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Content of Conjugated Linoleic Acid (CLA) and *Trans* Isomers of C18:1 and C18:2 Acids in Fresh and Stored Fermented Milks Produced with Selected Starter Cultures

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Abstract

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The effect of the applied starter cultures and storage time of fermented milk drinks on the content of *cis*-9, *trans*-11 octadecadienoic acid (CLA), as well as *trans* isomers of C18:1 and C18:2 acids were evaluated. The analysed fermented milk drinks were produced by the thermostat method and with three different starter cultures. Analyses were carried out for freshly produced fermented milk drinks and for stored drinks (analyses after 6, 13, and 21 days of storage). The study demonstrated that the type of applied starter culture and storage time affected the content of CLA and of *trans* isomers of C18:1 acid in fermented milk drinks. Among the starter cultures applied, only the Ceska-star Y508 culture caused a significant increase in CLA content in the stored fermented milk drinks. The mean content of CLA in fresh drinks reached to 3.60 mg/g fat. The significantly higher CLA contents (3.85 and 3.89 mg/g fat) were found in drinks after 6 and 13 days of storage, respectively. The content of *trans* isomers of C18:1 acid in fresh products was 15.92 mg/g fat. In drinks analysed after 6 days of storage it was 17.14 mg/g fat.

Keywords: *trans* fatty acids; milk drinks; storage time

Conjugated dienoic fatty acids in milk fat, overall called conjugated linoleic acids (CLAs), include positional and geometric isomers of linoleic acid in which two double bonds are separated with only one single bond. The conjugated bonds may attain *cis* or *trans* configuration and usually occur at positions 9 and 11 as well as 10 and 12. The *cis*-9, *trans*-11 octadecadienoic acid (CLA or rumenic acid) is the main representative of the group in milk fat. According to literature data (Chin *et al*. 1992; Jiang *et al*. 1998; Lin *et al*. 1998), the *cis*-9, *trans*-11 CLA constitutes from 75 to over 90% of the sum of these isomers in the fat of milk and dairy products. In the body of ruminants CLA is synthesised as the first intermediate compound in the process of linoleic acid biohydrogenation by isomerase produced by the rumen bacteria (Kelly *et al*. 1998; Palmquist 2001). Only part of CLA present in milk and meat of the ruminants is, however, synthesised in this way. Investigations conducted by Griinari *et al*. (2000) demonstrated that CLA is also produced endogenously from *trans*-vaccenic acid (*trans*-11 isomer of C18:1) with the share of Δ-9 desaturase. It was estimated that ca. 64% of CLA found in milk fat originates from endogenous synthesis. As reported by various authors (Pariza 1991; Parodi 1994, 1997, 2003; Molkentin 1999), CLA displays a number of health-positive properties including e.g. anticarcinogenic, antiatherosclerotic, antioxidative, and anti-inflammatory effects. In the literature, there are also reports that *trans*-vaccenic acid, the main *trans*-C18:1 isomer in milk fat, has anticancer and antiatherosclerotic effects (Przybojewska & Rafalski 2003; Cichosz 2007; Lim *et al*. 2014).

The content of CLA in milk fat may fluctuate in a wide range depending on many factors, e.g. animal feeding, breed, age, as well as lactation period (Precht

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& Molkentin 1997; Chouinard *et al*. 2001; Paszczyk *et al*. 2005; Collomb *et al*. 2006; Janeczek & Kupczyński 2006; Żegarska *et al*. 2006). In dairy products it may additionally be affected by the production process. According to some studies (Ha *et al*. 1989; Shantha *et al*. 1992; Lin *et al*. 1998; Seçkin *et al*. 2005; Bisig *et al*. 2007; Prandini *et al*. 2007; Salamon *et al*. 2009b; Santos Junior *et al*. 2012), technological treatments applied in the industry and additives used may influence CLA content in the fatty acid composition of dairy products. Some authors (Jiang *et al*. 1998; Kim & Liu 2002; Lin 2003; Sieber *et al*. 2004; Ogawa *et al*. 2005; Domagała *et al*. 2009; Hennessy *et al*. 2009; Salamon *et al*. 2009a) claimed that selected strains of bacteria are capable of CLA synthesis during fermentation. As reported by Jiang *et al*. (1998), the *Propionibacterium* strains applied as starter cultures in the dairy industry are capable of transforming free linoleic acid into CLA isomers. According to Kim and Liu (2002), nine out of thirteen bacterial strains analysed in their study were capable of synthesising this isomer, with *Lactococcus lactis* IO-1 being the most effective in this respect.

The objective of this study was to evaluate the effect of selected starter cultures and storage time of fermented milk drinks on the content of conjugated linoleic acid (*cis*-9, *trans*-11 CLA) as well as *trans* isomers of C18:1 and C18:2 acids.

