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CONTENT OF *CIS* 9 *TRANS* 11 C18:2 ACID (CLA) AND *TRANS* ISOMERS OF C18:1 ACID IN YOGHURTS AND BIOYOGHURTS

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The research was aimed at comparing contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 acid in commercial yoghurts and bioyoghurts as well as acquiring information whether CLA concentration in yoghurts and bioyoghurts differs from that in the initial material. Determinations were carried out with the method of gas chromatography using a 100-m capillary column with CP Sil 88 phase.

Investigations demonstrated that in commercial yoghurts and bioyoghurts analysed the content of *cis*9*trans*11 C18:2 acid (CLA) and the total content of *trans* isomers of C18:1 acid were alike. The mean content of CLA in fat isolated from the yoghurts examined ranged from 0.41% to 0.43% of the total fatty acid composition, whereas in fat extracted from the bioyoghurts under study it ranged from 0.41% to 0.44%. The total content of *trans* isomers of C18:1 acid in yoghurts oscillated between 1.66% and 2.34% of the total fatty acid composition, and that in the bioyoghurts – between 1.95% and 2.91%.

The content of *cis*9*trans*11 C18:2 acid in the total fatty acid composition of fat obtained from raw bulk milk examined did not differ from its content in fermented drinks produced from that milk. The mean content of CLA in fat of the yoghurts accounted for 0.53%, whereas that in fat of milk they were produced from – for 0.52%. Fat of the bioyoghurts analysed was characterised by a slightly lower content of *cis*9*trans*11 C18:2 acid as compared to the raw material. The mean content of *trans* isomers of C18:1 acid reached 2.25% in yoghurts, 2.00% in bioyoghurts and 2.13% of the total fatty acid composition in raw milk.

INTRODUCTION

In molecules of conjugated dienes of milk fat (CLA), double bonds (in *cis* or *trans* configuration) are separated only with one single bond. Even one bond of the *trans* configuration occurring in those acids allows most CLA isomers to be considered as *trans* isomers of polyene fatty acids. In that group of compounds, the highest biological activity has been ascribed to the *cis*9*trans*11 C18:2 acid which is a predominating constituent of conjugated dienes of milk fat (it constitutes from 83 to 93% of all CLA isomers [Chin *et al.*, 1992; Lin *et al.*, 1995; Jiang *et al.*, 1997]). It has been acknowledged to display antioxidant, anticarcinogenic, antiatherogenic and antimutagenic properties [Molkentin, 1999; Parodi, 1999]. In milk fat, the content of *cis*9*trans*11 C18:2 acid (CLA) is positively correlated with the content of *trans* isomers of C18:1 acid. Investigations of Paszczyk *et al.* [2005] have demonstrated that the correlation coefficient between CLA and total content of C18:1 *trans* isomers in milk fat accounted for 0.673. A positive correlation (r=0.78) between the content of *cis*9*trans*11 C18:2 acid and that of the main C18:1 *trans* isomer, vaccenic acid, has been reported in milk fat examined by Jiang *et al.* [1997]. Currently, over 60% of CLA present in milk fat is known to be synthesized from the vaccenic acid in mammary gland tissue [Griinari *et al.*, 2000].

A rich source of *cis*9*trans*11 C18:2 acid in a human diet is milk and dairy products. Its content in the total fatty acid composition in milk fat may fluctuate from 2.3 to 26 mg/g fat [Chin *et al.*, 1992; Lin *et al.*, 1995]. Those fluctuations are affected by a number of factors, among other, feeding regime of cows connected with season, composition of feed administered to cows, individual properties of bacterial flora in stomachs of ruminants and genetical traits of animals. The content of CLA in dairy products may differ from CLA concentration in milk. The CLA content of dairy products, as compared to the initial raw material, may be affected by conditions of technological processes (temperature, ripening time, packaging method) [Lin *et al.*, 1998; Shanta *et al.*, 1992] and capacity of some cultures of propionic and lactic bacteria for CLA synthesis under appropriate conditions of the fermentation process [Jiang *et al.*, 1998; Kim & Liu, 2002; Lin, 2003; Sieber *et al.*, 2004]. Investigations of Jiang *et al.* [1998] have indicated that out of seven strains of *Lactobacillus* bacteria, two *Streptococcus* strains and six strains of *Propionibacterium* cultured *in vitro*, only *Propionibacterium freudenreichii* was capable of transforming linoleic acid to *cis*9*trans*11 and *trans*9*cis*11 C18:2 isomers. Kim & Liu [2002] evaluated thirteen strains of lactic fermentation bacteria with sunflower oil, containing *ca.* 70% of linoleic acid, used as a substrate for CLA production. They reported that nine out of the thirteen bacterial strains examined

