-	0.62	
C18.0	17.82^{a}	16.09^{b}	14.44^{c}	0.625
C18.1 n-9 T	27.02^{a}	26.45^{a}	19.56^{b}	0.129
C18.1 n-9 C	1.78^{b}	1.83^{b}	3.06^{a}	0.018
C18:2 *trans*-10, *cis*-12	0.10^{b}	0.13^{b}	0.73^{a}	0.040
C18:2 *cis*-9, *trans*-11	0.18^{b}	0.18^{b}	3.85^{a}	0.027
Total CLA	0.28^{b}	0.30^{b}	4.58^{a}	0.036
C18.3 n-3	0.01^{c}	0.15^{b}	1.00^{a}	0.230
C18.3 n-6	0.27^{c}	0.73^{a}	0.82^{a}	0.066
n-6/n-3	27.0^{a}	4.86^{b}	0.82^{c}	0.129
C20.0	0.71^{a}	0.31^{b}	0.62^{a}	0.035
C20.1	0.19^{a}	-	-	0.430
Saturated fatty acids	69.32	67.62	68.93	0.863
Unsaturated fatty acids	30.67	32.38	31.07	0.957

Each value represents an average of four samples,
[a, b, c] means with different superscripts are significant (P<0.05) difference. (-): not detected.

Chemical Composition of Stirred Yoghurt

Data in Table 5 indicated that yoghurt produced from milk donated from buffaloes fed rations supplemented with flaxseed (T_1) contained higher total solid and fat contents compared with other treatments while, protein content was not affected by treatments. These results are in contrary with those obtained by Zhang et al. (2006) and Dhiman et al. (1999) who found fat, protein and dry matter contents in cheese were not affected by dietary oilseed supplementation. pH value was decreased with T_2 compared with other treatments. This decrease may be due to the acidic effect of fatty acids of ground flaxseed.

Table 5: Chemical composition of stirred yoghurt prepared from different skimmed milk samples

Items	*(C)*	*(T_1)*	*(T_2)*	*±SE*
Total solids	14.57^{c}	15.33^{a}	15.02^{b}	0.258
Protein	2.78	2.80	2.83	0.086
Fat	1.50^{b}	1.87^{a}	1.60^{b}	0.141
pH	4.59^{a}	4.53^{a}	4.38^{b}	0.138

[a, b] means with different superscripts are significantly (P<0.05) different.

Microbial Analysis of Stirred Yoghurt

Total Bacterial Count

The changes occurred in the mean log count of total bacteria are illustrated in Figure 1 and indicated that control yoghurt contained less count than that in both treatments (T_1 and T_2). Similar results were obtained by Bahagat (2010), who found that the increasing flaxseed concentration from 3 to 7 % increased the total bacteria count of Kareish cheese significantly compared to control samples. While, prolonging cold storage decreased the total counts significantly. This could be due to the presence of PUFA in flaxseed ground which promoted the growth of lactic acid bacteria (KankaanpÎÎ et al. 2001).

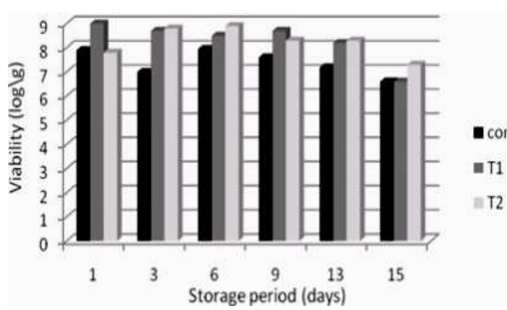

Fig. 1. Viability of total bacterial count (log cfu/g) during the cold storage

Detection of Moulds, Yeasts and Coliforms

Moulds and yeasts were appeared after nine days in the control and after 15 days in both treatments; while, coliforms were not detected in all samples either fresh or during the cold storage period. This may be attributed to increase the acidity during storage period. Xu et al. (2008) found complete inhibition of *F. graminerum* by different concentrations of flaxseed flour.

Detection of Proteolysis Bacteria

Various lactic acid bacteria have the proteolysis ability. So, data presented in Figure 2, showed that the mean log of proteolysis bacteria did not change significantly among both treatments (T_1), (T_2) and the control, either in fresh samples or during cold storage period. The presence of proteolysis' bacteria in stirred yoghurt could be due to the presence of starter culture of *Streptococcus thermophilus* which has proteolytic ability to grow and hydrolyze milk proteins (casein). *S. thermophilus* contains two unique peptidases, oligopeptidase and aminopeptidase PepS, which have multiple functions in bacterial growth (Fernandez et al. 1999).

