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EFFECT OF SELECTED STARTER CULTURES ON CONTENTS OF *cis*9*trans*11 C18:2 (CLA) AND *TRANS* C18:1 AND C18:2 ISOMERS IN FERMENTED MILK DRINKS

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Key words: fermented dairy drinks, yoghurt, starter cultures, *cis*9*trans*11 C18:2 (CLA), *trans* isomers.

Abstract

This study was aimed at evaluating the effect of selected starter cultures on the content of *cis*9*trans*11 C18:2 acid and contents of *trans* isomers of C18:1 and C18:2 acids in fermented milk drinks. Analyses were carried out for normalized milk used to produce fermented milk drinks and for fermented drinks produced from this milk with three different yoghurt starter (Ceska-star Y508, YC-X11 and ABT -1).

The study demonstrated that the type of starter culture applied affected the content of *cis*9*trans*11 C18:2 acid (CLA) as well as contents of *trans* isomers of C18:1 and C18:2 acids in the fermented dairy drinks.

In all analyzed fermented dairy drinks produced with various starter cultures, analyses showed slightly lower contents of conjugated linoleic acid that in normalized milk used as a raw material for their production.

WPŁYW WYBRANYCH KULTUR STARTEROWYCH NA ZAWARTOŚĆ KWASU *cis*9*trans*11 C18:2 (CLA) ORAZ IZOMERÓW *TRANS* C18:1 I C18:2 W FERMENTOWANYCH PRODUKTACH MLECZARSKICH

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Słowa kluczowe: fermentowane produkty mleczarskie, jogurt, kultury starterowe, CLA, izomery *trans*.

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Abstrakt

Przedmiotem badań była ocena wpływu wybranych kultur starterowych na zawartość kwasu *cis*9*trans*11 C18:2 oraz na zawartość izomerów *trans* kwasu C18:1 i C18:2 w mlecznych napojach fermentowanych. Analizie poddano mleko normalizowane przygotowane do produkcji mlecznych napojów fermentowanych oraz wyprodukowane z niego produkty fermentowane. Objęte badaniem mleczne napoje fermentowane zostały wyprodukowane przy użyciu trzech różnych starterowych kultur jogurtowych (Ceska-star Y508, YC-X11 oraz ABT-1).

Przeprowadzone badania wykazały, że rodzaj zastosowanej kultury starterowej wpływa na zawartość kwasu *cis*9*trans*11 C18:2 (CLA) oraz na zawartość izomerów *trans* kwasu C18:1 i kwasu C18:2 w wyprodukowanych jogurtach.

We wszystkich objętych badaniem napojach fermentowanych wyprodukowanych z udziałem różnych kultur starterowych stwierdzono nieco niższe zawartości sprzężonego kwasu linolowego niż w mleku normalizowanym stanowiącym surowiec do ich produkcji.

Introduction

Conjugated dienes of milk fat (CLA) constitute a group of positional and geometric isomers of linoleic acid (C18:2), in which two double bonds are separated with only one single bond. In fat of the ruminants, the main representative of this group of isomers is *cis*9*trans*11 C18:2 acid which in milk fat constitutes from 75 to over 90% of total isomers of C18:2 acids with conjugated bonds (CHIN et al. 1992, LIN et al. 1998, PRECHT and MOLKENTIN 2000). The *cis*9*trans*11 C18:2 acid displays a variety of health-promoting properties, including: antioxidative, anticarcinogenic and antimutagenic ones (MOLKENTIN 1999, PARIZA 1991, PARODI 1994, 1997, 1999, PRZYBOJEWSKA and RAFALSKI 2003).

An important source of CLA in man's diet is milk and dairy products (butter, cheeses and fermented products). The content of this acid in milk depends, most of all, on the feeding regime and rearing method as well as on the breed, age and lactation period of an animal. Its average content in milk fat ranges from 3 to 6 mg/g fat (CHIN et al. 1992, LIN et al. 1995, JIANG et al. 1997). CLA content in dairy products may, however, differ from its content in milk, as in dairy products it may be influenced by parameters of technological processes conducted, additives applied, or capability of some lactic fermentation bacteria to synthesize CLA under appropriate conditions of the fermentation process (strain dose and duration of its action, conditions of incubation and composition of milk) (JIANG et al. 1998, KIM and LIU 2002, LIN 2003, SIEBER et al. 2004, CIOŁKOWSKA et al. 2012). According to a study by KIM and LIU (2002), nine out of eleven analyzed strains of lactic fermentation bacteria were capable of synthesizing CLA, with *Lactococcus lactis* IO-1 strain found to be the most effective. In turn, a research by JIANG et al. (1998) shows that among seven *Lactobacillus* strains, two *Streptococcus* strains and six *Propionibacterium*

strains cultured in vitro, only *Propionibacterium freudenreichii* was capable to transform free linoleic acid to *cis*9*trans*11 and *trans*9*cis*11 C18:2 isomers. DOMAGAŁA et al. (2009) determined the effect of seven different starter cultures on the level of conjugated linoleic acid in fermented cream and demonstrated that only the addition of a yoghurt culture ABY-2 caused an increase in CLA content in the finished product.