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demonstrated the ability to synthesize CLA. The most effective proved to be strain *Lactococcus lactis* IO-1 at the optimal concentration of sunflower oil, *i.e.* 0.1 g/L of full-fat milk. According to those authors, the content of CLA in milk subjected to the fermentation process in affected by such factors as: bacterial strain, number of cells, appropriate concentration of a substrate, and time of incubation at neutral pH.

The objective of the reported research was to compare the contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 acid in fat of commercial fermented drinks (yoghurts and bioyoghurts), as well as to obtain information whether the content of *cis*9*trans*11 C18:2 acid in yoghurts and bioyoghurts differs from its concentration in the initial raw material.

MATERIAL AND METHODS

Experimental material. Material to be analysed were yoghurts and bioyoghurts purchased at a local market in Olsztyn between January and March 2005. In January, analyses were carried out on 3 bioyoghurts and 8 yoghurts, whereas in Febraury and March on 8 yoghurts and 8 bioyoghurts in each month. The products investigated originated from different producers and were examined over their shelf life.

In the second part of the study, the experimental material was milk fat obtained from bulk milk as well as yoghurts and bioyoghurts produced from that milk at the Institute of Dairy Industry Development, University of Warmia and Mazury in Olsztyn. Raw milk, collected from suppliers from milk collecting points in the area of Olsztyn, was transported to the Institute in one week intervals for six consecutive weeks, in the period from March to May. In the first week, the milk was used to produce a series of yoghurts, whereas in the second week – a series of bioyoghurts. Two samples were collected from each series of yoghurts and bioyoghurts produced for analytical examinations.

Analytical methods. Fat was obtained from the dairy products with the modified method of Folch [Christie, 1973]. According to this method, a product's sample to be analysed is homogenised first with methanol, then with chloroform, and finally with a chloroform:methanol mixture (2:1 v/v). Next, first chloroform and then methanol are added to the sample, which is then vigorously shaken after the addition of each reagent, fat separation and removal of non-fat substances with a water solution of sodium chloride.

Fat extraction from milk was carried out with the method of Röse-Gottlieb [IDF standard 1D:1996].

The extracted fat was used to prepare methyl esters according to the IDF method, using a methanolic solution of KOH [IDF standard 182: 1999].

Methyl esters of fatty acids of milk fat were separated with the method of gas chromatography, using a Hewlett Packard 6890 chromatograph with a flame ionization detector (FID), a capillary column with CP Sil 88 phase with the length of 100 m, internal diameter of 0.25 mm, and liquid phase film thickness of 0.20 m. Separations were carried out under the following conditions: column temp.: 60°C (1 min) – 180°C, t=5°C/min; injector temp.: 225°C; detector temp.: 250°C; carrier gas: helium, flow rate of 0.8 mL/min, injector: split 100:1. Identification of linoleic acid with a conjugated system of double bonds (CLA) and that of methyl esters of *trans* fatty acids were carried out by comparing their retention times with those of Sigma standards and on the basis of literature data. The percentage contribution of fatty acids was calculated as compared to the total fatty acids (% wt).

RESULTS AND DISCUSSION

The average, minimum and maximum content of CLA and that of the sum of *trans* isomers of C18:1 acid in the total fatty acid composition of yoghurts and bioyoghurts purchased in January, February and March were presented in Table 1. In the study, the major CLA isomer – *cis*9*trans*11 C18:2 acid – was identified based on Sigma standards.

Data on the average, minimum and maximum concentrations of CLA and *trans* isomers of C18:1 acid in the total fatty acid composition of fat from bulk milk as well as yoghurts and bioyoghurts made of it were compiled in Table 2.