MATERIAL AND METHODS

Experimental material. The experimental material included fermented milk drinks produced with selected starter cultures. Analyses were carried out for freshly produced fermented drinks and drinks stored, cooled to a temperature of 7 ± 1°C for 21 days (analysed after 6, 13, and 21 days of storage). The analysed fermented drinks were produced by the thermostat method according to the following technological scheme: raw milk was heated to the temperature of 45°C, centrifuged, and degassed (80 kPa; 60°C), subjected to HTST pasteurisation (72°C/15 s), and cooled to the temperature of 6°C. Afterwards, it was normalised to the fat content of 2 ± 0.1% (addition of skimmed milk). The normalised milk was then subjected to two-stage homogenisation (18/5 MPa, temperature 65°C) and long-term VHT pasteurisation (90°C/5 min). After cooling to the temperature of 45°C, the milk was divided into three batches and inoculated with three different starter cultures. For this purpose, unitary packages of the DVS vaccines were dissolved in the normalised and pasteurised milk and pre-incubated for 2 h at 45°C. The prepared vaccines were added in the amount of 1 ml/l of milk. The first batch was inoculated with Ceska-star Y508 starter (CSK Food Enrichment Poland, Toruń, Poland) containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacteria (CSK Food Enrichment, Poland). The second batch was inoculated with YC-X11 starter culture that contained *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacteria, whereas the third batch with ABT-1 starter culture contained *Streptococcus thermophilus, Lactobacillus acidophilus*, and *Bifidobacterium bifidum* (both Chr. Hansen, Hørsholm, Denmark). The fermented milk drinks produced with the addition of selected starter cultures were transferred to unitary packages and left to ripen in thermostats at a temperature of 43.5°C till they reached pH 4.6.

For each starter culture one production process was carried out. Every time, four samples of fermented milk drinks from each batch were collected for analyses. All determinations were conducted in two parallel replications.

Analytical methods. Fat from milk and from the analysed fermented milk drinks was isolated by the method of Folch *et al*. (1957).

Fatty acid methyl esters were prepared according to IDF 182:1999 (Milkfat: Preparation of fatty acid methyl esters) using a methanolic solution of KOH.

Contents of CLA and *trans* isomers of unsaturated fatty acids were determined by the GC-FID method (Hewlett Packard 6890 GC System; Hewlett Packard, Münster, Germany). Use was made of capillary column (100 m × 0.25 mm i.d., film thickness 0.20 µm) (Chrompack, Middelburg, The Netherlands) with CP Sil 88 stationary phase, and helium applied as a carrier gas at the flow rate of 1.5 ml/minutes. Sample injection volume was 0.4 µl (split ratio of 50 : 1). Determinations were carried out under the following conditions: column temperature 60°C (1 min) – 180°C, Δt = 5°C/min, detector and injection temperature 250 and 225°C, respectively.

The conjugated linoleic acid (*cis*-9, *trans*-11 CLA) and *trans* isomers of fatty acids were identified with the use of standards of fatty acid methyl esters from Sigma-Aldrich (St. Louis, USA) and Supelco (Bellefonte, USA).

Contents of CLA and *trans* isomers of C18:1 and C18:2 were calculated in mg/g fat in respect of the introduced standard (methyl ester of C21:0 acid).

Statistical analysis. The statistical analysis of results was carried out using STATISTICA Ver-

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sion 10 software based on the one-way analysis of variance (ANOVA) at the significance level of α = 0.05. Differences between mean values were evaluated by Duncan's test.

RESULTS AND DISCUSSION

Results obtained for contents of *cis*-9, *trans*-11 CLA, as well as *trans* isomers of C18:1 (*trans*-6 to *trans*-9, *trans*-10+11, *trans*-12, and *trans*-16) and C18:2 (*cis*-9, *trans*-13, *cis*-9, *trans*-12, *trans*-11, *cis*-15) acids in fresh and stored fermented milk drinks produced with selected starter cultures are presented in Table 1.

The conducted analyses demonstrated differences in CLA content between fresh fermented drinks and drinks analysed after prolonged times of storage. Only in the case of drinks produced with Ceska-star Y508 starter culture (CSK Food Enrichment, Poland) was the content of CLA significantly higher in the stored than in the fresh fermented milk drinks. The mean content of CLA in fresh drinks reached 3.60 mg/g fat. The significantly higher CLA contents (3.85 and 3.89 mg/g fat) were found in drinks after 6 and 13 days of storage, respectively. However, a decrease in CLA content was demonstrated in the drinks analysed after 21 days of storage (Table 1).