The aim of this study was to evaluate the effect of selected starter cultures on the content of conjugated linoleic acid (*cis*9*trans*11 C18:2) and contents of *trans* isomers of C18:1 and C18:2 acids in fermented milk drinks.

Material and Methods

Analyses were conducted for normalized milk prepared to produce fermented dairy drinks and for freshly-produced drinks. Three production series were run with three starter cultures: Ceska-star Y508 – containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* bacteria, produced by CSK Food Enrichment, Poland; YC-X11 – containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* bacteria, produced by Chr. Hansen; and ABT-1 – containing *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* bacteria, produced by Chr. Hansen.

Fermented drinks were produced with the thermostat method, according to the following technological scheme: after collection, raw milk was cooled to a temp. of 6°C and stored for 4 h, next after heating to 45°C it was subjected to centrifugation and degasing (80 kPa; 60°C), and to HTST pasteurization (72°C/15 s), and finally it was cooled to a temp. of 6°C. Afterwards, milk was normalized to fat content of ca. 2% (addition of skim milk). The normalized milk was subjected to two-stage homogenization (18/5 MPa, temp. 65°C) and long-term VHT pasteurization (90°C/5 min). After cooling to 45°C, it was divided into three portions (about 10 liters each one) and inoculated with three different starter cultures. The fermented dairy drinks produced in this way were poured into unitary packages (250 mL cups) and thermostated at 43.5°C for ca. 3.5h, to pH 4,65.

Three samples were collected from each production series for analyses. All determinations were carried out in two parallel replications.

Methods

Fat content in produced fermented milk drinks was determined with the Roese-Gottlieb's method (PN-75/A-86130).

To determine CLA and *trans* C18:1 and C18:2 isomers, fat of normalized milk and fermented milk drinks was isolated with the Folch's method [Christie, 1973].

Methyl esters of fatty acids were prepared acc. to the IDF method, using a methanol solution of KOH [IDF Standard 182: 1999].

Determinations of fatty acid composition and contents of CLA and *trans* isomers of unsaturated fatty acids were carried out with gas chromatography (GC) method using an HP 6890 chromatograph with a flame-ionization detector. Chromatographic separation of fatty acid methyl esters was carried out on a capillary column (100 m x 0.25 mm i.d., film thickness 0.20 μm) with CP Sil 88 phase. Separation conditions were as follows: column temp.: 60°C (1 min) – 180°C, Δt = 5°C/min; detector temp.: 250°C; injector temp.: 225°C; carrier gas: helium, flow rate: 1.5 mL/min, injector: split 50:1.

For identification of positional *trans* isomers of C18:1, used the standards of methyl esters of those isomers (*trans* 6, Supelco and *trans* 9 and *trans* 11, Sigma-Aldrich) and literature data. The *trans* isomers of C18:2 acid (*cis*,*trans* and *trans*,*cis*) were identified with the use of a mixture of standards of C18:2 isomers (Supelco), *cis*9, *trans*11 CLA – with a mixture of CLA methyl esters (Sigma-Aldrich).

