Middle East Journal of Applied Sciences
ISSN 2077-4613
Volume : 07 | Issue :04 |Oct.-Dec.| 2017
Pages: 827-839

Effect of Lactobacillus spp. and yogurt culture on conjugated Linoleic acid content of fermented cow Milk

Abeer E. A. Amer

Dairy Microbiology Department, Agriculture Research Center, Animal Production Research Institute, Dokki, Cairo, Egypt.

Received: 07 August 2017 / Accepted: 23 Oct. 2017 / Publication date: 16 Nov. 2017

ABSTRACT

This work aimed to investigate the effect of Lactobacillus spp. for their ability to produce conjugated linoleic acid in fermented cow milk supplementing form of linseed oil to lactating cow's ration on milk yield and milk composition, It was found that 8 multifarious Friesian cows gives 15±2.26 kg/d of milk, and averaging 500±15.26 kg/ body weight, were divided into two experimental feeding groups (4 each) for 180 day.There was no significant difference in milk composition among the two groups. From the results shows in table (1) it could be concluded that using (3%) linseeds oil in lactating cow's rations increased milk yield, milk fat yield/ day, and also increased CLA content in milk (Average CLA contents in milk fat through the 2 diet treatments were (1.03 mg CLA /g fat) in $control_1$ milk and 2.07 mg CLA /g fat in milk from cow fed with 3% linseed oil treatment. The effect of *Lactobacillus rhamnosus* TISTR 541 or *Lactobacillus plantarum* NRRL B – 4496 or *Lactobacillus acidophilus* TISTR 450 and yogurt cultures and storage time on conjugated linoleic acid (CLA) content and quality of fermented cow milk products was determined. Using of Yogurt culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*, 1:1 ratio, YC), *L. rhamnosus* TISTR 541, *L. plantarum* NRRL B – 4496 or *L. acidophilus* TISTR 450 alone or co-cultured with yogurt culture, were inoculated at 10^8 cfu/ml into a milk obtained from cow fed linseed oil as the lipid source. CLA content, microbial counts, acidity, and texture of the fermented milk products were stable during storage at 5+2◦C for 21 days. Total CLA contents ranged from 0.79 in C_1 ($Control_1$) to 2.96 (mg CLA/g lipid) in T4 treatment following 21 days of storage. *L. plantarum* with yogurt cultures or *L. acidophilus* and yogurt cultures or *L. rhamnosus* and yogurt cultures had significant effect on CLA content and texture, but affected acidity of the fermented milk products. The fermented cow milk products produced by *L. plantarum* co-cultured with yogurt culture with 10^8 cfu/ml total inoculation level gave a higher CLA content and desirable quality characteristics than all treatments (*L. Rhamnosus* or *L. acidophilus* alone or co-cultured with yogurt culture). This research demonstrated that the combination of *L. rhamnosus* or *L. plantarum* or *L. acidophilus* and yogurt cultures were important factors to produce fermented cow milk products with high CLA content. The highest CLA 2.96 (mg CLA/g lipid) content was observed in fermented milk containing *L. plantarum* with yogurt culture.

Key words: linseed oil conjugated linoleic acid (CLA), Cow fermented milks, *L. rhamnosus*, *L. plantarum*, *L. acidophilus,* yogurt culture.

Introduction

Recently there has been a renewed interest in using linseed meal in animal rations to alter the fatty acid composition of meat products and milk products and therefore, provide functional health benefits for the consumer.

Over a few past decades Interest in ruminant animal rations with various fat sources has increased (Hess *et al*., 2008). Initially, the primary aim of fat addition to the ruminant's ration was to provide concentrated energy. Presently, the increase interest in fat utilization in ruminant nutrition is mainly a possibility to modify fatty acid composition of animal origin food products, milk and meat (Jan *et. al.,* 2013).Traditional varieties forms of linseed, were used in dairy cows feeding are characterized by highly content of total fatty acids (over 50%) specially, linolenic acid C18:3(53% of total fatty acids (FA) (Chow, 1992) P18/6 and others healthy fatty acid profile. This fatty acid

Corresponding Author: Abeer E. A. Amer, Dairy Microbiology Department, Agriculture Research Center, Animal Production Research Institute, Dokki, Cairo, Egypt.

promotes increased omega-3 fatty acids and conjugated linoleic acid (CLA) content in milk (Chilliard *et al*., 2007).

Feeding dairy cows a ration contained rolled extruded linseed (meal) has beneficial effects on the fatty acids profile of cow's milk whereas, there was an increase in alpha-linolenic acid (ALA, conjugated linoleic acid (CLA) and the proportion of stearic acid relative to other saturated fatty acids, and there was decrease in the omega-6/omega-3 ratio and the overall saturated fat content. These enhancements give consumers value-added foods with good sensory qualities and a healthier fat profile (Diane and Essi, 2008).

Cow's milk naturally contains low amounts of omega-3 fats one cup of 2% milk contains only 20 mg of ALA and virtually no EPA or DHA and high levels of saturated fats, particularly palmitic acid (16:0), which raises blood cholesterol l. Milk and milk products like cheese and ice cream are relatively rich sources of conjugated linoleic acid (CLA). Natural cheeses, for example, contain 2.9 to 7.1 mg CLA/g of fat, while processed cheeses average about 5 mg CLA/g of fat. Milk contains about 5.5 mg CLA. The main CLA isomer in cow's milk is cis-9, trans-11-CLA (c9, t11-CLA), accounting for more than 82% of the total CLA (Chin *et al*., 1992). They described how the ALA and CLA content of milk can be increased, the omega-6/omega-3 ratio reduced, and the saturated fat content decreased by supplementing the rations of dairy cows with linseed.

The growing interest in functional foods has contributed to an increased interest in dairy products with increased conjugated linoleic acid (CLA) contents and added probiotic bacteria. CLAs, a mixture of conjugated positional and geometric isomers of linoleic acid, have demonstrated unique biological activities in invitro and animal studies, including an anticarcinogenic agent (Corl *et. al*., 2001), body-fat reducer (Park *et al*., 1999), antiatherogenicagent (Nicolosi *et al*., 1997), antidiabetic agent (Houseknecht *et al*., 1998), immune system modulator (Hayek *et al*., 1999), and body weight protector (Chin *et al*., 1994). However, the health benefits associated with CLA have yet to be definitively confirmed in human studies and other systems.

