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CONTENT OF *CIS9TRANS*11 C18:2 ACID (CLA) AND *TRANS* ISOMERS OF C18:1 AND C18:2 ACIDS IN BUTTERS

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K e y w o r d s: butter, fatty acids, *trans* isomers, CLA.

A b s t r a c t

The aim of this study was to evaluate the fatty acid composition, including the content of conjugated linoleic acid *cis*9*trans*11 C18:2 (CLA) and *trans* isomers C18:1 and C18:2 acids, in 17 butters come from different producers available on the Olsztyn market. Determinations were carried out with the GC method using a 100 m capillary column with CP Sil 88 phase.

In all the analysed butters *cis*9*trans*11 C18:1 acid and *trans* isomers of C18:1 and C18:2 acids were found. The percent content of CLA in total fatty acids in fat butters was ranged from 0.53 to 1.13%, *trans* C18:1 ranged from 2.12 to 3.72% and *trans* C18:2 from 0.62 to 1.17%. A short-chain fatty acids in fat examined butters were ranged from 4.93 to 10.40%, saturated fatty acids from 52.82 to 60.08%, monounsaturated from 26.84 to 36.62% and polyunsaturated from 2.74 to 6.29% of total fatty acids.

ZAWARTOŚĆ KWASU *CIS9TRANS*11 C18:2 (CLA) I IZOMERÓW *TRANS* KWASU C18:1 I C18:2 W MASŁACH

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S ł o w a k l u c z o w e: masło, kwasy tłuszczowe, izomery *trans,* CLA.

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Abstrakt

Przedmiotem badań była ocena składu kwasów tłuszczowych, ze szczególnym uwzględnieniem zawartości kwasu linolowego o wiązaniach sprzężonych *cis*9*trans*11 C18:2 (CLA) oraz izomerów *trans* kwasu C18:1 i C18:2 w 17 masłach dostępnych na rynku w Olsztynie. Oznaczenia przeprowadzono metodą GC na 100-metrowej kolumnie kapilarnej z fazą CP Sil 88.

We wszystkich badanych produktach stwierdzono obecność kwasu *cis*9*trans*11 C18:2 (CLA) oraz izomerów *trans* kwasu C18:1 i kwasu C18:2. Udział CLA w ogólnym składzie kwasów tłuszczowych tłuszczu badanych maseł wynosił od 0,53 do 1,13%, izomerów *trans* kwasu C18:1 od 2,12 do 3,72%, a kwasu C18:2 od 0,62 do 1,17%. W tłuszczu wydzielonym z badanych maseł udział kwasów krótkołańcuchowych w ogólnym składzie kwasów tłuszczowych wynosił od 4,93 do 10,40%, kwasów nasyconych od 52,82 do 60,08%, monoenowych od 26,84 do 36,62%, a polienowych od 2,74 do 6,29%.

Introduction

Butter is a high-fat product manufactured exclusively from cow's milk as a result of the so-called churning of specially-prepared sour or sweet cream. The quality of milk fat is determined, primarily, by the composition of its fatty acids, the properties of which depend on the length of the carbon chain and the presence of unsaturated bonds. Unlike other natural fats, the milk fat is a complex fat, for it contains over 400 different fatty acids, many of which exert beneficial effects on human health. The quantitative composition of fatty acids of cow;s milk is changing under the influence of multiple factors, including: the feeding system of animals, breed of cows, lactation period, individual characteristics and health status of cows, and others. Out of these factors, the most significant effect is ascribed to the feeding system (JAWORSKI 1978, 1995). Milk fat from the summer period is characterised by significantly higher contents of C18 group fatty acids, including mainly fats from the C18:1 group, and by significantly lower contents of palmitic and myristic acids than the milk from the winter period (JAWORSKI 1978, JENSEN 2002, ŻEGARSKA 1988). A unique characteristics of milk fat, contributing to its high digestibility, is the presence of short- and medium-chain fatty acids. Butyric acid, whose concentration in milk fat reaches 3–4% of total fatty acids, has been found effective in the treatment of nipple and colon cancer (CICHOSZ 2009, ŻEGARSKA 2005). In turn, vaccenic acid (*trans* 11) constituting over 50% of all *trans* isomers of C18:1 acid in milk fat, has been shown to exhibit anticarcinogenic and antiatherosclerotic actions (PRZYGOJEWSKA and RAFALSKI 2003). Milk fat constitutes the main, dietary source of conjugated linoleic acid (CLA) characterised by a variety of health-promoting properties, including: anticarcinogenic, antidiabetic, anti-inflammatory and antiatherosclerotic effects (BIAŁEK and TOKARZ 2009, PARIZA 1991, PARODI 1999). It is also a source of other components implicated to exert beneficial effects on human health, e.g. phospholipids, fat-soluble vitamins and β-carotene.