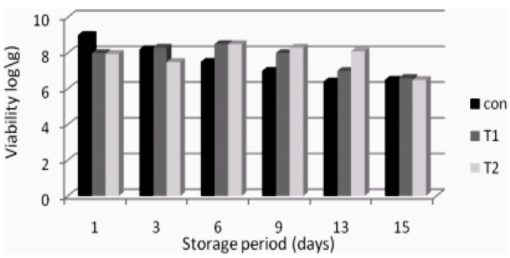

Fig. 2. Viability of proteolytic bacteria (log cfu/g) during the cold storage

Detection of Lipolytic Bacteria

Lipolytic bacteria were not detected in all samples either when fresh or during the cold storage period. This may be due to the absence of the lipolytic activity of the presence starter cultures bacteria.

The Viability of Lactic Acid Bacteria

Figure 3 shows the viability of *Streptococcus salivarius* subsp *thermophilus* in fresh sample (1 day) and during cold storage for 15 days. The mean log count of *Streptococcus salivarius* subsp *thermophilus* in (T_1) and (T_2) was increased after 6 days till 13 days by 0.5 and 1.0 log compared with control samples.

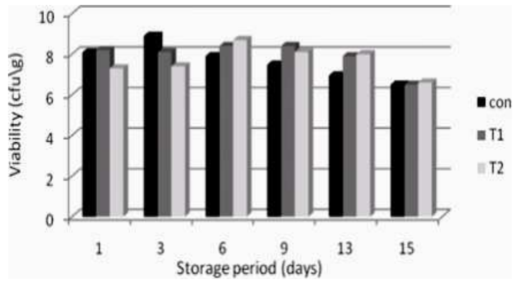

Fig. 3. Viability of *Streptococcus thermophillus* (log cfu/g) during cold storage

Viability of *Lactobacillus bulgaricus* (Fig. 4) was increased (T_1) and (T_2) by 1.0 log after 3 days to 13 days compared with control samples. These results could be due to the addition of flaxseed which known to increase concentrations of polyunsaturated fatty acid (PUFA) as reported by Kennelly (1996). William et al. (1947), who found several cultures of lactic acid bacteria require oleic acid, linoleic acid, or a combined source of these materials for growth. Both lactic acid bacteria counts were decreased in the end of the storage period. Ringo et al. (1998) reported that dietary lipid influences the gastrointestinal microbiology and especially the population level of lactic acid bacteria. According to KankaanpÎÎ et al. (2001), higher concentrations of PUFA inhibited the growth and mucus adhesion of selected lactobacilli, whilst growth and mucus adhesion of the lactobacillus casei shirota was promoted by low concentrations of α-linolenic acid and arachidonic acid. When lactobacilli were cultured in MRS broth supplemented with various free polyunsaturated fatty acids (PUFA), the incorporation of a given PUFA into bacterial fatty acids was clearly observed. Moreover, PUFA supplementation also resulted in PUFA-dependent changes in the proportions of other fatty acids. Kankaanpaa et al. (2004) demonstrated that free PUFA in the growth medium induces changes in bacterial fatty acids in relation to the regulation of the degree of fatty acid unsaturation, cyclization, and proportions of CLA and PUFA containing 20 to 22 carbons.

Sensory Evaluation of Stirred Yoghurt

The total acceptability score of stirred yoghurt prepared from milk sample donated from buffaloes fed rations supplemented with flax-

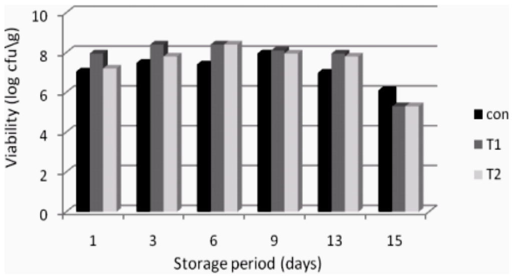

Fig. 4. Viability of *Lactobacillus bulgaricas* (log cfu/g) during cold storage

seeds and even all sensory items (Table 6) were the highest followed by the control. While, stirred yoghurt prepared from the direct addition of flaxseed to milk caused lower total acceptability scores. The same result was obtained with the sensory evaluation of milk samples (Table 3).

