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CONJUGATED LINOLEIC ACID (*cis*9*trans*11 C18:2, CLA) AND *trans* ISOMERS OF C18:1 AND C18:2 ACIDS IN YOGHURTS, KEFIRS AND MOLD CHEESES

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Key words: yoghurt, kefir, mold cheeses, CLA, *trans* isomers.

Abstract

The aim of this study was to determine contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in fat of yoghurts, kefirs, cheeses with bloomy molds and blue-veined cheeses. Fatty acid composition of the analyzed dairy products was assayed with the gas chromatography method using a 100-m capillary column with CP Sil 88 phase.

The study demonstrated that contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in the analyzed yoghurts and kefirs were very similar. Slightly higher contents of CLA and *trans* isomers were determined in fat isolated from the investigated mold cheeses.

SPRZĘŻONY KWAS LINOLOWY (*cis*9*trans*11 C18:2, CLA) ORAZ IZOMERY *trans* KWASU C18:1 I C18:2 W JOGURTACH, KEFIRACH I SERACH PLEŚNIOWYCH

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Słowa kluczowe: jogurt, kefir, sery pleśniowe, CLA, izomery *trans*.

Abstrakt

Celem pracy było określenie zawartości kwasu *cis*9*trans*11 C18:2 (CLA) oraz izomerów *trans* C18:1 i C18:2 w tłuszczu jogurtów, kefirów oraz serów z porostem i przerostem pleśni. Skład kwasów tłuszczowych tłuszczu badanych produktów oznaczano metodą chromatografii gazowej na 100 m kolumnie kapilarnej z fazą CP Sil 88.

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Przeprowadzone badania wykazały, że zawartość kwasu *cis*9*trans*11 C18:2 (CLA) oraz izomerów *trans* kwasu C18:1 i C18:2 w badanych jogurtach i kefirach była bardzo zbliżona. Nieco wyższą zawartość kwasu CLA i izomerów *trans* stwierdzono w tłuszczu wydzielonym z badanych serów pleśniowych.

Introduction

The term „linoleic acid with conjugated bonds" (CLA) refers to 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 highest concentration has been reported for *cis*9*trans*11 C18:2 acid which in milk fat constitutes from 75 to over 90% of the sum of C18:2 acid isomers with conjugated bonds (CHIN et al. 1992, PARODI 2003, PRECHT and MOLKENTIN 2000). This acid exhibits many activities beneficial to health like anticarcinogenic, anti-atherosclerotic, antioxidative and anti-inflammatory (CICHOSZ 2007, MOLKENTIN 1999, PARIZA 1991, PARODI 1994, PARODI 1997). A rich natural source of CLA in a human diet is milk and its products. CLA concentration in milk fat is highly diversified depending mainly on the feeding season of cows. According to ŻEGARSKA et al. (2006), the content of conjugated linoleic acid in milk originating from the pasture feeding (summer) ranged from 1.06% do 1.76% of total fatty acids and in milk from the stall feeding (winter) – from 0.32% to 0.52%. In turn, LIPIŃSKI et al. (2012) reported that the contribution of CLA in the total fatty acid composition ranged from 0.38% in March and April to 1.68% in August.

The content of CLA in dairy products may differ from that in milk. According to literature data (BZDUCHA-WRÓBEL and OBIEDZIŃSKI 2009, DOMAGAŁA et al. 2009, JIANG et al. 1998, KIM and LIU 2002, LIN et al. 1998, LIN 2003, SHANTHA et al. 1992, SIEBER et al. 2004), the level of CLA in dairy products (cheeses or fermented dairy drinks) may be influenced by parameters of technological processes and by the activity of starter cultures added.

Therefore, the aim of this study has been to determine contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in fat of yoghurts, kefirs, cheeses with bloomy molds and blue-veined cheeses.

Material and Methods

Material

The experimental material included fermented dairy drinks (6 yoghurts and 6 kefirs) and mold cheeses (7 cheeses with bloomy molds and 7 blue-veined cheeses). The analyzed products originated from various producers and were

purchased (one sample of each) between February and April in retail stores in Olsztyn. All analysed were performed in duplicate. All products were tested within their shelf life.

Analytical methods

Fat content in yoghurts and kefirs was determined with the Roese-Gottlieb's method (PN-75/A-86130), whereas in the analyzed mold cheeses – with the Schmidt-Bondzynski-Ratzlaff's method (PN-73/A-86232).

To determine fatty acid composition, fat of yoghurts and kefirs was isolated with the Roese-Gottlieb's method (PN-75/A-86130), and fat of cheeses – with a modified Folch's method (CHRISTIE 1973).

Methyl esters were prepared from the isolated fat acc. to the IDF method using a methanolic solution of KOH (IDF standard 182:1999).

