Original scientific paper - Izvorni znanstveni rad UDK: 637.072/637.142

Fatty acid composition of cream fermented by probiotic bacteria

Lutfiye Yilmaz-Ersan

Uludag University, Department of Food Engineering, 16059, Gorukle, Bursa-Turkiye

Received - Prispjelo: 24.04.2013.
Accepted - Prihvaćeno: 12.07.2013.

Summary

The production of fatty acids in cream containing one of the three probiotic microorganisms (*Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus)* was evaluated at 4±1 °C for up to 15 days. Gas chromatographic analysis of the fatty acid content showed that during storage the amount of linoleic and α-linolenic acids increased in the probiotic cream fermented with *B. lactis* compared to the control cream. Probiotic bacteria were all associated with increases in medium chain and polyunsaturated fatty acid content in fermented cream. The highest amounts of saturated fatty acids were found in cream fermented with *L. acidophilus*, while the highest amounts of monounsaturated and polyunsaturated fatty acids were found in cream fermented with *B. lactis*. The results of this study demonstrate that probiotic bacteria could improve the fatty acid profile of fermented creams and provide high value-added dairy products.

Key words: probiotic, cream, fatty acids

Introduction

The beneficial effects of foods with added nutritive value on human health are being highly promoted by health professionals. This awareness has led to an increased market demand particularly within children and other high-risk population for functional dairy products that contain beneficial components such as specific proteins, peptides and fatty acids produced via the metabolic activities of the added lactic starter cultures. There has been an increased interest in understanding the metabolism of milk lipids and their role in the human health and well-being (Lock and Bauman, 2003; Park, 2009).

Milk lipids represent a good dietary source of the liposoluble vitamins such as retinol, α-tocopherol and β-carotene, as well as the essential fatty acids. The fatty acid composition of milk is not only effective on physical properties, oxidative stability and organoleptic quality of dairy products, but also has positive effects on human health. Thus, recent clinical studies have focused on the bioactive fatty acids such as butyric acid, oleic acid and conjugated linoleic acid (CLA), which may show key roles in the prevention of certain diseases. The fatty acid content of milk is affected by numerous factors such as the geographical location, the quality of farming practices, the breed type and the genetic and physiological factors of the animals. In addition, milk processing conditions like the heat treatment, the added starter culture, the ripening period and the storage temperature also affect the fatty acid composition of different dairy products (Collomb and Bülher, 2000; Chilliard et al., 2001; Ledoux et al., 2005; Georgala et al., 2006; Tamime, 2009; Nunes and Torres, 2010).

Ripened/cultured/fermented/sour(ed) cream is manufactured by the fermentation of standardized, homogenized and heat-treated sweet cream using lactic acid bacteria. Cream may also be produced by using food grade acidulant like citric acid. This fermented product has more pleasant acidic taste, buttery aroma, higher yield and lower risk of contamination after heat treatment than sweet cream (Kurman et al., 1992; Law, 1997; Mattila-Sandholm and Saarela, 2003; Born, 2006; Ekinci et al., 2008; Tamime, 2009).

*Corresponding author/Dopisni autor: Phone/Tel: 00 90 224 29 41 505; E-mail: lutfiyey@uludag.edu.tr

The most endorsed definition of probiotics is "viable microbial dietary supplements which exert beneficiary impact on the health of the host". It is important to denote that they confer health benefits when administered alive in adequate amounts. The primary probiotic bacteria associated with dairy products have been *Bifidobacteria*, *Lactobacillus acidophilus* and *Lactobacillus casei*. The use of these bacteria as starter culture depends on their potential health or nutritional benefits following consumption (Naidu et al., 1999; Sanders, 2008). The composition of fermented dairy products such as yoghurt, cheese and butter have been investigated by many authors (Bergano et al., 2003; Coskun and Ondul, 2004; Ledoux et al., 2005; Georgala et al., 2006), however, there are limited data on the fatty acid profile of fermented cream (Ekinci et al., 2008; Domagala et al., 2009). It is well-documented that probiotics are able to change the fatty acid profile of milk as a result of forming biologically active fatty acids during fermentation (Mattila-Sandholm and Saarela, 2003; Yadav et al., 2007; Ekinci et al., 2008). Therefore, the objective of this study was to determine the fatty acid composition during cold storage of fermented creams produced by three strains of probiotic bacteria, namely *Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*.