Ruminant foods, especially dairy products, are the predominant sources of CLA (Chin *et al*., 1992). Fermented dairy foods generally contain higher CLA contents than nonfermented dairy products (Shantha *et al*., 1995). The cis-9, trans-11 CLA isomer is an intermediate in the biohydrogenation of linoleic acid by linoleic acid isomerase from the bacterium, Butyrivibriofibrisolvens, in the rumen (Kepler *et al*., 1966). Probiotic bacteria have also demonstrated linoleic acid isomerase activity and the ability to form CLA (Lin *et al*., 2003.)

Probiotic bacteria could actively enhance the health of consumers by improving the intestinal microbial balance. The positive effects associated with probiotics include antitumor activity, cholesterol reduction, protection against gastroenteritis, improvement of lactose tolerance, and stimulation of the immune system through nonpathogenic means (Rastall *et al*., 2000). Lactic acid bacteria (Lactobacilli, Streptococci, Lactococci, and Bifidobacteria) are the primary commercially available probiotics.

Several studies have shown that lactic acid bacteria can form CLA in model systems. Lin *et. al*., (1999) found that six lactic cultures *(Lactobacillus acidophilus, L. delbrueckii subsp. bulgaricus, L. delbrueckii subsp. lactis, Lactococcus lactis subsp. cremoris, Lc. lactis subsp. lactis, and Streptococcus salivarius subsp. thermophilus*) each increased CLA content by four-fold in a model system of sterilized skim milk and free linoleic acid.

Kishino *et al*. (2002) reported that *Propionibacterium freudenreichii subsp. freudenreichii and P. freudenreichii subsp. shermanii* produced CLA from free linoleic acid in in vitro systems. Probiotic bacteria, including *Lactobacillus rhamnosus, Propionibacterium freudenreichii* subsp. *shermanii* 56, P. *freudenreichii* subsp. *shermanii* 51 and *P. freudenreichii* subsp. *freudenreichii* 23, were able to produce CLA in a skim milk model system containing 1% hydrolyzed soy oil emulsified in nonfat dry milk (Xu *et al*., 2004).

Starter culture has been shown to affect the CLA content (Lin 2003; Xu *et al*., 2005) and sensory attributes of fermented dairy products (Penna *et al*., 1997; Xu *et al*., 2005). In study (Xu *et al*., 2005), only a small percentage of the free linoleic acid was isomerized to CLA. Thus, linoleic acid isomerase rather than the free linoleic acid may be the limiting factor in CLA formation. The aim of this study was to

1- Determine the CLA content of milk from cows offered diets rich in linoleic or linolenic acid. By feeding (Linseed oil) as a different source of energy (in concentrate feed mixture) for lactating cows.

2- Study the effect of using two different *Lactobacillus* spp. With or without yogurt culture for their ability to produce conjugated Linoleic acid and study increase the amount of CLA formation in fermented milk.

Materials and Methods

Materials

Milk:

Fresh cow milk was obtained from the herd supply of the Karada station, Animal Production Research Institute, Agriculture Research Center, Giza, Egypt.

This experiment was carried out at El-Karada. El-Karada Station is the experimental Station of Animal Production Research Institute which belonging to Agriculture Research Center during summer season 2014-2015.

Eight cows average 500±15.26 kg/body weight, were chosen and divided into two similar experimental feeding groups (4 in each). Animals in all of the experimental groups were housed under open loose system barns. The groups of animals were randomly assigned to receive four experimental ration containing concentrate feed mixture which included zero % and 3% linseed oil as source energy supply from the previous material was represent about 10% from total energy of concentrate feed mixture. All animals received concentrate feed mixture plus roughage with rate of 50:50 according NRC (2001). The feeding trial tested 180 days. During experimental trials, milk yield were determined and its composition were estimated.

Bacteria

Traditional yogurt culture (1:1 ratio of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; YC-180, Chr. Hansen Laboratories, Copenhagen, Denmark. *Lb. rhamnosus* TISTR 541 and *Lactobacillus acidophilus* TISTR 450 was kindly provided in freeze-dried form Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. *Lactobacillus plantarum* NRRL B – 4496 was obtained from the NRRL were selected for this study. Lactic acid bacteria were inoculated into Lactobacilli MRS broth (Difco, Detroit, Mich., U.S.A.) and incubated at 37◦C for 15h to reach a concentration of 10^8 cfu/ml. Individual starter cultures were prepared at concentrations of 10^8cfu/ml.

Growth and preservation media:

Cultures of lactobacilli were propagated for 24h at 37ºC in modified lactobacilli Difco MRS Broth. Stock cultures of lactobacilli was made by mixing a pure culture that had been grown over night with an equal volume of 10% glycerol solution and storing at -20ºC until experimental use (Van Den Berg *et al.,* 1995).The microbial count of the lactic acid bacteria in the fermented milk samples was determined using Lactobacilli MRS agar (Difco, Detroit,Mich., U.S.A.). Potato dextrose agar was using for yeasts and moulds count.

Chemicals:

Chemicals used in this study were of the analytical grade.

Methods:

Milk analysis, (total solids, solid not fat, protein, fat, Lactose) was determined using milkoscan (model 130 series – type 10900 FOSS electric – Denmark).

Viscosity and Syneresis Measurement:

The apparent viscosity was determined by using a RVDVII + Brookfield viscometer (Brook field Engineering Labs Inc., Stoughton, MA) in a 100 ml fermented milk products at room temperature (25 °C). Samples were stirred for 20 second before measurement. All viscosity values were measured at 10 rpm with spindle #5 Gassem AM, and Frank FJ. (1991). Readings were converted to centipoise units. Syneresis (%) was expressed as volume of drained whey per 100 ml sample Rodarte *et al*., (1993).

Physico-chemical analysis of fermented milk:

Titratable acidity, pH values, total solids and fat contents in fermented milk samples were determined according to AOAC, (2012). Acetaldehyde was estimated as mentioned by Lees and Jago (1969). The pH value was measured by using laboratory microprocessor pH meter model. Hanna HI 852.