Table 6: Effect of flaxseed on sensory evaluation of stirred yoghurt

Items	*(C)*	*(T_1)*	*(T_2)*	*± SE*
Taste (30 points)	17.74[b]	23.81[a]	14.30[c]	0.381
Appearance (30 points)	18.78 [b]	25.71 [a]	14.54 [c]	0.399
Oder (20 points)	15.09 [b]	17.23 [a]	12.81 [c]	0.234
Color (20 points)	15.78 [b]	18.62 [a]	12.59 [c]	0.239
Total acceptability	67.78 [b]	85.51 [a]	54.07 [c]	1.066

[a, b, c] means with different superscripts are significantly ($P <0.05$) different

CONCLUSION

Flaxseed supplements to either lactating buffaloes ration or milk can be used as a nutritional strategy to reduce concentrations of saturated fatty acids and increase concentrations of polyunsaturated fatty acids, CLA and n-3 fatty acids and the acceptability of milk and yoghurt. A significant decrease in the *trans* form of oleic acid and increased *Cis* form can be achieved by flaxseed supplementation to milk. Further studies are required to improve the acceptability of dairy products after flaxseed supplementation to milk such as adding fruits, chocolate or different kinds of flavors.

REFERENCES

AOAC (Association of Official Analytical Chemists) 1998. *Official Methods of Analysis.* 16th Edition. Arlington, VA: AOAC.

AOAC (Association of Official Analytical Chemists) 2000. *Official Methods of Analysis.* 17th Edition. Washington, DC: AOAC.

APHA (American Public Health Association) 1992. *Compendium of Methods for the Microbiological Examination of Foods.* 3th Edition. Washington, DC, USA: American Public Health Association.

Bahagat K W 2010. *Utilization of Some Functional Foods for Lowering Blood Lipids*. PhD Thesis. Faculty of Agriculture, Ain Shams University, Shobra El-Khema, Egypt.

Baldwin N, Rice R 2004. *Eating Long Chain Omega-3 Polyunsaturated Fatty Acids, as Part of a Healthy Lifestyle, Has Been Shown to Help Maintain Heart Health*. UK: Joint Health Claims Initiative.

Breslow JL 2006. N-3 fatty acids and cardiovascular disease. *Am J Clin Nutr*, 83 Suppl: 1477- 1482.

Caroprese M, Marzano A, Marino R, Gliatta G, Muscio A, Sevi A 2010. Flaxseed supplementation improves fatty acid profile of cow milk. *J Dairy Sci,* 93: 2580-2588

Choo WS, Birch EJ, Dufour JP 2007. Physico-chemical and quality characteristics of cold pressed flaxseed oils. *J Food Compos Anal,* 20: 202-211

Dave RT, Shah NP 1996. Evaluation of media for selective enumeration of *streptococcus thermophilus*, *lactobacillus delbrueckii spp. bulgaricus*, *lactobacillus acidophilus* and bifidobacteria *J Dairy Sci*, 79: 1529-1536.

Dhiman TR, Satter LD, Pariza MW, Galli MP, Albridht K, Tolosa MX 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. *J Dairy Sci*, 83: 1016-1027.

Dhiman TR, Helmink ED, Mcmahon DJ, Fife RL, Pariza MW 1999. Conjugated linolenic acid content of milk and cheese from cows fed extruded oilseeds. *J Dairy Sci*, 82: 412-419.

Duncan DB 1955. Multiple range and multiple F- tests. *Biometrics*, 11: 1-42.

El-Beltagi HS, Salama ZA, El-Hariri DM. 2007. Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars (linum usitatissimum L). *Gen Appl Plant Physiol*, 33: 187-202.

FDA(Food and Drug Administration) 2002. *Bacteriological Analytical Manual*. 9th Edition. Arlington, VA, USA.: AOAC International.

Fernandez-Espla, Dolores M, Françoise R 1999. FEBS PepS from Streptococcus thermophilus: A new member of the aminopeptidase T family of thermophilic bacteria. *Eur J Biochem*, 263: 502- 510.

Frank JF, Christen GL, Bullerman LB 1992. Tests for groups of microorganisms. In: RT Marshall (Ed.): *Standard Methods for the Examination of Dairy Products*. Washington, DC: Am Publ Health Assn, pp. 271-282.

Haug A, Hotmark AT, Harstad OM. 2007. Bovine milk in human nutrition-a review. *Lipids Health Dis*, 6: 25-31.

Kankaanpaa PE, Salminen SJ, Isolauri E , Lee YK 2001. The influence of polyunsaturated fatty acids on probiotic growth and adhesion. *FEMS Microbiology Letters*, 194: 149–153.

Kankaanpaa P, Yang B, Kallio H, Isolauri E, Salminen S 2004. Effects of polyunsaturated fatty acids in growth medium on lipid composition and on physicochemical surface properties of lactobacilli**.** *Appl Environ Microbiol*, 70: 129-136.