Materials and methods

Cream sample and cultures

Commercial UHT cream samples for this study were provided by the SEK Dairy Plant Bursa, Turkey. Concentrated and freeze-dried probiotic strains *Bifidobacterium lactis (*HOWARU tm Bifido), *Lactobacillus acidophilus* (HOWARU tm Acidophilus NCFM) and *Lactobacillus rhamnosus* (HOWARU tm Rhamnosus) were obtained from Deutschland GmbH, Niebüll, Germany. Each lyophilized strain were prepared according to Ozcan et al. (2010), using 1 g of lyophilized culture in 100 mL 12 % (w/v) reconstituted sterile non-fat milk (121 °C for 15 min). The probiotic cultures were incubated at 37±1 °C for 18 h under anaerobic conditions by the Anaerobic System Anaerogen (Oxoid). The necessary inoculum, to give approximately 7.0 $\log_{10}$ colony forming units (CFU) mL^{-1} in cream after inoculation, was calculated.

Fermented cream production and analysis

As soon as the cream reached the desired temperature, calculated amounts of probiotic cultures were inoculated to the cream at initial counts of approximately 7.0 $\log_{10}$ colony forming units (CFU)·mL^{-1} at the beginning of the fermentation time. After inoculation of the cultures, the cream samples were incubated overnight at 25 °C until the pH reached 4.7 and anaerobic conditions were ensured by the use of AnaeroGen (Oxoid Ltd., Basingstoke, UK) during fermentation. After the fermentation, the cream samples were cooled down to 5±2 °C and stored at 4 °C for 15 days. A non-cultured cream was chosen as the control sample. The fatty acid analyses were carried out after 2, 8, and 15 days of storage at the refrigerator temperature (4±1 °C).

Lipid extraction

Lipid extraction have been carried out after digestion with HCl according to AOAC (2000) methods. Portions of 2-5 g of the homogenized sample were weighed with an electronic scale (Sartorius-4102D, Denver Instrument Co., Denver, CO) and placed in a thimble for instrumental extraction. The fat content of the samples was extracted at 50 °C for 3 h using a solvent extractor (SER 148, Velp Scientifica, Usmate, Italy) with diethyl ether as the solvent.

Fatty acid profile analysis

The fatty acid (FA) contents were determined using fatty acid methyl esters (FAMEs), which were prepared according to the IUPAC method II.D.19. In brief, 0.1 g extracted milk fat was placed into a test tube with a screw cap. Then, 0.5 mL 2.0 N KOH and 5 mL heptane were added and vortexed followed by addition of anhydrous sodium sulfate for drying. After 1 minute, the standing solution was used directly for gas chromatography (GC, Perkin Elmer, Auto System GLX, Shelton, U.S.A.). Chromatographic separation was performed using a Supelco SP™ -2380 (30 m x 0.25 mm i.d., 0.25 μm film thickness) column equipped with a flame ionization detector (FID). Table 1 shows the instrument settings. The data were collected and quantified with a Total Chrom Navigator. The results were expressed as percent concentration.

Table 1. The instrument settings of gas chromatography for fatty acid composition

Apparatus	Perkin Elmer Auto System
Experiment time	32 min
Carrier Gas	Helium 0.5 mL/min
Oven Temperature programme	
Rate 1	120 °C for 2 min isotherm
Rate 2	5 °C/min to 220 °C hold for 10 min isotherm
Injection volume	1.0 µL split
Injection temperature	280 °C
Split control	
Mode	Flow
Flow rate	50 mL/min
Split ratio	1:50

Identification of the peaks was achieved based on the retention times and by referencing the GC/MS spectra of the authentic standards and the identified compounds in the TUBITAKMRC, NIST and WILEY Libraries. The peak areas of triplicate injections were measured with an HP computing integrator. The fatty acid concentration of the cream samples were expressed as g/100 g of lipids, as calculated with peak areas that were corrected by factors according to the AOAC 963.22 method (AOAC, 2000).

Statistical analysis

A factorial experiment was designed with the product type and the storage time as the main factors. The results were submitted to variance analysis (ANOVA), at the 0.1 %, 1 % and 5 % significance levels, using the Statistica software package (StatSoft, USA, 1996). Analysis of variance with mean separations using the LSD multiple range test as the level of significant difference was used to determine the effect of starter culture differences and storage time on fatty acid contents. Different letters were used to label values with statistically significant differences among them.