Lipid analysis in milk

Milk samples containing no preservative were analyzed for fatty acid composition, including CLA content. To determine fatty acid composition in milk, milk fat was extracted by boiling in a detergent solution as described by Hurley *et al.,* (1987), weighed, capped under argon gas, and stored if needed at –20°C until further analysis. Fat samples were analyzed for fatty acids in a gas chromatograph as described by Dhiman *et al.,* (1999). Relative yield of CLA was calculated by multiplying the fat yield with CLA concentration of the respective week on an individual cow basis.

Lipid analysis in fermented milk

Lipids were extracted from fermented milk products using a modified Bligh and Dyer chloroform-methanol extraction method (Lin *et. al.,* 1995). Lipids were hydrolyzed with 1 N NaOH in methanol, followed by methylation of the fatty acids at room temperature with boron trifluoride (Xu *et al.* (2004). Heptadecanoic acid (C17:0, Sigma Chemical Co., St. Louis, Mo., U.S.A.) was added to the lipid extracts as an internal standard. Gas chromatography (GC) and GC-mass spectrometry were used for the quantitation and confirmation of the identification of the fatty acid methyl esters (Xu *et al.* (2004). The fatty acid methyl esters samples were analyzed for CLA content in fermented milk.

Microbiological analysis

Yeasts & moulds were determined according to Blanchette *et al.* (1996). Lactobacilli count was determined using MRS agar according to De Man *et al.* (1960).

Viscosity and syneresis measurement

Apparent viscosity was determined by using a RVDVII + Brookfield viscometer (Brookfield Engineering Labs Inc., Stoughton, Mass., U.S.A.) in a 100-ml fermented milk product at room temperature (25 °C). Samples were stirred for 20 s before measurement. All viscosity values were measured at 10 rpm with spindle #5, (Gassem and Frank 1991). Readings were converted to centipoise units. Syneresis (%) was expressed as volume of drained whey per 100 ml sample Rodarte *et al.* (1993).

Organoleptic properties were evaluated by panelists according to the scoring sheet outlined by Nelason and Trout (1956). All the treatments analysed when fresh and every week during storage period (for 4 weeks) at 5ºC±2ºC.

Statistical analysis

The study was designed as a two-way factorial experiment with bacterial treatment, and storage time as the main factors. Each treatment was replicated three times. The experimental data was analyzed using Duncan multiple range test (SAS version 8.2, Cary, N.C., USA, 2004) with a significance level of 0.

Experimental procedures:

Fermented cow milk Preparation:

Eight treatments of fermented cow milk was manufactured from two kinds of cow's milk obtained from cows fed control diet or diet contain 3% linseed oil as a lipid source (containing 8% (w/v) milk solids non-fat (MSNF) and 3.28% (w/v) fat, the milk solids-not-fat content was adjusted to 12% (w/w) through the addition of nonfat dry milk), 3.28% (w/v) fat. The cow's milk heat treated at 90ºC for 10 min then cooled to 42°C before inoculation. The first cow's milk was served as $control_1$ (cow milk was obtained from cows fed with control diet) of yogurt by using 3% of yogurt mixed culture with *S. thermophilus* and *L. bulgaricus*. The cow's milk (which was obtained from cows fed Linseed oil) was divided to seven portions. The first portion was served as $control_2$ was inoculated with 3% of yogurt mixed culture (*S. thermophilus* and *L. bulgaricus)*, the second portion was inoculated with 3% *L. rhamnosus* TISTR 541 alone, the third portion was inoculated with 1.5% of *L. rhamnosus* TISTR 541 and 1.5% of yogurt starter culture (1:1). The fourth portion was inoculated with 3% of *L. plantarum* NRRL B – 4496 alone and the fifth portion was inoculated with 1.5% of *L. plantarum* NRRL B – 4496 and 1.5% of yogurt starter culture (1:1), the sixth portion was inoculated with 3% *L. acidophilus* TISTR 450 alone and the seventh portion was inoculated with 1.5% *L. acidophilus* TISTR 450 and 1.5% of yogurt starter culture (1:1). All cultures were inoculated at approximately 1×10^8 cfu /g into 3 kg milk. Once inoculated, the yoghurts were incubated at 40°C, The Fermented cow milks were then stored at 5+2°C for 21 days. Viable lactobacilli in the Fermented cow milks were enumerated weekly using MRS agar. Cow milk with yogurt starter culture added was used as control. The fermented milk samples were analysed for Physico-chemical analysis, microbiological analysis and organoletic properties during storage period.

Results and Discussion

Daily milk yield and its composition:

Data presented in table (1) revealed that the actual milk yield was 12.06 and 12.76 Kg with animals fed rations A (control diet) and B (3% Linseed oil), respectively. The corresponding values as FCM yield were 10.61 and 11.38 Kg for respective rations.

The results showed that the milk yield either actual or corrected milk appeared to significant ($P<0.05$) higher than the control milk as shown in table (1) Increasing milk yield and FCM yield with animals fed ration B (3% Linseed oil) might be due to increase energy content of that ration.

On the other hand, all component of milk composition showed no significant difference among different experimental rations. On the other hand, all component of milk composition showed no significant difference among different experimental rations. Fat, protein, T.S, SNF and lactose percentage and their yield were no significant affect by different experimental rations. However somewhat higher in fat percentage was recorded with animals fed ration B (containing linseed oil) with animals fed ration A tended to higher of protein and T.S percentage. The results were in agreement with those reported by Abu Ghazalehal *et al.* (2007), Bu *et al.* (2007) and Chilliard *et al.* (2001).

Table (1) shows that unsaturated fatty acids composition in milk, fatty acids (C18:2) was higher (2.5%) in milk from cows were fed a control diet than milk obtained from cows were fed a diets containing linseed oil (2.1%) but fatty acids C18:0 was higher (12%) in milk from cows were fed a diets containing linseed oil than milk obtained from cows were fed a control diet (8.7%), also fatty acids C18:3 was higher (0.79%) in milk from cows were fed a diets containing linseed oil than milk obtained from cows were fed a control diet (0.6%).

Table (1) also shows that the conjugated linoleic acid (CLA yield g/d) was higher in milk fat (0.87 g/d and in milk obtained from cows fed with linseed oil than CLA yield in milk obtained from cows fed with a control diet (0.4 g/d), Average CLA contents in milk fat in week 25 of the experimental time through the 2 diet treatments were (1.03 mg CLA /g fat) in control milk and (2.07 mg CLA /g fat) in milk from cows fed 3 % linseed oil treatments, respectively.