In the fermented drinks produced with the use of YC-X11 starter culture by Chr. Hansen Co. (Denmark), which were analysed after 6, 13, and 21 days of storage, there were similar values of CLA content (Table 1).

The mean content of CLA in the analysed fresh drinks produced with ABT-1 starter culture (Chr. Hansen, Denmark) reached 3.64 mg/g fat. The fermented drinks analysed after 6 and 13 days of storage were characterised by the lower contents of conjugated linoleic acid than the freshly produced drinks. Approximate values of CLA were determined in the products after 21 days of storage (Table 1).

It is likely that the starter cultures applied in the study could cause an increase in the content of CLA in the analysed fresh and stored fermented milk drinks. As reported by Kim and Liu (2002), CLA content in fermented milk is affected by the type of bacterial strain applied, cell count, appropriate concentration of the substrate, and incubation conditions (time and pH).

The diversified content of CLA in the investigated fermented dairy drinks produced with various starter cultures could be influenced by various conditions of incubation and storage. According to Cais-Sokolińska *et al.* (2004, 2009), yoghurt bacteria are characterised by various acidifying, proteolytic, and lipolytic activity, owing to which they affect changes in yoghurt acidity during ripening and storage. Investigations carried out by Pawlos *et al.* (2014) demonstrated that the type of applied starter culture and time of its action had a significant effect on yoghurt acidity. The effect of storage time on CLA

Table 1. The content of CLA, C18:1, and C18:2 *trans* isomers in stored fermented milk drinks [mg/g fat]

Type of starter culture	*Trans* isomers ($\bar{x}$ ± SD)	Time of storage [days]			
		0	6	13	21
Lactobacillus delbrueckii subsp. *bulgaricus* *Streptococcus thermophilus* Ceska-star Y508 (CSK Food Enrichment, Poland)	*cis*-9, *trans*-11 CLA	$3.60^{a} \pm 0.08$	$3.85^{b} \pm 0.22$	$3.89^{b} \pm 0.15$	$3.68^{a,b} \pm 0.08$
	Σ *trans* C18:1	$15.92^{a} \pm 0.76$	$17.14^{b} \pm 0.32$	$16.65^{a,b} \pm 0.61$	$16.63^{a,b} \pm 0.27$
	Σ *trans* C18:2	$4.24^{a} \pm 0.11$	$4.49^{a} \pm 0.46$	$4.26^{a} \pm 0.30$	$4.55^{a} \pm 0.39$
Lactobacillus delbrueckii subsp. *bulgaricus* *Streptococcus thermophilus* YC-X11 (Chr. Hansen, Denmark)	*cis*-9, *trans*-11 CLA	$3.60^{a} \pm 0.06$	$3.76^{a} \pm 0.12$	$3.74^{a} \pm 0.28$	$3.66^{a} \pm 0.17$
	Σ *trans* C18:1	$16.24^{a} \pm 0.27$	$16.63^{a} \pm 0.73$	$15.86^{a} \pm 1.12$	$16.29^{a} \pm 0.26$
	Σ *trans* C18:2	$4.30^{a} \pm 0.31$	$4.59^{a} \pm 0.66$	$4.58^{a} \pm 0.35$	$4.57^{a} \pm 0.23$
Streptococcus thermophilus, *Lactobacillus acidophilus*, *Bifidobacterium bifidum* ABT-1 (Chr. Hansen, Denmark)	*cis*-9, *trans*-11 CLA	$3.64^{a} \pm 0.16$	$3.59^{a} \pm 0.19$	$3.48^{a} \pm 0.24$	$3.62^{a} \pm 0.12$
	Σ *trans* C18:1	$15.84^{a} \pm 0.57$	$16.47^{b} \pm 0.79$	$15.88^{a} \pm 0.47$	$15.77^{a} \pm 0.23$
	Σ *trans* C18:2	$4.32^{a} \pm 0.49$	$4.58^{a} \pm 0.30$	$4.32^{a} \pm 0.12$	$4.41^{a} \pm 0.23$

$\bar{x}$ ± SD – mean value ± standard deviation; (n = 4); ^{a,b}values in the rows denoted by different letters differ statistically significantly ($P < 0.05$)