Owing to a high intake of butter by Polish consumers and to a rich assortment of butter products available on the Polish market, the assessment of their quality is of great importance. In view of this, the objective of this study was to determine the composition of fatty acids, with special emphasis put to concentrations of conjugated linoleic acid *cis*9*trans*11 C18:2, CLA) and *trans* isomers of C18:1 and C18:2 acids, in extra butters available on the market of the city of Olsztyn.

Material and Methods

Material

The study comprised 17 butter samples purchased in the retail shops in Olsztyn, in October and November. The butters, containing 82–83% fat come from different producers.

Analytical methods

Fat from the butters was separated by melting, decantation and filtering through anhydrous sodium sulfate.

Methyl esters of fatty acids were prepared according to the IDF method using a methanol solution of KOH (*IDF Standard 182...* 1999).

The total composition of fatty acids of the isolated fat was determined by gas chromatography (GC) using a Hewlett Packard 6890 chromatograph (Palo Alto, CA), with flame-ionization detector and capillary column CP Sil 88 (100 m, 0.25 mm I.D., 0.20 μm film thickness); the column temperature was 60°C (1 min) – 180°C, Δt=5°C/min; split ratio:1:100; the injector and detector temperature: 225°C and 250°C, respectively; carrier gas: helium, flow 0.8 mL /min.

Identification of methyl esters of the *trans* fatty acids and linoleic acid with conjugated bonds was carried out by comparing their retention times with those of standards (Sigma and Supelco) and literature date (PRECHT and MOLKENTIN 1994, PRECHT and MOLKENTIN 1997b, PRECHT and MOLKENTIN 1999, RATNAYAKE et al. 1992, WOLFF and BAYARD 1995). The content of fatty acids was expressed as a percentage (weight %) of the total fatty acids. All measurements were performed in duplicate.

Results and Discussion

The content of particular groups of fatty acids in fat extracted from the butter samples examined was presented in Table 1. Results of the determination of *cis*9*trans*11 C18:2 acid (CLA) and the total content of *trans* isomers of C18:1 and C18:2 acids were presented in Figure 1. The exemplary chromatogram separation of *trans* isomers C18:1 and C18:2 acids of butter is presented in Figure 2.

Table 1
Percentage of some groups of fatty acids (% of total fatty acids) in fat of analysed butters

Product	Σ short-chain fatty acids	Σ saturated fatty acids	Σ monounsaturated fatty acids	Σ polyunsaturated fatty acids	Σ *trans* fatty acids
1	5.42	55.10	33.20	6.29	3.41
2	9.68	58.18	28.68	3.46	5.76
3	7.17	56.54	31.66	4.63	5.43
4	10.09	58.64	27.93	3.34	5.58
5	4.93	52.82	36.62	5.65	6.72
6	9.38	58.86	28.48	3.28	5.03
7	9.17	59.12	28.59	3.12	4.77
8	10.18	58.88	27.68	3.26	5.20
9	9.87	58.58	28.30	3.25	4.41
10	10.33	58.55	27.75	3.37	4.99
11	8.53	57.64	29.57	4.26	3.40
12	10.38	58.86	27.68	3.08	4.78
13	9.58	59.30	27.74	3.38	4.66
14	9.88	59.15	27.33	3.64	5.63
15	10.35	58.12	28.15	3.38	5.66
16	10.40	59.64	26.84	3.12	4.56
17	9.84	60.08	27.34	2.74	4.02
Mean ± SD	9.13 ± 1.69	58.12 ± 1.80	29.03 ± 2.54	3.72 ± 0.96	4.82 ± 0.72

In fat extracted from the butter samples the short-chain fatty acids (C4:0 – C10:0) ranged from 4.93 to 10.40% of the total fatty acids composition (Table 1). In all butter samples examined, the highest content in that group of fatty acid was noted for butyric acid (C4:0), *i.e.* from 1.58 to 3.38%.