Diets rich in linoleic or linolenic acid can increase CLA content of milk when dietary oil is accessible to the rumen microorganisms. There results were agreement with the finding of Chin *et al.* (1992). They described how the ALA and CLA content of milk can be increased, the omega-6/omega-3 ratio reduced, and the saturated fat content decreased by supplementing the rations of dairy cows with linseed.

Table 1: Average daily actual and fat corrected fat milk (FCM) and milk yield, milk composition, and milk fatty acid composition of cows fed control or linseed meal diets rich in linoleic and linolenic acid.

Items	A	B
Av. Milk yield (Kg/head/day)		
Av. Actual Milk yield	12.06[c]	12.76[b]
Av. 4% FCM yield	10.61[b]	11.38[b]
Av. Milk composition and its yield		
Fat%	3.21 [c]	3.28 [a]
Fat yield (gm/cow/day)	387 [c]	419 [b]
Protein%	2.67 [c]	2.60 [c]
Protein yield (gm/cow/day)	322 [c]	332 [c]
Lactose%	4.71 [c]	4.74 [b]
Lactose yield (gm/cow/day	568 [c]	605 [b]
Total solids%	11.30 [a]	11.28 [b]
Total solids yield (gm/cow/day)	1363 [b]	1439 [c]
Solids not fat%	8.09 [b]	8.00 [c]
solids not fat yield (gm CLA yield mg/d /cow/day)	976 [c]	1020 [c]
CLA mg/g fat	1.03[c]	2.07[a]
CLA yield g/d	0.4[c]	0.87[a]
Milk fatty acid, % of total fatty acids reported		
C16:0	39.8 [a]	36.3 [b]
C18:0	8.7 [b]	12.0 [a]
C18:2	2.5 [b]	2.1 [b]
C18:3	0.60 [b]	0.79 [a]

a, b and c: Means of different letter in the same raw are significant different.
FCM yield: were 10.61and 11.38 Kg for respective rations. *A: 4 Cows were fed a control diet*
B: 4 Cows were fed a diets containing linseed oil at 10% of diet. *CLA mg/g fat: conjugated linoleic acid content in milk milligram /g fat.*
CLA yield g/d: conjugated linoleic acid yield gram /day (CLA) concentration in milk fat.

Chemical composition of fermented milk:

Total solids and fat content were slightly decreased in all treatments throughout the storage period (Table 2). Tamime and Deeth (1980) explained this effect to lactose fermentation as well as protein and fat hydrolysis with the formation of volatile substances. Furthermore these values of total solids and fat contents were different among the treatments as a result of using different types of milk. Data revealed also that the type of starter culture had no obvious effect on the total solids and fat content of all treatments.

The general trend of pH values of all treatments during storage were oppositely to the corresponding values of acidity. The production of lactic acid by all bacterial treatments during incubation resulted in pH of the fermented milk ranging from 4.70 to 4.34.

Acetaldehyde content of all treatments were increased up to the first week of storage then decreased after that. This decrease is presumably due to not only to the numerous lactic acid producing microorganisms to utilize acetaldehyde (Bills & Day, 1966) but also, could be attributed to the volatile effect of acetaldehyde. Similar findings were reported by Bills *et al.* (1972) and Magdoub *et al.* (1992). Fermented cow milk with yohgurt culture and *L. plantarum* (T4 treatment) had relatively higher values in Acetaldehyde content during the storage period than the other ones. The highest value of Acetaldehyde content was found in (T4) treatment and the lowest value of Acetaldehyde content was to control$_1$ (C_1). Acetaldehyde level also changed according to the type of culture during storage period of fermented milk, where it was higher in (T4), (T3), (T5), (T2), (T1) treatments and then (T2) tre In general it is worth to note that type of diet of cow milk used had affected the T.S, fat, acidity, pH and acetaldehyde contents of all treatments. Moreover the type of starter was also affected the acidity and acetaldehyde contents of treatments along the storage period. atment after one week of storage, respectively.

Table 2: Effect of bacterial cultures, and storage period on the chemical composition of fermented milk treatments during storage for 3 weeks.

Items	Storage period (weeks)	Treatments							
		YC		*L. rah.*	YC+*L. rah.* (1:1)	*L. pl.*	YC+*L. pl.* (1:1)	*L. acid.*	YC+*L. acid.* (1:1)
		cow 's milk (C1)	cow 's milk (CO)	(T1)	(T2)	(T3)	(T4)	(T5)	(T46)
Total solids %	0	11.31 ^{d}	11.30^{d}	11.30^{d}	11.31^{d}	11.31^{d}	11.31^{d}	11.30^{d}	11.31^{d}
	1	11.33 ^{b}	11.31^{d}	11.32^{c}	11.32^{c}	11.32^{c}	11.32^{c}	11.32^{c}	11.32^{c}
	2	11.33 ^{b}	11.32^{c}	11.33^{b}	11.33^{b}	11.33^{b}	11.32^{d}	11.32^{b}	11.33^{b}
	3	11.35^{a}	11.33^{b}	11.35^{a}	11.34^{a}	11.33^{b}	11.34^{a}	11.33^{a}	11.34^{a}
Fat %	0	3.10^{d}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}
	1	3.11^{d}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}	3.28^{b}
	2	3.12^{c}	3.29^{a}	3.28^{b}	3.28^{b}	3.28^{b}	3.29^{b}	3.29^{b}	3.2^{b}
	3	3.12	3.29^{a}	3.29^{a}	3.29^{a}	3.29^{a}	3.29^{a}	3.29^{a}	3.29^{a}
pH value	0	4.64^{a}	4.7^{a}	4.7^{a}	4.7^{a}	4.7^{a}	4.7^{a}	4.56^{a}	4.6^{a}
	1	4.61^{a}	4.61^{a}	4.61^{a}	4.65^{a}	4.64^{a}	4.65^{a}	4.51^{a}	4.58^{a}
	2	4.53^{a}	4.39^{a}	4.60^{a}	4.60^{a}	4.44^{a}	4.54^{a}	4.43^{a}	4.52^{a}
	3	4.40^{a}	4.37^{a}	4.36^{a}	4.36^{a}	4.30^{a}	4.37^{a}	4.34^{a}	4.35^{a}
Acetaldehyde content (M mol / 100ml)	0	15.51d	14.50e	17.20^{b}	17.50^{b}	17.37^{a}	18.28^{a}	17.30^{b}	17.53^{b}
	1	19.62e	17.40f	21.75^{b}	21.82^{b}	21.89^{a}	21.96^{a}	20.85^{b}	21.62^{b}
	2	13.43c	13.41d	14.61^{b}	14.38^{b}	14.95^{a}	15.97^{a}	14.71^{b}	14.98^{b}
	3	13.25c	12.21c	14.37^{b}	14.29b	15.77a	14.80a	14.38b	14.49b