Results and discussion

To our knowledge, this is the first study that reports the differences in fatty acid concentrations of cream fermented with the probiotic bacteria namely *B. lactis*, *L. acidophilus* and *L. rhamnosus* with respect to control. The gas chromatography analysis of the fatty acids from the lipids of cream samples revealed the presence of 24 fatty acids. The major differences in the fatty acid profiles among each of the four types of cream samples were related to the degree of saturation of the compounds, depending on the type of probiotic bacteria. The major differences between fermented creams were observed in myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (*c* C18:1n-9), elaidic (*t* C18:1n-9), linoleic (*c* C18:2n-6) and α-linolenic acid (18:3n-3, ALA) (Table 2). The cream fermented with *B. lactis* had significantly higher ($P<0.001$) mean values for oleic acid compared with the other samples. Oleic acid content was higher in cream fermented with *B. lactis*, vice versa to caprylic acid and capric acid contents. With regard to the profiles of the most important fatty acids, the addition of a probiotic culture did not lead to any significant variations ($P>0.05$) in the average values of butyric (4:0), caproic (6:0), caprylic (8:0) or conjugated linoleic (*c*9*t*11 C18:2, CLA) acids (Table 2). The saturated fatty acids C14:0, C16:0, C18:0 and the monounsaturated fatty acid *c* C18:1n-9 were predominant fatty acids in all samples. Palmitic acid was the most abundant fatty acid in the cream fermented with *L. acidophilus*.

Acids 18:2n-6 and 18:3n-3 are the main PUFAs in milk fat as reported by Dewhurst et al. (2006). In our experiment, the cream fermented

with *B. lactis* had the highest content of 18:2n-6 (P<0.001), which is an important precursor of CLA through the biohydrogenation pathway during lactic acid fermentation (Ekinci et al., 2008). However, in milk fat, the presence of other interesting compounds such as butyric acid (C4:0) and CLA should be noted from a nutritional point of view. Both compounds were detected in fermented creams at levels of approximately 2.20 and 0.84 g/100 g, respectively (Table 2). Nevertheless, in the present study, no differences in the levels of CLA in probiotic fermented cream as well as control were detected (P>0.05). CLA content of fermented were shown to differ within strains used depending on substrate concentration, culture media, temperature and time of fermentation (Nieuwenhove et al., 2012). Lin et al. (1995) demonstrated that the CLA content of sour cream ranged from 0.38 to 0.47 g/100 g fat. In general, the CLA concentrations are in agreement with the values reported by Ekinci et al. (2008) and Domagala et al. (2009) for cream containing various probiotic bacteria.

The contents of ALA, the precursor of the longer chain omega-3 fatty acids (Simopoulos, 2009), were approximately 0.18 g/100 g of lipids in the control cream and ranged from 0.14 g to 0.19 g/100 g of lipids in the fermented creams. The higher ALA concentration in the cream fermented with *B. lactis*, strongly suggests a high nutritional value for fermented cream (P<0.001). These observations are noteworthy because the dietary ingestion of ALA, which may prevent cardiovascular disease, is an im-

Table 2. Fatty acid composition of cream (control) and fermented cream samples with various probiotic bacteria (g/100 g of lipids)

Fatty acid[a]	Control	*B. lactis*	*L. acidophilus*	*L. rhamnosus*	SD[b]	P-value[c]
SCFA						
C4:0	2.17	2.20	2.18	2.21	0.057	ns
C6:0	1.57	1.57	1.59	1.59	0.042	ns
MCFA						
C8:0	0.99	0.98	1.01	0.99	0.028	ns
C10:0	2.29[c]	2.28[c]	2.33[a]	2.31[b]	0.042	*
C12:0	2.78	2.77	2.83	2.81		ns
C14:0	10.30[b]	10.24[b]	10.45[a]	10.43[a]	0.099	***
C15:0	1.09	1.09	1.10	1.10	0.127	ns
C14:1	1.46	1.45	1.47	1.47	0.085	ns
LCFA						
C16:0	30.50[b]	30.20[c]	30.75[a]	30.68[a]	0.240	***
C17:0	0.65[b]	0.65[b]	0.66[a]	0.66[a]	0.107	*
C18:0	11.76[b]	11.67[c]	11.83[a]	11.84[a]	0.028	***
C16:1	1.30[a]	1.30[a]	1.31[b]	1.31[b]	0.014	*
c C 18:1n-9	22.83[b]	23.18[a]	22.37[d]	22.48[c]	0.184	***
t C 18:1n-9	2.46[a]	2.29[b]	2.51[a]	2.48[a]	0.240	***
tt C 18:2n-6	0.23[b]	0.31[a]	0.22[b]	0.27[ab]	0.001	*
C18:2n-6	2.52[b]	2.64[a]	2.31[c]	2.33[c]	0.057	***
VLCFA						
C20:0	0.16	0.16	0.16	0.16	0.023	ns
C22:0	0.08	0.08	0.08	0.08	0.002	ns
C23:0	0.04[a]	0.04[a]	0.03[b]	0.03[b]	0.001	*
C20:3n-6	0.10	0.11	0.11	0.10	0.003	ns
C20:3n-3	0.04[b]	0.04[b]	0.05[a]	0.04[b]	0.001	*
C20:4n-6	0.18[a]	0.18[a]	0.18[a]	0.17[b]	0.005	*
CLA	0.84	0.84	0.84	0.84	0.010	ns
ALA	0.18[b]	0.19[a]	0.14[d]	0.15[c]	0.007	***