Karagul Y, Wilson C, White H 2004. Formulation and processing of yoghurt. *J Dairy Sci*, 87: 543-550.

Kennelly JJ 1996. The fatty acid composition of milk as influenced by feeding oilseeds. *Anim Feed Sci Technol,* 60: 137-152.

Kholif SM, Morsy TA, Abedo AA, El-Bordeny N, and Abdo MM 2011. Milk production and composition, milk fatty acid profile, nutrients digestibility and blood composition of dairy buffaloes fed crushed flaxseed in early lactation. *Egy J Nutri Feeds*, (in press).

Luck H 1981. Quality control in the dairy industry. In: R K Robinson (Ed.): *Dairy Microbiology*. London: Applied Science Publishers, pp. 313-315.

Marona D, Pedrigon G 2004. Yoghurt feeding inhibits promotion and progression of cancer. *Med Sci Monit,* 10: 96-104.

McKinley Health Center 2006. Health Center Flax seeds and Nutritional Needs. The Board of Trustees of the University of Illinois, HED. III-236, 02-03-06, pp 1-2.From <http://www.mckinley.illinois.edu/handouts/heart_healthy_eating.htm> (Retrieved May, 2006).

Mihaiu R, Mihaiu M, Pintea A, Bele C, Lapusan A, Dan SD, Taulescu C, Ciupa A 2010. Comparative study on the fat acids profile from buffalo milk as quality and traceability markers. *Vet Med,* 67: 161-165.

Mustafa AF, Chouinard PY, Christensen DA 2003. Effects of feeding micronised flaxseed on yield and composition of milk from Holstein cows. *J Sci Food Agric,* 83: 920-926.

Nykter M, Kymalainen HR, Gates F, Sjoberg AM 2006. Quality characteristics of edible linseed oil. *Agric Food Sci,* 15: 402-413.

Oba M G, Dehghan-banadaky TM, Ambrose DJ 2009. Unprocessed whole flaxseed is as effective as dry-rolled flaxseed at increasing a-linolenic acid concentration in milk of dairy cows. *Livest Sci,* 122: 73-76.

Paschos GK, Magkos F, Panagiotakos BD, Votteas V, Zampelas A 2008. Dietary supplementation with flaxseed oil lowers blood pressure in dyslipidemic-patients. *Eur J Clin Nutr,* 61: 1201-1206.

Rebolé A, Rodriguez ML, Ortiz LT, Alzueta C, Centeno C, Trevino C 2002. Mucilage in linseed: effects on the intestinal viscosity and nutrient digestion in broiler chicks. *J Sci Food Agric,* 82(10): 1171-1176.

Ringø E, Bendiksen HR, Gausen SJ, Sundsfjord A, Olsen RE 1998. The effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa and from faecalia of Arctic charr, Salvelinus alpinus (L.). *J Appl Microbiol,* 85: 855-864.

Ruxton CHS, Reed SC, Simpson MJA, Millington KJ 2004. The health beneûts of omega-3 polyunsaturated fatty acids: A review of the evidence. *J Human Nutr Diet,* 17: 449-459.

SAS 2000. *User's Guide Statistics. Version 8.1 Edition.* SAS Inst. Inc. Cary, NC.

Vijaimohan K, Jainus M, Sabitha KE, Subramaniyam S, Anandhan C, Shyamala Devi CS 2006. Beneficial effects of α-linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. *Life Sci,* 79: 448-454.

Willam LW, Broquist HP, Snell EE 1947. Oleic acid and related compounds as growth factors for lactic acid bacteria. *J Biol Chem,* 170: 619-630.

Wright T, McBride B, Holub B 1998. Docosahexaenoic acid enriched milk. *World Rev Nutr Diet,* 83: 160-165

Xu Y, Hall C, Wolf-Hall C 2008. Fungistatic activity of heat-treated flaxseed determined by response surface methodology. *J Food Sci,* 73: 250-256.

Zhang R, Mustafa AF, Zhao X 2006. Effects of flaxseed supplementation to lactating ewes on milk composition, cheese yield, and fatty acid composition of milk and cheese. *Small Rum Res,* 63: 233–241.

Zhang W, Wang X, Liu Y, Tian H, Flickinger B, Empie MW, Sun SZ 2008. Dietary flaxseed lignan extract lowers plasma cholesterol and glucose concentrations: Hypercholesterolaemic subjects. *Brit J Nutr,* 99: 1301-1309.