a Means in the same column followed by the same letter (a to c) are not significantly different (p > 0.05).
C1: fermented milk made from yogurt culture and Normal Cow milk from Cows fed with control diet. (control 1) .
C2: fermented milk made from yogurt culture and Cow milk from Cows fed with Linseed oil (control 2).
T1: fermented milk made from L. rahmnosus and Cow milk from Cows fed with Linseed oil.
T2: fermented milk made from yogurt culture+ L. rahmnosus and Cow milk from Cows fed with Linseed oil.
T3: fermented milk made from L. plantarum and Cow milk from Cows fed with Linseed oil.
T4: fermented milk made from yogurt culture+ L. plantarum and Cow milk from Cows fed with Linseed oil.
T5: fermented milk made from L. acidophilus and Cow milk from Cows fed with Linseed oil.
T6: fermented milk made from yogurt culture+ L. acidophilus and Cow milk from Cows fed with Linseed oil.
YC. : Yoghurt culture. L. rah: Lactobacillus rahmonosis. L. pl: Lactobacillus plantarum. L. acid.: Lactobacillus acidophilus .

Bacterial viability of fermented cow milk products

The microbial counts of all fermented cow milk products increased significantly during incubation at 40◦C and storage for 21 days at 5+2 ◦C, resulting in microbial counts of 1.84×10^{8} to 1.650×10^{9}cfu/ml (Table 3). Since a majority of the health benefits associated with probiotic bacteria depend on cell viability, it is important to maintain high levels of viable bacteria in probiotic foods. The bacterial counts should exceed the suggested minimum count (10^{7}cfu/ml), which is recommended to affect the gut environment and provide health benefits (Vinderola and Reinheimer 1999). All fermented milk samples had high viable lactic acid bacteria counts (>10^{8} cfu/ml) at the end of the 21 days storage period.

Type of culture had an obvious correlation with the counts of organisms during storage. Generally, it was concluded that the viability of strains after the storage period was sufficient to yield numbers of beneficial organisms that were higher than the accepted threshold (10^{6}cfu /g) for a probiotic effect (Sammona& Robinson, 1994 and Gomes & Malcata, 1998).

Table 3: Effect of bacterial cultures, and storage period on microbial counts of fermented cow milk products.

Culture[1]	Inoculation level cfu/ml	Storage period (weeks)	Lactobacilli counts (log10 cfu/ml)	Molds & Yeasts counts (log10 cfu/ml)
YC (3%) C1	10^8	0	8.47 [a]	ND
		1	9.10 [a]	ND
		2	9.05[a]	1 [b]
		3	8.71[ab]	2.47 [a]
YC (3%) C2		0	8.47 [a]	ND
		1	9.10 [a]	ND
		2	9.05[a]	1 [b]
		3	8.74[ab]	2.30 [a]
L. rah. (3%) (T1)	10^8	0	8.66 [a]	ND
		1	9.14 [a]	ND
		2	8.91 [a]	1 [b]
		3	8.97 [a]	1 [b]
YC+*L. rah*. (1:1) (T2)	10^8	0	8.16[b]	ND
		1	8.24[b]	ND
		2	8.46 [a]	1 [b]
		3	8.11[b]	1.5 [a]
L. pl. (3%) (T3)	10^8	0	8.16[b]	ND
		1	8.56[a]	ND
		2	8.52[a]	ND
		3	8.31[ab]	1 [b]
YC+*L. pl.* (1:1) (T4)	10^8	0	7.16[b]	ND
		1	8.81 [a]	ND
		2	8.96[a]	1 [b]
		3	8.76[a]	1.3 [a]
L. acid.. (3%) (T5)	10^8	0	8.14[b]	ND
		1	8.47[a]	ND
		2	8.37[a]	ND
		3	8.30[ab]	1.5 [b]
YC+*L. acid..* (1:1) (T6)	10^8	0	7.65[b]	ND
		1	8.90 [a]	ND
		2	8.97[a]	1.0 [b]
		3	8.71[a]	1.3 [a]

a *Means in the same column followed by the same letter (a to c) are not significantly different (p > 0.05).*
ND: not detected *Culture[1]: See table 2 for details.*

Table (4) shows that titratable acidity of all treatments was also increased as the storage period progressed. This result was in agreement with Shah (2000) who found that *L. delbrueckii ssp. bulgaricus* produces lactic acid during fermentation and refrigerated storage. The highest value was noticed for fermented cow milk with *L. rahmnosus* (T1) whereas the lowest one was for fermented cow milk with yogurt culture and *L. plantarum* (1:1) (T4). The values of acidity were relatively higher in (T1) treatment than in the other treatments along the storage period. Also, type of cow's diets milk markedly affected these values.

The acidity of fermented cow milk products has a significant impact on the flavor of the titratable acidities of the fermented milk products in this study were below the titratable acidity at which unpleasant acid tastes are detected.

Yogurt cultures, *L. rhamnosus, L. acidophilus* and *plantarum* belong to the genus Lactobacillus, which produce lactic acid as its major product by the fermentation of hexoses. The production of lactic acid by all bacterial treatments during incubation resulted titratable acidities ranging from 0.56 to 1.15 g lactic acid/100 g fermented milk.

Table (4) also show that production of lactic acid during fermentation reduces the pH of the milk to the isoelectric point (pH 4.6) of casein and contributes to the formation of a stable gel network with minimal syneresis. In general, bacterial culture treatments had no significant effect on the viscosity or degree of syneresis of the fermented milk products. Table (4) also shows that the lower viscosity for the *L. rahmnosus* treatments is attributed to the higher pH and the incomplete acid precipitation of the casein. The results of this study were agreement with research of (Xu *et al*., 2005).