[a]Different lowercase superscript in a same row denote significant differences between cream samples
[b]SD; Standard deviation of means
[c]P-values are: *P<0.05; **P<0.01; ***P<0.001; ns, non-significant (P>0.05)
Abbreviations: Short-chain fatty acids (SCFA, C4:0 to C6:0); Medium-chain fatty acids (MCFA; C8:0 to C15:1); Long-chain fatty acids (LCFA; C16:0 to C18:3); Very long chain fatty acids (VLCFA, longer than 19 carbons), α-Linolenic acid (ALA; C18:3n-3), Conjugated linoleic acid (CLA; C18:2c9t11)

Table 3. Fatty acid profiles (g/100 g of lipids), according to the carbon chain length, observed in control and fermented creams by different probiotics

Fatty acid[a]	Control	*B. lactis*	*L. acidophilus*	*L. rhamnosus*
SCFA	3.73±0.042	3.77±0.056	3.76±0.127	3.75±0.028
MCFA	18.99±0.099[ab]	18.87±0.046[b]	19.28±0.056[a]	19.19±0.123[a]
LCFA	72.00±0.268[b]	72.25±0.169[a]	71.98±0.142[b]	72.07±0.240[b]

[a]Different lowercase superscript in a same row denote significant differences between cream samples (P<0.05)
Abbreviations: Short-chain fatty acids (SCFA, C4:0 to C6:0); Medium-chain fatty acids (MCFA; C8:0 to C15:1); Long-chain fatty acids (LCFA; C16:0 to C18:3)

portant factor for the human nutrition and health (Zhao et al., 2007).

Compared to the other cream samples, as well as to the control sample, the content of the MCFA was significantly higher (P<0.05) in cream samples fermented with *L. acidophilus*, and the content of LCFA was higher in cream samples fermented with *B. lactis* (Table 3). The contents of MCFA in probiotic cream samples ranged from 18.87 to 19.28 g/100 g of lipids and were higher than in the control sample (P<0.05). The LCFA content was 71.98-72.25 g/100 g of lipids and constituted the highest lipid fraction in all samples. It was observed that the probiotic bacteria promoted the LCFA contents of with the exception of cream containing *L. acidophilus* (P<0.05) (Table 3). There was no significant difference (P>0.05) between the total short-chain fatty acid contents of the fermented cream samples (Table 3). Das and Fams (2002) stated that both, long chain poly-unsaturated fatty acids and probiotics, have similar beneficial actions such as anti-inflammatory actions and allergic inflammation. Futhermore, long chain poly-unsaturated fatty acids, especially α-linolenic acid, promote the adhesion of *Lactobacillus casei* to mucosal surfaces, potentiating beneficial action of the lactobacilli (Kankaanpäa et al., 2001).