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content in dairy products is disputable. Shantha *et al.* (1995) demonstrated no significant changes in CLA content in yoghurts stored for 6 weeks at a temperature of 4°C. Also Boylston and Beitz (2002) reported that the production of yoghurt and its storage for 7 days had no effect upon its CLA content. According to a study by Domagała *et al.* (2009), the type of applied starter culture and storage time affected the CLA level in cream subjected to fermentation. Only one of the seven starter cultures used by these authors (yoghurt culture ABY-2) caused an increase in CLA content in fermented cream. Changes in CLA content in yoghurts produced from cow's milk and goat milk stored for 14 days at a temperature of 5°C were also demonstrated by Serafeimidou *et al.* (2013). According to their research, after 7 days of storage the yoghurts made of cow's milk were characterised by a higher content of CLA than the products analysed on day 1. A significantly lower content of this acid was found by these authors in yoghurts analysed after 14 days of storage. Different tendencies were observed by these authors in yoghurts made of goat milk. A lower CLA content was determined in yoghurts analysed after 7 days than in the products analysed on day 1. A significantly higher content of this isomer was demonstrated in yoghurts produced from goat milk after 14 days of storage. Changes in CLA content in organic and conventional fermented milk stored for 7 days at a temperature 4°C were also reported by Florence *et al.* (2012). Organic and conventional milk produced with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* TA040 LB340 analysed on the 7th day of storage had a significantly lower content of CLA than the milk tested on the first day of storage. Significantly lower levels of CLA were observed in fermented milk made from organic milk produced with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* TA040 LB340 and *Bifidobacterium animalis* subsp. *lactis* HN019 tested on the 7th day of storage compared to that tested on the 1st day after fermentation. In conventional fermented milk produced with the same bacterial strains, no significant changes in CLA content were observed during refrigerated storage.

Differences in CLA content in stored fermented drinks may also result from various transformations taking place in these products during storage. As reported by Shantha *et al.* (1995), a decrease in CLA content in some dairy products may be due to oxidising reactions that cause damage to the conjugated system of double bonds, thus inducing a decrease in CLA content in stored products. According to literature data (Yang *et al.* 2000), owing to the presence of conjugated unsaturated bonds, the CLA is more susceptible to processes of oxidation and isomerisation than linoleic acid.

The total content of *trans* isomers of C18:1 acid and *trans* isomers of C18:2 acid in the analysed fermented milk drinks was also subjected to changes (Table 1). In the fermented drinks produced with Ceska-star Y508 starter culture (CSK Food Enrichment, Poland), the lowest content of *trans* isomers of C18:1 acid (15.92 mg/g fat) was determined in fresh products. A significantly higher content of these isomers was found in drinks analysed after 6 days of storage. Further storage affected a decrease in the content of these isomers in the analysed fermented drinks (Table 1). In the fermented drinks produced with the use of YC-X11 starter culture, the *trans* isomers of C18:1 acid constituted 16.24 mg/g fat in fresh products. In the stored drinks, the content of these isomers had similar values. In the fresh fermented drinks produced with ABT-1 starter (Chr. Hansen, Denmark), the content of C18:1 *trans* isomers reached 15.84 mg/g fat. A significantly higher content of these isomers was found in the fermented drinks analysed after 6 days of storage. Further storage caused a significant decrease in the total content of *trans* isomers of C18:1 acid (Table 1). The decrease in the total content of *trans* isomers in stored fermented drinks, such as the decrease in CLA content, may be a result of oxidative changes that occur in these products during storage (Shantha *et al.* 1995; Yang *et al.* 2000). As reported by Fritsche and Steinhart (1998), there is a high positive correlation (r = 0.806) between CLA content and the content of *trans* isomers in the fat of milk and dairy products. According to the study by Żegarska *et al.* (2008), CLA content in fermented drinks (yoghurts, kefirs, and acidophilous milks) was strongly and positively correlated with the content of *trans* C18:1 isomers, the correlation coefficient was above 0.95.

The study carried out by Florence *et al.* (2012) showed that during the storage of fermented milks at 4°C for 7 days, the content of *trans*-C18:1 was stable, regardless of the type of milk and type of starter culture. According to a study by Domagała *et al.* (2009), the type of applied starter culture and storage time affected the *trans*-vaccenic acid level in cream subjected to fermentation. The content of *trans*-vaccenic acid was lower in most cases after fermentation and storage in comparison with the content of these components in cream before fermentation.

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The total content of *trans* isomers of C18:2 acid in all analysed fermented milk drinks produced with various starter cultures, analysed after 6, 13, and 21 days of cold storage, was subjected to a small change (Table 1).

CONCLUSIONS

The study demonstrated that the type of applied starter culture and storage time affects the content of CLA as well as the content of *trans* isomers of C18:1 acid in fermented milk drinks. Among the starter cultures applied in the study, only Ceska-star Y508 (CSK Food Enrichment, Poland) caused a significant increase in CLA content in the stored fermented milk drinks. Whereas a significant increase in the content of *trans* isomers of C18:1 acid was caused by applying Ceska-star Y508 (CSK Food Enrichment, Poland) as well as ABT-1 starter culture (Chr. Hansen, Denmark).

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