Table 4: Effect of bacterial cultures, and storage period on titratable acidity, syneresis and viscosity in fermented cow, s milk products.

Culture[1]	Sample	Acidity (g lactic acid/100g)				degree of syneresis (%)				Viscosity (centipoise)			
		1 Day	7 Day	14 Day	21 Day	1 Day	7 Day	14 Day	21 Day	1 Day	7 Day	14 Day	21 Day
YC	C1	0.8[a]	0.85[a]	0.87[a]	0.9[a]	6a	10a	12a	15[b]	225000[a]	225000[a]	224000[a]	223000[a]
	C2	0.7[a]	0.74[a]	0.76[a]	0.82[a]	6a	10a	12a	16[b]	226000[a]	226000[a]	225000[a]	224000[a]
L. rah. (3%)	T1	1.11[a]	1.12[a]	1.13[a]	1.15[a]	15[b]	19[b]	21[b]	23[a]	237000[a]	237000[a]	236000[a]	235000[a]
L. rah. + YC (1:1)	T2	0.56[b]	0.66[b]	0.76[b]	0.86[b]	12[b]	15[b]	19[b]	21[a]	57300[bc]	57300[bc]	57200[bc]	57100[bc]
L. pl. (3%)	T3	0.61[b]	0.66[b]	0.69[b]	0.78[b]	44[a]	47[a]	49[a]	50[a]	224000[a]	224000[a]	223000[a]	222000[a]
YC+*L. pl.* (1:1)	T4	0.83[a]	0.84[a]	0.87[a]	0.88[a]	16[b]	19[b]	21[b]	23[a]	227000[a]	227000[a]	226000[a]	225000[a]
L. acid. (3%)	T5	0.60[b]	0.64[b]	0.67[b]	0.75[b]	44[a]	45[a]	47[a]	49[a]	234000[a]	234000[a]	221000[a]	220000[a]
YC+*L. acid.* (1:1)	T6	0.80[a]	0.82[a]	0.85[a]	0.86[a]	15[b]	18[b]	20[b]	22[a]	226000[a]	226000[a]	225000[a]	224000[a]

a Means in the same column followed by the same letter (a to c) are not significantly different (p > 0.05).
Culture[1]: See table 2 for details.

Table (5) shows that yogurt culture, *L. rhamnosus, L. acidophilus* and *L. plantarum* belong to the genus Lactobacillus, which produces lactic acid as its major product by the fermentation of hexoses. The production of lactic acid by all bacterial treatments during incubation resulted in pH of the fermented milk products ranging from 5.90 to 4.17 during incubation at 40°C for 3.5 hour of incubation time with pH and titratable acidity inversely related.

Table (6) shows that using of *L. plantarum* as a co-cultured with yogurt culture (T4) or *L. plantarum* alone in fermented cow's milk was more effective in the formation of CLA than using *L. rahmnosus* alone or L. acidophilus used alone or as a co-culture with yogurt culture. This was agreement with (Xu *et al.*, 2005).

Results in Table (6) show that the highest value of CLA content was noticed for fermented cow milk with yogurt culture and *L. plantarum* (T4 treatment) followed by (T4, T1, T5, T3, T6, T2 and C_2 treatments respectively) whereas the lowest one was for fermented cow milk cultured with yogurt culture alone (C_1 treatment as a $control_1$ it was made from milk from cows fed a control diet). The type of culture markedly affected the values of CLA content. There was significant different of CLA content during storage period for all fermented cow milk treatments. Total CLA contents ranged from 0.79 in C_1 to 2.96 (mg CLA/g fat) in T4 treatment following 21 days of storage.

Table 5: Effect of bacterial cultures on pH in fermented cow milk Products during incubation time at 40°C.

Sample	Culture1	pH 1h	pH 2h	pH 2.5h	pH 3h	pH 3.5h
C1	YC (3%)	6.1[a]	5.2[a]	4.8[a]	4.77[a]	4.70[a]
CM	YC (3%)	6.2[a]	5.6[a]	4.9[a]	4.81[a]	4.17
(T1)	L. rah. (3%)	6.2[a]	5.2[a]	4.8[a]	4.77[a]	4.70[a]
(T2)	L. rah. + YC (1:1)	6.1[a]	5.1[a]	4.6[a]	4.76[a]	4.68[a]
(T3)	L. pl. (3%)	6.10[b]	5.2[a]	4.8[a]	4.77[a]	4.74[a]
(T4)	YC+L. pl. (1:1)	5.9[a]	4.8[a]	4.77[a]	4.74[a]	4.31
(T5)	L. acid. (3%)	6.10[b]	5.3[a]	4.7[a]	4.70[a]	4.72[a]
(T6)	YC+L. acid. (1:1)	6.0[a]	4.9[a]	4.75[a]	4.73[a]	4.33

a Means in the same column followed by the same letter (a to c) are not significantly different ($p > 0.05$).
h: hour Culture[1]: See table 2 for details.

Table 6: Effect of bacterial cultures on concentration of CLA (mg /g fat) produced in fermented cow milk Products.

Sample	Culture[1]	CLA (mg /g fat)			
		1 Day	7 Day	14 Day	21 Day
C1	YC (3%)	1.03[c]	0.91[c]	0.88[c]	0.79[d]
C2	YC (3%)	2.07[b]	2.61[b]	2.55[b]	2.46[b]
(T1)	*L. rah*. (3%)	2.07[b]	2.72[a]	2.70[a]	2.68[c]
(T2)	*L. rah*. + YC (1:1)	2.07[b]	2.64[b]	2.61[b]	2.56[b]
(T3)	*L. pl.* (3%)	2.07[b]	2.68[ab]	2.46[ab]	2.43[c]
(T4)	YC+*L. pl.* (1:1)	2.07[b]	2.96[a]	2.79[a]	2.65[ab]
(T5)	*L. acid.* (3%)	2.07[b]	2.70[ab]	2.45[ab]	1.40[c]
(T6)	YC+*L. acid.* (1:1)	2.07[b]	2.66[b]	2.67[a]	2.63[ab]

a Means in the same column followed by the same letter (a to c) are not significantly different ($p > 0.05$).
CLA: (mg /g fat): conjugated linoleic acid(mg/g fat). Culture1: See table 2 for details.