According to the degree of saturation of the carbon chain as shown in Table 4, the content of SFA in the cream samples ranged from 64.05-65.15 g/100 g of lipids and was the highest in cream samples fermented with *L. acidophilus* (65.15 g/100 g of lipids; P<0.001). Yadav et al. (2007) reported that the addition of probiotic bacteria to Dahi, an important fermented milk product highly consumed throughout India, increased saturated fatty acid content of fermented milk in comparison to the control sample. The highest levels for monounsaturated fatty acids and polyunsaturated fatty acids were detected in cream samples fermented with *B. lactis* (28.27 g /100 g and 4.35 g /100 g, respectively) (P<0.001). The fatty acid *c* C18:1n9 predominated as the MUFA with the highest content in the fermented cream samples and in cream sample fermented with *B. lactis* was higher than in the other creams. Since PUFAs have been shown to confer a number of benefits in humans, particularly reduction of the risk of coronary heart disease, research have mainly focused on these fatty acids. However, high level of unsaturation increases lipid oxidation which could affect the quality of the dairy products due to the development of the off-flavour. Ekinci et al. (2008) determined similar results for SFA, MUFA and PUFA contents in the fermented creams. Kankaanpäa et al. (2004) showed that PUFA in the growth medium

Table. 4. Fatty acid profiles (g/100 g of lipids), according to the saturation degree of the carbon chain, observed in control and fermented creams by different probiotics

Fatty acid[a]	Control	*B. lactis*	*L. acidophilus*	*L. rhamnosus*
SFA	64.52±0.152[b]	64.05±0.182[c]	65.15±1.739[a]	65.02±1.028[a]
MUFA	28.09±0.139[a]	28.27±0.064[a]	27.72±0.506[b]	27.79±0.313[b]
PUFA	4.13±0.826[b]	4.35±0.997[a]	3.87±0.437[c]	3.96±0.023[c]

[a]Different lowercase superscript in a same row denote significant differences between cream samples (P<0.05)
Abbreviations: Saturated Fatty Acid (SFA); Monounsaturated Fatty Acid (MUFA); Polyunsaturated Fatty Acid (PUFA)

Table5. Fatty acid composition of cream samples during storage (g/100 g of lipids)

Fatty acid[a]	Storage time (days)			SD[b]	P-value[c]	Sample x storage time
	2nd	8th	15th			
SCFA						
C4:0	2.23[a]	2.19[ab]	2.15[b]	0.013	*	ns
C6:0	1.60	1.57	1.56	0.009	ns	ns
MCFA						
C8:0	1.01	0.99	0.98	0.002	ns	ns
C10:0	2.32	2.29	2.29	0.021	ns	**
C12:0	2.82	2.79	2.79	0.003	ns	ns
C14:0	10.43[a]	10.32[b]	10.29[c]	0.007	**	**
C15:0	1.09	1.09	1.09	0.001	ns	ns
C14:1	1.47	1.46	1.48	0.018	ns	ns
LCFA						
C16:0	30.73[a]	30.50[b]	30.36[c]	0.375	**	***
C17:0	0.66	0.66	0.65	0.007	ns	***
C18:0	11.85[a]	11.78[b]	11.70[c]	0.046	**	***
C16:1	1.31[a]	1.30[b]	1.30[b]	0.002	*	***
c C 18:1n-9	22.43[c]	22.74[b]	22.97[a]	0.731	***	***
t C 18:1n-9	2.46	2.39	2.46	0.042	ns	ns
tt C 18:2n-6	0.23	0.28	0.26	0.001	ns	**
C18:2n-6	2.31[c]	2.48[b]	2.56[a]	0.173	***	***
VLCFA						
C20:0	0.16	0.16	0.16	0.001	ns	ns
C22:0	0.07[b]	0.09[a]	0.08[a]	0.003	*	ns
C23:0	0.03[b]	0.04[a]	0.03[b]	0.001	*	ns
C20:3n-6	0.10	0.10	0.10	0.003	ns	ns
C20:3n-3	0.04[b]	0.04[b]	0.05[a]	0.001	*	**
C20:4n-6	0.18[a]	0.17[b]	0.17[b]	0.002	*	**
CLA	0.84[a]	0.83[b]	0.83[b]	0.010	*	**
ALA	0.15[b]	0.17[a]	0.18[a]	0.002	*	***

[a]Different lowercase superscript in a same row denote significant differences between storage days
[b]SD; Standard deviation of means
[c]P-values are: *P<0.05; **P<0.01; ***P<0.001; ns, non-significant (P>0.05)
Abbreviations: Short-chain fatty acids (SCFA, C4:0 to C6:0); Medium-chain fatty acids (MCFA; C8:0 to C15:1); Long-chain fatty acids (LCFA; C16:0 to C18:3); Very long chain fatty acids (VLCFA, longer than 19 carbons), α-Linolenic acid (ALA; C18:3n-3), Conjugated linoleic acid (CLA; C18:2c9t11)

of lactobacilli could induce changes in fatty acids, such as the degree of fatty acid unsaturation and the proportions of PUFAs. These observations suggest a strain-dependent effect of lactobacilli on the unsaturated fatty acid profile of fermented creams.