From the nutritional viewpoint, the unbeneficial characteristics of milk fat is a high content of saturated fatty acids that constitute ca. 56% of the total fatty acids of milks fat (JAWORSKI 1978, STANIEWSKI 2000). The total content of saturated fatty acids in fat extracted from butter samples ranged from 52.82%

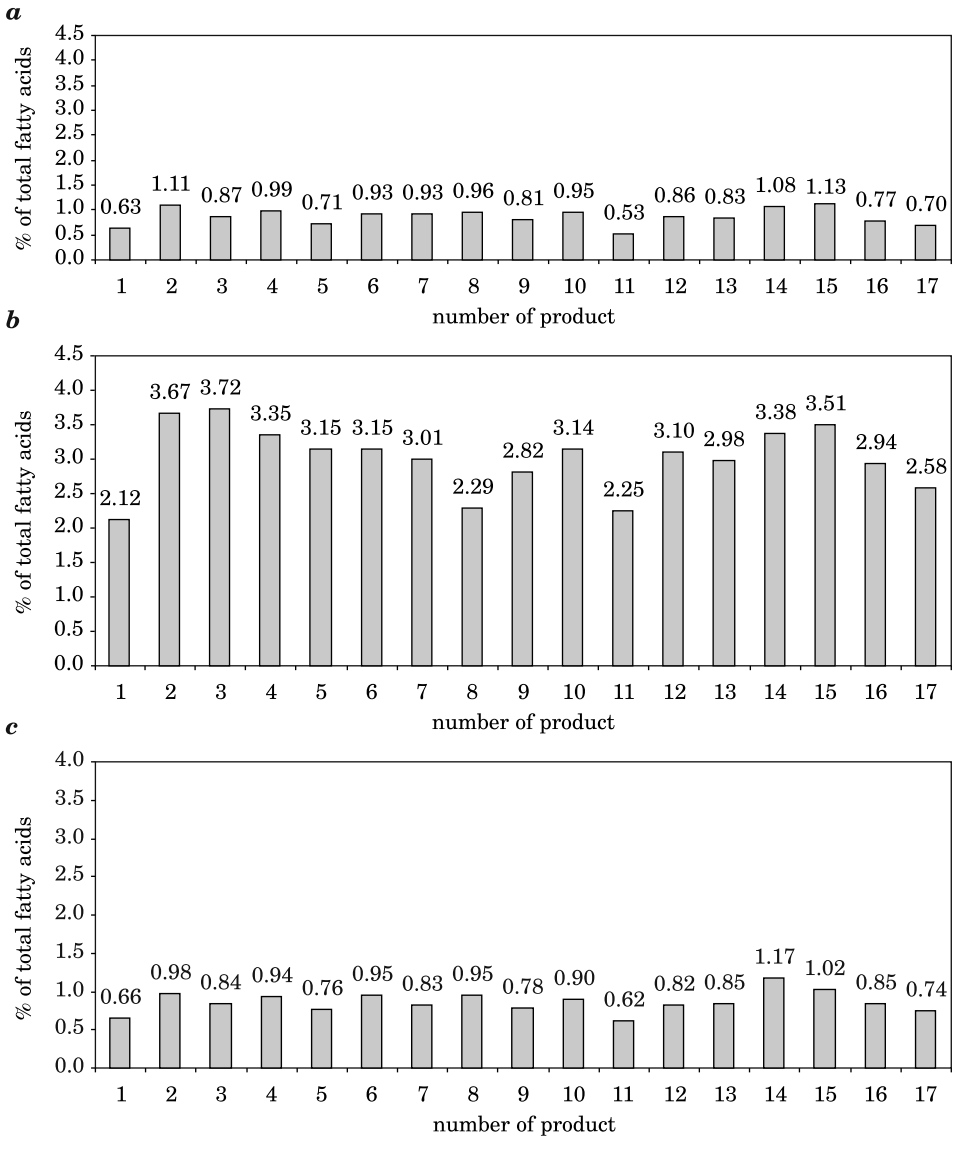

Fig. 1. The content of: *a* – cis9*trans*11 CLA, *b* – *trans* C18:1, *c* – *trans* C18:2 in the total fatty acids composition of fat isolated from the analysed butters

to 60.08% (Table 1). Those acids were mainly represented by palmitic acid (C16:0) which constituted from 27.84 to 36.46%, and by stearic acid (C18:O) which constituted from 7.25 to 10.50% of the total fatty acids composition.