Sensory evaluation

Data revealed that the fermented milk samples made from cow's milk from cows fed with Linseed oil (T4 treatment) had the highest total sensory score throughout the storage compared to the other treatments. This is attributed to the difference in chemical composition and total solids of used milks. While, the lower total sensory score were given to the fermented milk (T3 treatment contain *L. plantarum* culture alone) when fresh and throughout the storage period. No foreign or undesirable flavour was detected in all treatments.

However, the body and texture of the fermented milk treatments made from cow's milk with *L. plantarum* culture alone (T3 treatment) had the lowest scores compared to all other treatments along the storage. Also, no acid flavor (below pH 4.3) was noticed in all treatments during storage**.** The flovour of fermented milk made with *L. plantarum* as a co culture with yogurt culture (T4 treatment) was to some extent less pronounced as yoghurt culture fermented milk. Most judgers found pleasant flovour of fermented milk made with *L. plantarum* as a co culture yogurt culture and *L. acidophilus* with yogurt culture (T6 treatment) as compared with other fermented milk treatments and it had a good texture. On the other hand no effect on the appearance of the product.

Table 7: Effect of type of milk and starter culture on sensory evaluation of fermented milk during storage for 21 days.

Items	Storage period (weeks)	Treatments[1]							
		Yoghurt culture		*L. rah.*	YC+*L. rah.* (1:1)	*L. pl.*	YC+*L. pl.* (1:1)	*L. acid.* (3%)	YC+*L. acid.* (1:1)
		cow 's milk (C_1)	cow 's milk (C_2)	(T1)	(T2)	(T3)	(T4)	(T5)	(T6)
Flavour (45)	0	44^a	44^b	43^a	44^b	42^a	44^a	42^a	44^b
	1	43^a	35^{ab}	38^b	41^a	38^b	39^b	38^b	40^a
	2	39^a	30^b	34^b	38^{ab}	35^a	36^a	33^b	38^{ab}
	3	38^{ab}	29^b	33^a	37^{ab}	34^a	35^a	32^a	37^{ab}
Body &Texture (30)	0	28^a	27^a	27^a	28^a	27^b	29^a	27^a	28^a
	1	25^a	25^a	26^a	27^a	27^b	27^b	26^a	26^a
	2	23^a	23^a	25^a	25^a	24^b	25^b	24^a	24^a
	3	22^a	22^a	23^b	24^a	23^b	24^b	23^b	23^a
Acidity (10)	0	9^a	8^a	8^a	9^a	8^a	9^a	8^a	8^a
	1	8^a	7^a	8^a	8^a	8^a	9^a	8^a	7^a
	2	7^a	6^a	6^a	7^a	6^a	7^a	6^a	6^a
	3	6^a	5^a	5^a	6^a	5^a	6^a	4^a	5^a
Appearance (15)	0	14^a	14^a	14^a	14^a	13^b	14^b	13^a	13^a
	1	13^a	13^a	12^a	13^a	12^b	13^b	11^a	12^a
	2	11^a	10^a	11^a	12^a	11^b	12^b	10^a	11^a
	3	10^a	10^a	10^a	11^a	10^b	11^b	9^a	10^a
Total (100)	0	95^a	93^a	92^a	95^a	82^b	96^a	91^a	93^a
	1	89^a	80^{ab}	88^a	89^{ab}	67^c	89^{ab}	83^a	85^{ab}
	2	80^a	69^b	76^a	82^{ab}	61^b	76^b	73^a	79^{ab}
	3	76^b	66^b	71^c	78^b	61^b	72^b	68^c	75^b

a Means in the same column followed by the same letter (a to c) are not significantly different (p > 0.05). Treatments[1]: See table 2 for details.

Conclusions

The incorporation of probiotic bacteria into dairy products to provide nutritional benefits represents challenging opportunities for the food industry. Inoculation of the yogurt culture with or without *L. rhamnosus* or *L. acidophilus* or *L. plantarum* did not significantly affect the microbial counts of the fermented milk products but *L. plantarum* as a co culture with yoghurt culture (T4) or *L. plantarum* culture alone (T3) was significantly affect CLA content of the fermented milk products. It was found that using *L. plantarum* as a co culture with yoghurt culture (T4) had the highest CLA content (2.96 mg/g fat) than the other treatments. However, all treatments resulted in microbial counts greater than the recommended 10^7 cfu /ml needed to affect the gut environment and provide health benefits. So it was concluded that *L. plantarum* or *L. rhamnosus* or *L. acidophilus* culture combination with yoghurt culture was important to Produce fermented cow milk products with enhanced CLA concentration and good quality comparable to the control. The success of new functional foods will not only depend on the enhanced nutritional value, but also desirable sensory quality of the product to meet consumers' demands.

References

AOAC. 2012. Association of Official Analytical Chemists, Official methods of analysis 19th(Ed), vol. (2), Arlington, VA. USA.

Abu Ghazaleh, A. A. and L. D.Holmes, 2007. Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. J. Dairy Sci.90:2897–2904.

Bills, D. D., and E .A. Day, 1966. Dehydrogenase activity of lactic streptococci. J. Dairy Sci., 49 (12) 1473.

Bills, D. D., C. S. Yang, M. E. Morgan, and F.W. Body Flet, 1972. Effect of sucrose on the production of acetaldehyde and acids by yoghurt culture bacteria. J. Dairy Sci., 55(11):1570.

Blanchette, L., D.Ray, G.Belanger, and S. F. Gauthier, 1996. Production of Cottage cheese using dressing fermented by Bifidobacterium. J. Dairy Sci., 79: 8-13.

Bu, D. P., J. Q. Wang, T. R. Dhiman and S. J. Liu, 2007. Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugatedlinoleic acid in milk from dairy cows. J. Dairy Sci. 90:998–1007.

Chilliard, Y. F., F.Glasser, A.Ferlay, L.Bernard, J.Rouel, M.Doreau, 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat: A review. Eur. J. Lipid Sci.Tec. 109, 828–855.

Chilliard, Y., A.Ferlay, and M.Doreau, 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livestock Production Science, 70:31.

Chin, S. F., W. Liu, and J. M. Storkson, 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Anal. 5: 185-197.