The fatty acid content of cream samples during storage is given in Table 5. No detailed research is available concerning the fatty acid profile of fermented cream during storage. The storage conditions and time employed in this experiment affected the levels of P<0.05 significantly some fatty acids such as butyric, stearic, oleic, CLA and ALA (Table 5). Domagała et al. (2009) stated a negligible effect of the storage time on CLA concentration in cream samples fermented using different starter cultures. After 15-days of storage at 4 °C, *c* C 18:1n-9, C18:2n-6 and ALA contents of the cream samples were slightly increased. A significant interaction of sample and storage time was also noted in relation to the content of linoleic, ALA and some fatty acids such as C16:0, C17:0 and C16:1 (P<0.001).

Conclusion

In this study, probiotic fermented cream was found to contain a high amounts of short chain fatty acids and polyunsaturated fatty acids; which could

be important for the production of functional dairy products in which probiotic bacteria is incorporated. The content of CLA was not significantly increased by adding probiotic cultures opposite to the content of α-linolenic acid by the addition of *B. lactis*. Further studies are required to understand whether fatty acid levels in fermented cream could be enhanced by the addition of probiotic bacteria with higher potential for producing biologically active fatty acid isomers in combination with prebiotics during fermented cream processing.

Sastav masnih kiselina vrhnja fermentiranog probiotičkim bakterijama

Sažetak

Proizvodnja masnih kiselina u vrhnju, koji sadrži jednu od tri vrste probiotičkih mikroorganizama (*Bifidobacterium lactis*, *Lactobacillus acidophilus* i *Lactobacillus rhamnosus*) istraživana je na 4±1 ° C u trajanju do 15 dana. Analiza masnih kiselina plinskom kromatografijom pokazala je da udjeli linolne i α-linolenske kiseline rastu tijekom skladištenja u probiotičkom vrhnju fermentiranom s *B. lactis* u usporedbi s kontrolnim vrhnjem. Probiotičke bakterije povezane su s povećanjem udjela masnih kiselina srednjeg lanca i polinezasićenih masnih kiselina u fermentiranom vrhnju. Najveći udio zasićenih masnih kiselina utvrđen je u vrhnju fermentiranom s *L. acidophilus*, dok je najveći udio mononezasićenih i polinezasićenih masnih kiselina utvrđen u vrhnju fermentiranom s *B. lactis*. Rezultati ovog istraživanja pokazuju da probiotičke bakterije mogu poboljšati profil masnih kiselina u fermentiranom vrhnju i osigurati veću dodanu vrijednost mliječnih proizvoda.