Determinations of fatty acid composition, CLA and *trans* isomers of C18:1 and C18:2 acid were carried out with gas chromatography method using a Hewlett Packard 6890 chromatograph with a flame-ionization detector and capillary column with CP Sil 88 phase (100 m x 0.25 mm i.d., liquid phase film thickness 0.20 mm). Separation conditions were as follows: column temp.: 60°C (1 min) – 180°C, Δt = 5°C/min; injector temp.: 225°C; detector temp.: 250°C; carrier gas: helium, flow rate: 1.5 cm^3/min, injector: split 50:1.

Peaks of individual fatty acids were identified by comparing their retention times with those of methyl esters of reference fat with known fatty acids profile (BCR Reference Materials, symbol CRM 164). For identification of positional *trans* isomers of C18:1, use was made of the standards of methyl esters of those isomers (*trans* 6, *trans* 9, *trans* 11) (Sigma) and literature data. In turn, 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) and literature data.

Percentage contents of conjugated linoleic acid *cis*9*trans*11 C18:2 and assayed *trans* isomers of C18:1 acid and C18:2 acid were calculated relative to the total content of fatty acids (weight %). Statistical calculations were made using STATISTICA PL software.

Results and discussion

The analyzed products were characterized by diversified contents of fat. In yoghurts its content ranged from 1.1% to 3.4% and in kefirs – from 1.3% to 2.0%. The percentage content of fat in cheeses with bloomy mold ranged from 19.3% to 31.5%, whereas in blue-veined cheeses – from 27.9% to 33.6%.

The examined yoghurts and kefirs were characterized by similar contents of particular groups of fatty acids. The content of short-chain fatty acids ranged from 10.00% to 11.33% of the total fatty acid composition in fat of yoghurts, and from 10.02% to 11.05% in fat of kefirs. The contents of saturated fatty acids ranged from 59.66% to 63.14% in fat of yoghurts, and from 60.40 to 62.47% in fat of kefirs. In fat of yoghurts, the mean content of monoenoic fatty acids accounted for 25.37%, and that of polyenoic fatty acids for 3.12% of the total fatty acid composition. In fat of kefirs, the respective values reached 25.45% and 3.05%. Greater differences in contents of the particular groups of fatty acids were determined in the analyzed mold cheeses. In fat extracted from cheeses with bloomy mold, the content of short-chain fatty acids ranged from 7.26% to 12.40%, that of saturated fatty acids from 58.95% to 81.56%, that of monoenoic acids from 8.81% to 27.39%, and that of polyenoic fatty acids from 2.39% to 3.48%. In the blue-veined cheeses, the respective values were as follows: from 9.67% to 16.29%, from 57.20% to 67.36%, from 18.54% to 25.32%, and from 2.52% to 4.19%.

In this study, we paid special attention to contents of CLA and *trans* isomers of C18:1 and C18:2 acids in fat separated from the analyzed dairy products.

Contents of *cis*9*trans*11 C18:2 acid (CLA) and assayed *trans* isomers of C18:1 acid and C18:2 acid in the total fatty acid composition of fat isolated from the analyzed fermented drinks and mold cheeses were presented in Table 1.

Data in Table 1 demonstrate that CLA content in fat of the analyzed yoghurts ranged from 0.38% to 0.46% of total fatty acids. The mean content of this isomer was at 0.42%. The mean content of this acid in kefirs available on the market in the same period was at the same level. These results are similar to findings reported by PASZCZYK et al. (2006), who showed that in commercial yoghurts originating from January to March the mean CLA content reached 0.42% (range: from 0.35 to 0.50% of total fatty acids). Similar contents of CLA in yoghurts and kefirs were also determined by ŻEGARSKA et al. (2008). Kefirs purchased by these authors in winter (from January to February) contained from 0.34 to 0.48%, and yoghurts from 0.37 to 0.49% of CLA. Kefirs and yoghurts analyzed by these authors in June and July were characterized by a higher CLA content.

Compared to fermented dairy drinks, a significantly ($p \geq 0.05$) higher content of CLA was reported in the analyzed cheeses with bloomy mold, i.e. on average 0.71% of total fatty acids (range: from 0.54 to 0.88%) in cheeses produced from milk from the winter feeding period.

The analyzed blue-veined cheeses were characterized by the greatest differences in CLA content. In fat of this group of cheeses, the content of *cis*9*trans*11 C18:2 acid ranged from 0.35% to 1.10% of total fatty acid composition. Its mean content in fat of these cheeses reached 0.56% of total fatty acids

and did not differ significantly from values reported in the analyzed fermented dairy drinks and cheeses with bloomy molds (Table 1).