In milk fat, the monoenoic acids constitute *ca.* 30% of the total fatty acids (JAWORSKI 1978, STANIEWSKI 2000). In the analysed butter samples, the total

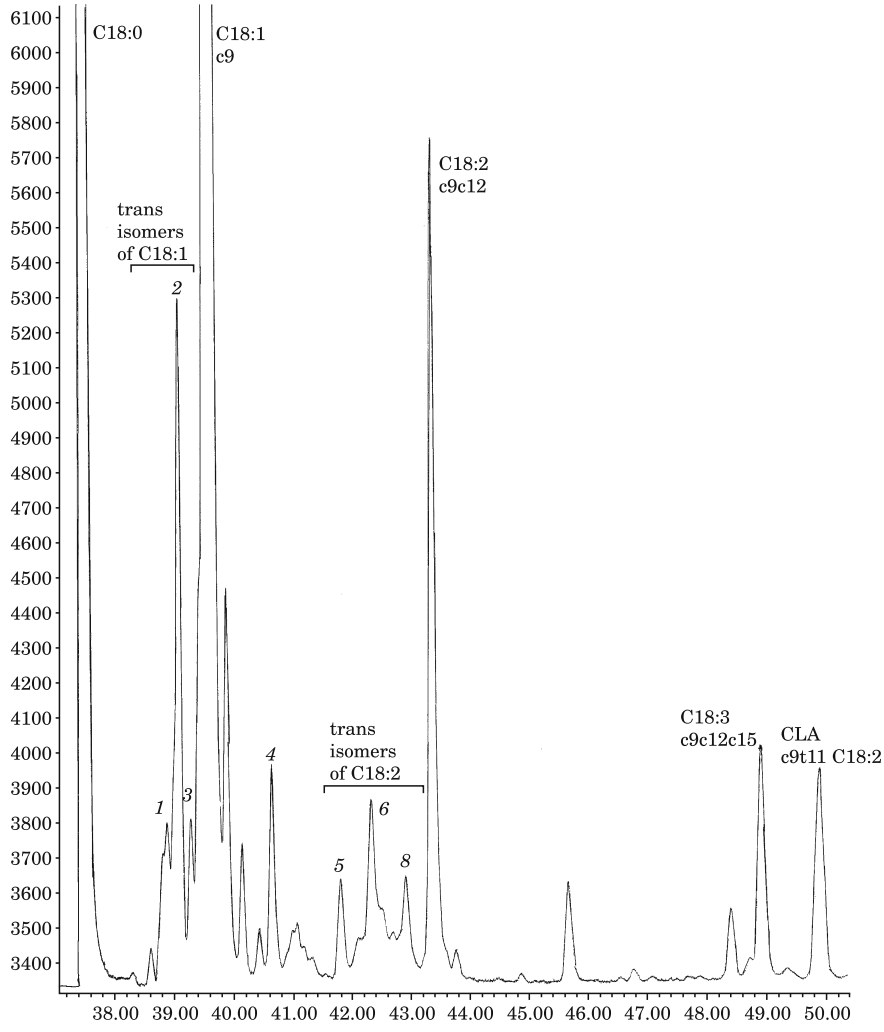

Fig. 2. Separation of *trans* isomers of C18:1 and C18:2 fatty acids of butter by GC. Identyfication: *trans* isomers of C18:1 (*1* – *trans* 6–9; *2* – *trans* 10+11; *3* – *trans* 12; *4* – *trans* 16); *trans* isomers of C18:2 (*5* – *cis*9*trans*13; *6* – *cis*9*trans*12; *7* – *trans*9*cis*12; *8* – *trans*11*cis*15)

content of those acids ranged from 26.84 to 36.62% of the total fatty acids composition. They were the most frequently represented by oleic acid (*cis* 9 C18:1), whose content reached 18.72 to 23.35% of the total fatty acids composition. Worthy of notice are antiatherosclerotic properties of this acid (ZIEMLAŃSKI and BUDZYŃSKA-TOPOLOWSKA 1991).

Milk fat is a poor source of polyenoic fatty acids (JAWORSKI 1978, STANIEWSKI 2000, ŻEGARSKA 1988). In fat extracted from the butter samples

examined the content of those acids ranged from 2.74 to 6.29% of the total fatty acids composition (Table 1). In all butter samples the predominating turned out to be linoleic acid (*cis*9*cis*12 C18:2) which constituted from 1.20 to 4.90%, linolenic acid (*cis*9*cis*12*cis*15 C18:3) which constituted from 0.39 to 0.76% of the total fatty acids composition.

Fat extracted from all butter samples contained conjugated linoleic acid (*cis*9*trans*11 C18:2, CLA), *trans* isomers of C18:1 acid as well as *cis,trans* / *trans,cis* isomers of C18:2 acid.