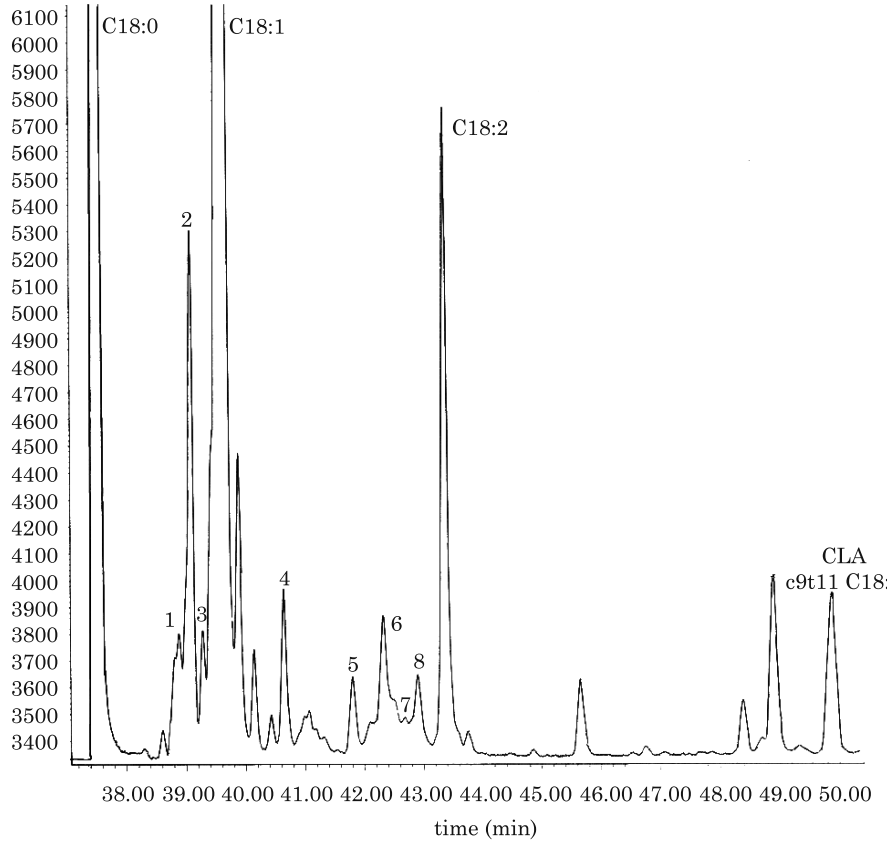
Quantitative computations of *cis*9*trans*11 C18:2 acid and *trans* isomers of C18:1 and C18:2 acids were made against the introduced standard (methyl ester of C21:0 acid). Statistical calculations were conducted with STATISTICA 10 software.

Results and Discussion

All analyzed fermented milk drinks were characterized 2 ± 0.1% fat content.

Results obtained for contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in normalized milk and fermented milk drinks produced using different starter cultures are presented in Table 1. The exemplary chromatogram separation of CLA and *trans* isomers C18:1 and C18:2 acids of fermented milk drink produced with YC-X11 starter is presented in Figure 1.

The content of *cis*9*trans*11 C18:2 acid in normalized milk prepared for the production of fermented dairy drinks reached 3.75 mg/g fat. LINA et al. (1995)



1 – *trans* 6-9 C18:1; 2 – *trans* 10+11 C18:1; 3 – *trans* 12 C18:1; 4 – *trans* 16 C18:1; 5 – *cis*9*trans*13 C18:2; 6 – *cis*9*trans*12 C18:2; 7 – *cis*9*trans*12 C18:2; 8 – *trans*11*cis*15 C18:2;
Fig. 1. Separation of CLA and *trans* isomers of C18:1 and C18:2 fatty acids of fermented milk drink produced with YC-X11 starter

reported that CLA content in full-fat milk was at 4.49 mg/g fat. In milk with 2% fat content, the level of this acid was at 4.14 mg/g fat. Based on investigations of other authors, KOWALSKA and CICHOSZ (2013) state that CLA content in full-fat milk may range from 3.4 to 6.8 mg/g fat. In milk with fat content of 2%, the CLA content accounts for 4.1 mg/g fat, whereas in condensed milk it may range from 6.3 to 7.0 mg/g fat. The fermented milk drinks produced with various starter cultures were characterized by a slightly lower content of this acid than milk (Table 1.). The content of *cis*9*trans*11 C18:2 acid in the analyzed fermented drinks reached: 3.68 mg/g fat in drinks produced with YC-X11 starter, 3.62 mg/g fat in drinks produced with ABT -1 starter, and 3.61 mg/g fat in drinks produced with Ceska-star Y508 starter culture.