Ključne riječi: probiotik, vrhnje, masne kiseline

References

1. AOAC (2000): Official methods of analysis of AOAC International (17th ed.). Gaithersburg, MD, USA: AOAC International.
2. Bergano, P., Fedele, E., Iannibelli, L., Marzillo, G. (2003): Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. *Food Chemistry 82*, 625-631.
3. Born, B. (2006): Cultured/Sour Cream. In Manufacturing Yogurt and Fermented Milks, Ed: Ramesh C. Chandan, Blackwell Publishing, USA, p 285-293.
4. Chilliard, Y., Ferlay, A., Doreau, M. (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*, 31-48.
5. Collomb, M., Bülher, T. (2000): Analyse de la composition en acides gras de la graisse de lait. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene 91*, 306-332.
6. Coskun, H., Ondul, E. (2004): Free fatty acid accumulation by mesophilic lactic acid bacteria in cold stored milk. *Journal of Microbiology 42*, 133-138.
7. Das, U.N., Fams, M.D. (2002): Essential fatty acid as possible enhancers of the beneficial actions of probiotics. *Nutrition 18*, 786-789.
8. Dewhurst, R.J., Shingfield, K.J., Lee, M.R.F., Scollan, N.D. (2006): Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology 131*, 168-206.
9. Domagala, J., Sady, M., Najgebauer-Lejko, D., Czernicka, M., Wieteska, I. (2009): The content of conjugated linoleic acid (CLA) in cream fermented using different starter cultures. *Biotechnology in Animal Husbandry 25*, 745-751.
10. Ekinci, F.Y., Okur, O.D., Ertekin, B., Guzel-Seydim, Z. (2008): Effects of probiotic bacteria and oils on fatty acid profiles of cultured cream. *European Journal of Lipid Science and Technology 110*, 216-224.
11. Georgala, A.K., Kaminarides, S.E., Anifantakis, E.M. (2006): Free fatty acid content of some traditional Greek cheese varieties. *Australian Journal of Dairy Technology 61*, 26-31.
12. Kankaanpäa, P.E., Salminen, S.J., Isolauri, E., Lee, Y.K. (2001): The influence of polyunsaturated fatty acid on probiotic growth and adhesion. *FEMS Microbiology Letters 194*, 149-153.
13. Kankaanpäa, P.E., Yang, B., Kallio, H., Isolauri, E., Salminen, S. (2004): Effects of polyunsaturated fatty acid in growth medium on lipid composition and on physicochemical surface properties of Lactobacilli. *Applied and Environmental Microbiology 70*, 129-136.
14. Kurmann, J.A., Rasic, J.L., Kroger, M. (1992): Encyclopedia of fermented fresh milk products: An international inventory of fermented milk, cream, buttermilk, whey, and related products. *Springer*, 368 p.
15. Law, B.A. (1997): Microbiology and biochemistry of cheese and fermented milk. *Springer,* 365 p.

16. Ledoux, M., Chardigny, J.M., Darbois, M., Soustre, Y., Sébédio, J.L., Laloux, L. (2005): Fatty acid composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers. *Journal of Food Composition and Analysis 18*, 409-425.
17. Lin, H., Boylston, T.D., Chang, M.J., Luedecke, L.O., Schultz, T.D. (1995): Survey of the conjugated linoleic acid contents of dairy products. *Journal of Dairy Science 78*, 2358-2365.
18. Lock, A.L., Bauman, D.E. (2003): Dairy products and milk fatty acids as functional food components. *Proceedings of the Cornell Nutrition Conference*, pp. 159-173.
19. Mattila-Sandholm, T., Saarela, M. (2003): Functional Dairy Products. Woodhead Publishing Limited and CRC Press, 313 p.
20. Naidu, A.S., Bidlack, W.R., Clemens, R.A. (1999): Probiotic spectra of Lactic Acid Bacteria (LAB). *Critical Reviews in Food Science and Nutrition 3*8 (1), 13-126.
21. Nieuwenhove, C.P.V., Terán, V., González, S.N. (2012): Probiotics, (Ed. Rigobelo, E.C.), Conjugated linoleic and linolenic acid production by bacteria: Development of functional foods, Chapter 3. *InTech*, pp 55-88.
22. Nunes, J.C., Torres, A.G. (2010): Fatty acid and CLA composition of Brazilian dairy products and contribution to daily intake of CLA. *Journal of Food Composition and Analysis 23*, 782-789.
23. Ozcan, T., Yilmaz-Ersan, L., Akpinar-Bayizit, A., Sahin, O.I., Aydinol, P. (2010): Viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 in rice pudding. *Mljekarstvo 60* (2), 135-144.
24. Park, Y.W. (2009): Bioactive components in milk and dairy products. Wiley-Blackwell, USA, 426 p.
25. Sanders, M.E. (2008): Probiotics: Definition, Sources, Selection, and Uses. *Clinical Infectious Diseases 46*, 58-61.
26. Simopoulos, A.P. (2009): Human requirement for n-3 polyunsaturated fatty acids, *Symposium: Role of poultry products in enriching the human diet with N-3 PUFA*, 961-970.
27. Tamime, A.Y. (2009): Dairy fats and related products, *John Wiley & Sons*, 344 p.
28. Yadav, H., Jain, S., Sinha, P.R. (2007): Production of free fatty acids and conjugated linoleic acid in probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* during fermentation and storage. *International Dairy Journal 60*, 1006-1010.
29. Zhao, G., Etherton, T.D., Martin, K.R., Gillies, P.J., West, S.G., Kris-Etherton, P.M. (2007): Dietary α-linolenic acid inhibits proinflammatory cytokine production by peripheral blood mononuclear cells in hypercholesterolemic subjects. *American Journal of Clinical Nutrition 85*, 385-391.