The linoleic acid with conjugated bonds (*cis*9*trans*11 C18:2) is a predominating constituent of conjugated dienes of milk fat, and is implicated to exert anticarcinogenic, antiatherosclerotic, immunomodulating and antioxidative effects. In fat extracted from the studied butter samples its content ranged from 0.53 to 1.13% (Figure 1). The content of CLA in milk varied in a wide range depending on the period of cows feeding. Higher contents of this acid are reported in milk fat originating from the period of pasture feeding, whereas lower ones from the period of stall feeding (PRECHT and MOLKENTIN 1997a, ŻEGARSKA et al. 1996). Results achieved in this study indicate that the butters examined were produced from milk originating from pasture or intermediate (October, November) feeding period (ŻEGARSKA et al. 2006). According to a study by BARTNIKOWSKA et al. (1999), in samples of butter produced in the winter (December, January and February) the mean content of CLA reached 0.45%, whereas in those produced from milk originating from the summer period (June, July, August) it constituted 1.2% of the fatty acids pool. A research by ŻEGARSKA et al. (2005) indicates that in samples of butters purchased in the summer the content of CLA ranged from 0.8 to 1.6% of the total fatty acids composition.

The total content of *trans* isomers of C18:1 acid in the butter samples examined fluctuated between 2.12 and 3.72% of the total fatty acids composition (Figure 1). In fat of all analysed butter samples, in the group of *trans* isomers of C18:1 acid the highest contents were reported for *trans*10 + *trans*11 isomers. Their contents were summarised due to their incomplete separation in some butter samples. Following literature data, the major *trans* isomer of C18:1 acid in milk fat is vaccenic acid (*trans*11), which constitutes over 50% of the total *trans* isomers of C18:1 acid, on average (PARODI 1976, ŻEGARSKA et al. 1996). This acid exhibits antiatherosclerotic properties and exerts a positive effect by inhibiting the growth of cancer cells of colon and other organs. Vaccenic acid is a substrate to the endogenous synthesis of CLA (BARTNIKOWSKA 2001, GRINARI et al. 2000, SANTORA et al. 2000). It has been estimated that *ca.* 64% of CLA in milk fat originated from the endogenous synthesis from *trans*11 acid mediated by delta-9 desaturase. According to literature data (PARODI 1976, PRECHT and MOLKENTIN 1997b, PRECHT and

MOLKENTIN 2000), the contribution of *trans*10 acid in milk fat reaches as little as *ca.* 5.5%. In butter samples analysed in this study, the total content of *trans*10 + *trans*11 isomers made up from 53% to over 70% of the total content of *trans* isomers of C18:1 acid.

According to a research by WOLF (1994), French butters from October-November contained 3.22% and those from May–June – 4.28% of *trans* isomers of C18:1 acid, on average. In turn ARO et al. (1998) report that the content of *trans* isomers of C18:1 acids in butters originating from different European countries ranged from 2.16 to 3.64%. BARTNIKOWSKA et al. (1999) demonstrated that in samples of butter produced in the winter (December, January, February) the mean content of *trans* isomers of C18:1 acid accounted for 1.4%, whereas in those of butter produced in the summer (June, July, August) for 3.55 of the fatty acids pool. Higher contents of those isomers in samples of butter purchased in the summer were reported by ŻEGARSKA et al. (2005). The samples of butters analysed in this study contained from 4.6 to 5.8% of those isomers. A research by DANIEWSKI et al. (1998) indicates that the total content of *trans* isomers of C18:1 acid in butters reached 0.59% in "Masło śmietankowe" and 3.72% in "Masło ekstra".

In fat extracted from the analysed butter samples, the content of *cis,trans* and *trans,cis* isomers of C18:2 acid ranged from 0.62 to 1.17% (Figure 1). A similar content of *trans* isomers of C18:2 acid, reaching from 0.8 to 1.3%, was demonstrated in butters investigated by ŻEGARSKA et al. (2005). According to data from another study of those authors (ŻEGARSKA et al. 2006), the content of *trans* isomers of C18:2 acid in milk fat from the summer period ranged from 0.65 to 1.19%, in milk fat from the intermediate period – from 0.51 to 1.07%, and in milk fat from the winter period – from 0.29 to 0.61% of the total fatty acids composition.

Conclusions

Contents of conjugated linoleic acid (*cis*9*trans*11 C18:2) and *trans* isomers of C18:1 and C18:2 acids in fat extracted from the investigated butters available on the Olsztyn market approximated respective contents demonstrated in milk fat. This may indicate that the level of *trans* isomers in butter is affected, to a significant extent, by the quality of raw material used for its production.

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