Table 1

Content of CLA and C18:1 and C18:2 *trans* isomers in milk and fermented milk drinks (mg/g fat)

Trans isomers	Normalized milk prepared for production $\bar{x}$ ±s /SD	Type of starter culture		
		Ceska-star Y508 $\bar{x}$ ± s /SD	YC-X11 $\bar{x}$ ± s /SD	ABT-1 $\bar{x}$ ± s /SD
*cis*9*trans*11 C18:2 (CLA)	3.75^{a} ± 0,04	3.61^{a} ± 0.08	3.68^{a} ± 0.06	3.62^{a} ± 0.16
t6 – t9 C18:1	2.80 ± 0.04	2.83 ± 0.19	2.74 ± 0.08	2.71 ± 0.12
*t*10+*t*11 C18:1	9.16 ± 0.01	8.77 ± 0.07	8.99 ± 0.13	8.72 ± 0.34
t12 C18:1	1.83 ± 0.19	1.95 ± 0.12	1.95 ± 0.04	1.87 ± 0.08
t16 C18:1	2.60 ± 0.03	2.35 ± 0.45	2.46 ± 0.09	2.54 ± 0.14
Σ *trans* C18:1	16.39^{a} ± 0.16	15.90^{a} ± 0.76	16.14^{a} ± 0.27	15.85^{a} ± 0.57
c9 t13 C18:2	1.59 ± 0.24	1.40 ± 0.11	1.41 ± 0.08	1.51 ± 0.31
t9 c12 C18:2	2.41 ± 0.06	2.12 ± 0.08	2.19 ± 0.26	2.02 ± 0.20
t11 c15 C18:2	0.77 ± 0.07	0.62 ± 0.03	0.60 ± 0.03	0.70 ± 0.13
Σ *trans* C18:2	4.77^{a} ± 0.11	4.14^{b} ± 0.11	$4.20^{a,b}$ ± 0.31	$4.22^{a,b}$ ± 0.49

^{a,b} – values in the rows denoted by the same letter are not significantly different ($p > 0.05$).

Lower concentrations of CLA in yoghurts compared to milk they were made of were also reported by SANTOS JUNIOR et al. (2012). These authors conducted their study in Brazil in the summer period and demonstrated that CLA content reached 6.22 mg/g fat in pasteurized milk and 5.41 mg/g fat in yoghurts. Differences in *cis*9*trans*11 C18:2 acid content in Italian fermented milk drinks produced with the use of various starter cultures were reported by PRANDINI et al. (2007). In products analyzed by these authors, the mean content of CLA ranged from 4.42 mg/g fat in probiotic yoghurts to 6.15 mg/g fat in fermented milk produced from milk of cows grazed on a mountain pasture. A research by DOMAGAŁA et al. (2009) indicated that CLA content in fermented cream depended on the type of starter culture applied in the fermentation process. The CLA content in the samples analyzed by these authors ranged from 3.33 mg/g fat in fermented cream produced with YC-180+*Propionibacterium* starter to 4.03 mg/g fat in the product made with ABY-2 starter culture. Yoghurts analyzed by Lin *et al.*, (1995) contained CLA at 3.82 mg/g fat, and buttermilk – at 4.66 mg/g fat. Based on investigations of other authors, KOWALSKA and CICHOSZ (2013) state the CLA content may range from 3.8 to 8.8 mg/g fat in yoghurts, from 5.4 to 6.7 mg/g fat in buttermilk, and from 4.6 to 7.5 mg/g fat in sour cream.

The total content of *trans* isomers of C18:1 acid in normalized milk prepared for production reached 16.39 mg/g fat. The production process and starter cultures applied caused a decrease in the content of these isomers. The

lowest decrease (to the value of 16.14 mg/g fat) was reported in drinks produced with YC-X11 starter culture. In the fermented drinks produced with Ceska-star Y508 and ABT-1 starter cultures, the levels of C18:1 *trans* isomers were at 15.90 mg/g fat and 15.85 mg/g fat, respectively.

In both milk and fermented milk drinks, among *trans* isomers of C18:1 acid the highest contents were noted for *trans* 10 + *trans* 11 isomers of C18:1. The contribution of the total sum of these isomers in the total fatty acid composition of normalized milk reached 9.16 mg/g fat. In the fermented dairy drinks, the total contents of these isomers were slightly lower (Table 1).

The content of *trans* isomers of C18:2 acid in normalized milk reached 4.77 mg/g fat. A significantly lower (4.14 mg/g fat) total content of these isomers was determined in fermented milk drinks produced with Ceska-star Y508 starter. Lower than in milk contents of C18:2 *trans* isomers acid were also noted in the other analyzed fermented drinks, however they were not statistically significant.

Conclusions

The study demonstrated that the type of the applied starter culture affected the content of *cis*9*trans*11 C18:2 acid (CLA) and contents of *trans* isomers of C18:1 and C18:2 acids in fermented products.

In all analyzed fermented dairy drinks produced with various starter cultures, analyses showed slightly lower contents of conjugated linoleic acid that in normalized milk used as a raw material for their production.

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