As shown by data presented in Table 1, the average content of CLA acid in fat extracted from the yoghurts examined reached 0.41% of the total fatty acid composition (for yoghurts from February and March) and 0.43% (for yoghurts purchased in Janury). Bioyoghurts available on the market in the same months were characterised by a similar content of *cis*9*trans*11 C18:2 acid, ranging from 0.41% (bioyoghurts from Janury) to 0.44% of the total fatty acid composition (bioyoghurts from March). Alike concentra-

TABLE 1. Content of CLA and the sum of *trans* isomers of C18:1 acid in fat of yoghurts and bioyoghurts purchased in January, February and March (% of the total fatty acid composition).

Trans isomers of fatty acids	Yoghurts				Bioyogurts			
	Min.	Max.	$\bar{X}$	± s	Min.	Max.	$\bar{X}$	±s
				January				
CLA	0.38	0.50	0.43[a]	± 0.03	0.35	0.45	0.41[a]	± 0.05
Σ *trans* C18:1	1.93	2.34	2.08[a]	± 0.23	1.97	2.91	2.39[a]	± 0.49
				February				
CLA	0.35	0.47	0.41[a]	± 0.04	0.40	0.46	0.42[a]	± 0.02
Σ *trans* C18:1	1.70	2.24	2.02[a]	± 0.56	1.96	2.21	2.13[a]	± 0.23
				March				
CLA	0.38	0.43	0.41[a]	± 0.02	0.38	0.50	0.44[a]	± 0.04
Σ *trans* C18:1	1.66	2.18	2.00[a]	± 0.28	1.95	2.82	2.21[a]	± 0.42

Values in lines denoted with the same letters are not significantly different

TABLE 2. Content of CLA and the sum of *trans* isomers of C18:1 acid in fat of bulk milk as well as yoghurts and bioyoghurts made of it in the period from March to May (% of the total fatty acid composition).

Trans isomers of fatty acids	Min.	Max.	$\bar{X}$	± s	Min.	Max.	$\bar{X}$	± s
	Bulk milk				Bioyoghurts			
CLA	0.38	0.70	0.52[a]	± 0.16	0.45	0.67	0.53[a]	± 0.12
Σ *trans* C18:1	1.87	2.84	2.27[a]	± 0.51	1.91	2.79	2.25[a]	± 0.47
	Bulk milk				Bioyoghurts			
CLA	0.37	0.52	0.43[a]	± 0.08	0.38	0.45	0.42[a]	± 0.04
Σ *trans* C18:1	1.53	2.40	2.00[a]	± 0.44	1.75	2.24	2.00[a]	± 0.25

Values in lines denoted with the same letters are not significantly different

tion of linoleic acid with a conjugated system of double bonds, accounting for 0.38% of the total fatty acids, was observed in yoghurts analysed by Lin *et al.* [1995]. According to a study by Fritsche & Steinhart [1998], the contribution of CLA acid in fat of yoghurts reached 0.69%, whereas in fat of probiotic yoghurts it was higher and accounted for 1.05%. Those authors investigated products available on the market from April to December, hence a higher content of *cis*9*trans*11 C18:2 acid in those products was likely to result from different contents of that acid in milk used for their production.

The results collected in Table 1 indicate that the concentration of *cis*9*trans*11 C18:2 acid in the yoghurts examined was not statistically different from that reported for bioyoghurts available on the market in the same period of the year.

The total content of *trans* isomers of C18:1 acid in the total fatty acid composition of fat of the yoghurts analysed ranged from 1.66% (yoghurts from March) to 2.34% (yoghurts from January), (Table 1). In fat extracted from bioyoghurts, the concentration of those isomers fluctuated between 1.95% (bioyoghurts from March) and 2.91% (bioyoghurts from January). According to a research by Fritsche & Steinhart [1997], the average content of C18:1 *trans* isomers in four German yoghurts purchased in the period from April to December accounted for 1.81%, whereas in fat of two probiotic yoghurts it appeared to be higher and reached 3.48%. Substantially lower concentrations of *trans* isomers of C18:1 acid in fat of yoghurts were reported by Daniewski *et al.* [1998]. In different types of yoghurts, they observed the content of those isomers to range from 0.46 to 1.21%, however the authors did not provide any information on the period of year the yoghurts examined originated from.

As indicated by data presented in Table 1, the average percentage of the sum of *trans* isomers of C18:1 acid in the total fatty acid composition of fat extracted from the bioyoghurts under study was slightly higher as compared to their concentration in fat of yoghurts originating from the same period of the year. The differences were, however, not significant.