In various mold cheeses analyzed by ŻEGARSKA et al. (2006), the mean CLA content accounted for 0.48% of total fatty acids (from 0.42 to 0.54%) in fat of cheeses purchased in February and March, and for 0.89% of total fatty acids (from 0.59 to 1.24%) in fat of cheeses bought in November. As demonstrated by PASZCZYK et al. (2012), mold cheeses originating from different EU Member States (Poland, Germany, Italy and France) available on the Olsztyn market in January and February were characterized by a similar mean content of *cis*9*trans*11 C18:2 acid (CLA), ranging from 0.43 (in Polish cheeses) to 0.58% of total fatty acids (in German cheeses). According to a study by LIN *et al*. (1995), the content of conjugated linoleic acid varied in different types of cheeses, i.e. the highest CLA content was found by these authors in Blue and Brie cheeses (mold cheeses) and the lowest one in processed melted cheeses. In turn, when investigating German mold cheeses, FRITHE and STEINHART (1998) showed CLA content to reach 0.55% of total fatty acids in Blue cheese and 0.49% in Brie cheese.

The total content of *trans* isomers of C18:1 acid in fat extracted from the analyzed yoghurts ranged from 1.99 to 2.61% of total fatty acids (Table 1). The mean content of these isomers was at 2.25% and did not differ significantly ($p \geq 0.05$) from the value reported in the analyzed kefirs. In fat of kefirs the *trans* isomers of C18:1 acid constituted on average 2.08% (i.e. from 1.82% to 2.32% of total fatty acids) (Table 1). Similar contents of these isomers, i.e. from 1.66 to 2.34%, were determined by PASZCZYK et al. 2006) in yoghurts purchased from January till March. According to research by ŻEGARSKA et al. (2008), yoghurts purchased in January and February contained on average 1.64% (from 1.24 to 1.87%), and kefirs 1.54% of *trans* isomers of C18:1 acid (from 1.18 to 1.98%). Higher contents of these isomers were determined by these authors in yoghurts and kefirs analyzed in June and July.

In the analyzed cheeses with bloomy molds, the content of *trans* isomers of C18:1 acid ranged from 2.32 to 3.34% of total fatty acids. The mean summary content of these isomers in these cheeses reached 2.92% and was significantly higher than their content in the analyzed kefirs ($p \geq 0.01$) and yoghurts ($p \geq 0.05$) (Table 1). The content of these isomers in the investigated blue-veined cheeses ranged from 1.71 to 3.45% of total fatty acids. Their average total content (2.36% of total fatty acids) did not differ significantly from respective values determined in fermented dairy drinks and cheeses with bloomy molds (Table 1). The mean content of C18:1 acid *trans* isomers in various mold cheeses analyzed by ŻEGARSKA et al. (2008) accounted for 1.88% (i.e. from 1.40 to 2.47%) in cheeses from January and February, and for 3.08% (i.e. from 2.52 to 3.91%) in cheeses from November. Mold cheeses from the winter period

Table 1

The content of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in fat of analyzed dairy products (% of total fatty acids)