Chin, S. F., J. M. Storkson, K. J. Albright, M. E. Cook and M.W. Pariza, 1994. Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. J. Nutr. 124:2344–9.

Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips and D. E. Bauman, 2001. The role of Δ9– desaturase in the production of cis-9, trans-11 CLA. J.Nutr. Biochem.

De Man, J. C., M.Rogosa and M.E. Sharp, 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 22, 130-136.

Dhiman, T. R. Helmink, E. D. McMahon, D. J. Fife, R. L. and Pariza, M. W. 1999. Conjugated linoleic acid content of milkand cheese from cows fed extruded oilseeds. J. Dairy Sci.82:412–419.

Gassem, A.M. and F.J. Frank, 1991. Physical properties of yogurt made from milk treated with proteolytic enzymes. J Dairy Sci 74:1503–11.

Hess, B.W., G. E. Moss and D.C. Rule, 2008. A decade of development in the area of fat supplementation research with beef cattle and sheep. J. Anim. Sci. 86: 188–204.

Houseknecht, K., J. Helvel, S. Moya-Camarena, C. Portocarrero, L. Peck, K. Nickel and M.Belury, 1998. Dietary conjugated linoleic acid normalize impaired glucose tolerance in zucker diabetic fatty fa/fa rat. Biochem. Biophys. Res. Commun. 244:678–82.

Hurley, W. L., G. J. Warner and R. R. Grummer, 1987. Changesin triglyceride fatty acid composition of mammary secretionsduring involution. J. Dairy Sci. 70:2406–2410.

Jan, M., K. Renata, K.Edyta and B.Maciej, 2013. Effect of the variety of linseed and its form (rolled or extruded) in dairy cow diets on nutrient digestibility. Annales Universitatis Mariae Curie-Sklodowska – Polonia, Vol. (2) Sectio EE.

Kepler, C.R., K.P. Hirons, J.J. McNeill and S.B. Tove, 1966. Intermediates and products of the biohydrogenation of linoleic acid by Butyrivibriofibri solvens. J. Biol. Chem. 241:1350–4.

Kishino, S., J.Ogawa, Y.Omura, K. Matsumura and S.Shimizu, 2002. Conjugated linoleic acid production from linoleic acid by lactic acid bacteria. J. Am. Oil Chem. Soc. 79:159–63.

Lees, G.J. and G. R. Jago, 1969. Methods for the estimation of acetaldehyde in cultured dairy products. Aust. J. Dairy Tech. 24:181.

Lin, H., T. D. Boylston, M. J. Chang, L. O. Luedecke and T. D. Shultz, 1995. Survey of conjugated linoleic acid contents of dairy products. J. Dairy Sci. 78:2358–65.

Lin, T. Y., 2003. Influence of lactic cultures, linoleic acid and fructo-oligosaccharides on conjugated linoleic acid concentration in non-fat set yogurt. Austral. J. Dairy Technol. 58(1):12–4.

Lin, T.Y., C.W. Lin and Y. J. Wang, 2002. Linoleic acid isomerase activity in enzyme extracts from *Lactobacillus acidophilus* and *Propionibacterium freudenreichii ssp. shermanii*. J. Food Sci. 67:1502–5.

Lin, T. Y., C.W. Lin and C. H. Lee, 1999. Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. Food Chem. 67:1–5.

Magdoub, M. N., E. O. Fayed, Nargis H. Mohamed and M.M.Salem, 1992. Utilization of soy protein in the manufacture of zabadi. Egypt. J. Food Sci. 20: 253-262.

Nelason, J. A. and G. M. Trout, 1956. Judging Dairy production 4th Ed. The Oleson Publishing Co. MilwoukeeWis. 53212.

Nicolosi, R.J., E. J.Rogers, D.Kritchevsky, J.A. Scimeca, and P.J. Huth, 1997. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery 22:266–77.

NRC., 1989. Nutrient Requirements of Dairy Cattle.National Academy Press, Washington, DC.

Penna, A. L. B., R.Baruffaldi and M.N. Oliveira, 1997. Optimization of yogurt production using demineralized whey. J. Food Sci 62:846–50.

Rego, O. A., H. J. D. Rosa, P. Portugal, T. Franco, C. M. Vouzela, A. E. S. Borba, and R. J. B. Bessa, 2005. The effects of supplementation with sunflower and soybean oils on the fatty acid profile of milk fat from grazing dairy cows. Anim. Res. 54:17–24.

Rodarte, C. W., M. V.Galvan, A.Farres, F. Gallardo, V. E. Marshall and M. G. Garibar, 1993. Yogurt production from reconstituted skim milk powders using different polymer and nonpolymer forming starter cultures. J Dairy Res 60:247–54.

SAS, 2004. Statistical Analysis System SAS User᾿s Guide Statistics SAS Institute Inc. Editors, Cary, NC.

Shah, N. J., 2000. Probiotic bacteria; Selective enumeration and survival in dairy foods. J. Dairy Sci., 83:894.

Shantha, N. C., L. N. Ram, J.O'Leary, C. L. Hicks and E. A. Decker, 1995. Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. J. Food Sci. 60:695–7.

Tamime, A. Y. and H. C. Deeth, 1980. Yoghurt: Technology and biochemistry . J. Food Protection. 43: 939.

Van den berg, D. , J. C. Rabijin, G.W. Jenssen, M. L. F. Giuseppin, R. Vereekev, J.P. Kamerling, J. F. G. Vliegentfort, A. M. Ledeboer and C. T. Verrips, 1995. Production of a novel extracellular polysaccharide by Lactobacillus sake O-1 and characterization of the polysaccharides. Appl. Environ. Microbiol., 61, 2840-2845.

Vinderola, C. G. and J. A., Reinheimer, 1999. Culture enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yogurt bacteria. Int. Dairy J 9:497–505.

Xu S., T. D. Boylston and B. A. Glatz, 2004. Effect of lipid source on probiotic bacteria and conjugated linoleic acid formation in milk model systems. J Am Oil Chem. Soc 81:589–95.

Xu S., T. D. Boylston and B. A. Glatz, 2005. Conjugated linoleic acid content and organoleptic attributes of fermented milk products produced with probiotic bacteria. J Ag Food Chem. 53:9064–72.