In the second part of the experiment, the concentration of *cis*9*trans*11 C18:2 acid (CLA) was compared in raw milk as well as yoghurts and bioyoghurts made of it. According to literature data [Jiang *et al.*, 1998; Lin, 2003; Sieber *et al.*, 2004], the fermented dairy drinks were expected to be characterised by higher CLA contents compared to the raw milk. The content of CLA may be higher due to the presence of lactic fermentation bacteria, some strains of which are capable of CLA synthesis under appropriate conditions of the fermentation process [Jiang *et al.*, 1998; Kim & Liu, 2002; Lin, 2003]. The results provided in Table 2 indicate that the content of *cis*9*trans*11 C18:2 acid in the total fatty acid composition of fat extracted from the examined raw milk and fermented dairy beverages made of it was very alike. The average concentration of CLA in fat of yoghurts accounted for 0.53%, and in fat of milk they were produced from – for 0.52%. Fat of the bioyoghurts analysed appeared to be characterised by a slightly lower average content of *cis*9*trans*11 C18:2 acid compared to the raw material (Table 2). The results obtained in this study may indicate that the major source of *cis*9*trans*11 C18:2 acid (CLA) in fermented dairy drinks is milk used for their production.

The total content of C18:1 *trans* isomers in the fatty acid composition of fat extracted from bulk milk and fermented dairy drinks made of it was also alike (Table 2). The average concentrations of those isomers accounted for 2.25% in yoghurts, 2.00% in bioyoghurts, and 2.13% in raw milk. Such a content of the *trans* isomers of C18:1 acid is typical of milk fat originating from the winter feeding of cows [Precht & Molkentin, 1997; Żegarska *et al.*, 1996].

CONCLUSIONS

1. The study demonstrated that the percentage of CLA and total content of *trans* isomers of C18:1 acid in the fatty acid composition of fat extracted from yoghurts and bioyoghurts available on the market in the same period of the year were not statistically different.

2. The content of *cis*9*trans*11 C18:2 acid (CLA) in fat of yoghurts and bioyoghurts was similar to its concentration in the fat of bulk milk they were made of.

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ZAWARTOŚĆ KWASU *CIS* 9 *TRANS* 11 C18:2 (CLA) I IZOMERÓW *TRANS* KWASU C18:1 W JOGURTACH I BIOJOGURTACH

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Przedmiotem badań było porównanie zawartości kwasu *cis*9*trans*11 C18:2 (CLA) i izomerów *trans* kwasu C18:1 w rynkowych jogurtach i biojogurtach, a także uzyskanie informacji, czy zawartość kwasu CLA w jogurtach i biojogurtach rożni się od zawartości tego kwasu w surowcu wyjściowym. Oznaczenia przeprowadzano metodą chromatografii gazowej stosując 100 m kolumnę kapilarną z fazą CP Sil 88.

Badania wykazały, że w rynkowych jogurtach i biojogurtach zawartość kwasu *cis*9*trans*11 C18:2 (CLA) oraz sumaryczna zawartość izomerów *trans* kwasu C18:1 była na zbliżonym poziomie. Średnia zawartość CLA w tłuszczu wydzielonym z badanych jogurtów wynosiła od 0.41% do 0.43% ogólnego składu kwasów tłuszczowych, a w tłuszczu wydzielonym z badanych biojogurtów od 0.41% do 0.44%. Łączna zawartość izomerów *trans* kwasu C18:1 w jogurtach kształtowała się w przedziale od 1.66% do 2.34% ogólnego składu kwasów tłuszczowych, a w biojogurtach od 1.95% do 2.91%.

Zawartość kwasu *cis*9*trans*11 C18:2 w ogólnym składzie kwasów tłuszczowych tłuszczu badanego mleka surowego nie różniła się od zawartości tego kwasu w napojach fermentowanych z niego wyprodukowanych. Średnia zawartość CLA w tłuszczu jogurtów wynosiła 0.53%, a w tłuszczu mleka, z którego jogurty te zostały wytworzone 0.52%. W tłuszczu badanych biojogurtów stwierdzono nieco niższą zawartość kwasu *cis*9*trans*11 C18:2 w porównaniu do zawartości tego kwasu w surowcu. Średnia zawartość izomerów *trans* kwasu C18:1 w jogurtach wynosiła 2.25%, w biojogurtach 2.00%, a w mleku surowym 2.13% ogólnego składu kwasów tłuszczowych.