Trans isomers	Products							
	Yoghurts (n=6)		Kefirs (n=6)		Cheese with bloomy mold (n=7)		Blue-veined cheese (n=7)	
	Min. – Max.	$\bar{x} \pm s$	Min. – Max.	$\bar{x} \pm s$	Min. – Max.	$\bar{x} \pm s$	Min. – Max.	$\bar{x} \pm s$
*cis*9*trans*11 C18:2(CLA)	0.38 – 0.46	$0.42^{a,A} \pm 0.03$	0.37 – 0.48	$0.42^{a,A} \pm 0.04$	0.54 – 0.88	$0.71^{b,A} \pm 0.15$	0.35 – 1.10	$0{,}56^{a,b,A} \pm 0.25$
t6 – t9	0.38 – 0.46	$0.42^{a,A} \pm 0.04$	0.35 – 0.43	$0.40^{a,A} \pm 0.03$	0.31 – 0.43	$0.40^{a,A} \pm 0.05$	0.32 – 0.58	$0.39^{a,A} \pm 0.09$
*t*10 ±*t*11	1.10 – 1.48	$1.26^{a,A} \pm 0.14$	1.02 – 1.37	$1.15^{a,A} \pm 0.14$	1.55 – 2.28	$1{,}94^{b,B} \pm 0{,}28$	0.97 – 1.53	$1.43^{a,A,B} \pm 0.47$
t12 C18:1	0.24 – 0.34	$0.28^{a,A} \pm 0.04$	0.20 – 0.26	$0{,}25^{a,A} \pm 0{,}02$	0,20 – 0,28	$0.26^{a,A} \pm 0.03$	0.17 – 0.33	$0.23^{a,A} \pm 0.06$
t16 C18:1	0.27 – 0.33	$0.30^{a,A} \pm 0.02$	0.25 – 0.30	$0.28^{a,A} \pm 0.02$	0.26 – 0.36	$0.32^{a,A} \pm 0.03$	0.24 – 0.38	$0.30^{a,A} \pm 0.06$
Σ *trans* C18:1	1.99 – 2.61	$2.25^{a,A,B} \pm 0.23$	1.82 – 2.32	$2.08^{a,A} \pm 0.18$	2.32 – 3.34	$2.92^{b,B} \pm 0.36$	1.71 – 3.45	$2.36^{a,b,A,B} \pm 0.54$
c9 t13	0.14 – 0.21	$0{,}18^{a,A} \pm 0{,}03$	0,11 – 0,20	$0.16^{a,A} \pm 0.03$	0.15 – 0.22	$0.19^{a,A} \pm 0.03$	0.12 – 0.31	$0.19^{a,A} \pm 0.06$
c9 t12	0.24 – 0.30	$0{,}27^{a,A} \pm 0{,}02$	0.23 – 0.28	$0.25^{a,A} \pm 0.02$	0.22 – 0.30	$0.27^{a,A} \pm 0.03$	0.13 – 0.35	$0.25^{a,A} \pm 0.08$
t9 c12	0.01 – 0.04	$0.02^{a,A} \pm 0.01$	0.01 – 0.03	$0.01^{a,A} \pm 0.01$	0.02 – 0.04	$0.03^{a,A} \pm 0.01$	0.01 – 0.14	$0.05^{a,A} \pm 0.08$
t11 c15	0.09 – 0.12	$0.11^{a,A} \pm 0.01$	0.08 – 0.15	$0.12^{a,A} \pm 0.03$	0.13 – 0.25	$0.19^{a,A} \pm 0.05$	0.06 – 0.40	$0.16^{a,A} \pm 0.11$
Σ *trans* C18:2	0.51 – 0.61	$0.57^{a,A} \pm 0.04$	0.51 – 0.63	$0.54^{a,A} \pm 0.05$	0.55 – 0.80	$0.76^{a,A} \pm 0.09$	0.48 – 1.06	$0.66^{a,A} \pm 0.19$

a,b – Values in rows denoted with the same letter are not significantly different ($p>0.05$)

A,B – Values in rows denoted with the same letter are not significantly different ($p>0.01$)

analyzed by PASZCZYK et al. (2012) contained on average from 2.34% (Polish cheeses) to 3.79% (Italia cheeses) of these isomers.

The mean content of the analyzed *trans* isomers of C18:2 acid in fat of kefirs and yoghurts was alike. In fat isolated from yoghurts these isomers constituted on average 0.57% (from 0.51 to 0.61% of total fatty acids) and in fat of kefirs – 0.54% (from 0.51 to 0.63%) (Table 1). Their similar contents were reported by ŻEGARSKA et al. (2008) in fat of commercially-available kefirs and yoghurts purchased in winter, i.e. 0.47% in both. Slightly higher contents of these isomers were determined by these authors in fermented dairy drinks purchased in June and July, i.e. 0.74% in fat from kefirs and 0.72% in fat from yoghurts.

A slightly higher mean content of *trans* isomers of C18:2 acid was determined in mold cheeses. In cheeses with bloomy molds they constituted 0.76% (from 0.55 to 0.80%) and in blue-veined cheeses – 0.66% (from 0. 48 to 1.06%) of total fatty acids. Slightly lower sums of these isomers were reported by ŻEGARSKA et al. (2008) in different mold cheeses purchased since February till March, i.e. from 0.39 to 0.69% with an average content of 0.54% of total fatty acids. According to PASZCZYK et al. (2012), in mold cheeses originating from different EU Member States and purchased in January-February the mean content of *trans* isomers of C18:2 acid ranged from 0.63% (cheeses from Poland) to 0.80% (cheeses from Italy).

Conclusions

The content of conjugated linoleic acid *cis*9*trans*11 C18:2 in the total fatty acid composition of fat isolated from the analyzed yoghurts ranged from 0.38% to 0.46%. The content of this isomer in fat of the investigated kefirs available on the market in the same period was at the same level.

The analyzed yoghurts and kefirs were also characterized by similar contents of *trans* isomers of C18:and C18:2 acids.

The content of CLA in the investigated mold cheeses was higher than in yoghurts and kefirs. In fat of cheeses with bloomy mold it ranged from 0.54% to 0.88%, whereas in fat of blue-veined cheeses it ranged from 0.35% to 1.10% of the total fatty acid composition.

The analyzed mold cheeses were additionally characterized by higher contents of *trans* isomers of C18:1 and C18:2 acids compared to yoghurts and kefirs.

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