

**CONJUGATED LINOLEIC ACID (CLA)
AND TRANS ISOMERS OF C18:1 AND C18:2
ACIDS IN MOULD CHEESES**

Beata Paszczyk, Zbigniew Borejszo, Joanna Łuczyńska

Chair of Commodity Science and Food Analysis
University of Warmia and Mazury in Olsztyn

Key words: mould cheeses, conjugated linoleic acid (CLA), *trans* isomers.

Abstract

This study was aimed at determining the content of *cis9trans11* C18:2 (CLA) acid and of *trans* isomers of C18:1 and C18:2 acids in fat of mould cheeses originating from various producers (from different countries) available on the market in the city of Olsztyn.

Analyses were carried out for 20 types of mould cheeses originating from different EU Member States, i.e. Poland (8 types) as well as Denmark, Germany, France and Italy (3 types from each country).

The content of *cis9trans11* C18:2 acid in fat of the analyzed mould cheese originating from Polish producers ranged from 0.25 to 0.64% and did not differ significantly ($p > 0.05$) from that noted in fat extracted from cheeses originating from the other EU Member States. The content of this acid in cheeses from Denmark ranged from 0.50 to 0.54%, in those from Germany – from 0.44 to 0.81%, in those from Italy – from 0.48 to 0.51%, and in those from France – from 0.41 to 0.54% of the total fatty acid composition.

Contents of *trans* isomers of C18:1 acid in cheeses from Poland accounted for 1.94 to 2.64% of the total fatty acid composition. A similar content of those isomers was reported in the French cheeses. In turn, in the cheeses originating from Denmark, Germany and Italy the total content of C18:1 *trans* isomers was found to be significantly ($p \leq 0.05$) higher.

Trans isomers of C18:2 acid analyzed in the Polish cheeses constituted from 0.57 to 0.73%. In the cheeses from Denmark and France they were at a similar level, whereas in the cheeses from Germany and Italy the total content of those isomers was significantly ($p \leq 0.05$) higher than in the Polish cheeses.

The study demonstrated that the investigated mould cheeses originating from various producers and from various countries, and available on the Olsztyn market, were characterized by similar contents of CLA and diversified contents of *trans* isomers of C18:1 and C18:2 acids.

SPRZĘŻONY KWAS LINOŁOWY (CLA) I IZOMERY TRANS KWASU C18:1 I C18:2 W SERACH PLEŚNIOWYCH

Beata Paszczyk, Zbigniew Borejszo, Joanna Łuczynska

Katedra Towaroznawstwa i Badań Żywności
Uniwersytet Warmińsko-Mazurski w Olsztynie

Słowa kluczowe: sery pleśniowe, sprzężony kwas linolowy (CLA), izomery *trans*.

Abstrakt

Celem badań była ocena poziomu kwasu *cis9trans11* C18:2 (CLA) oraz izomerów *trans* kwasu C18:1 i kwasu C18:2 w tłuszczu serów pleśniowych pochodzących od różnych producentów (z różnych krajów), dostępnych w Olsztynie.

Oceniano 20 rodzajów serów pleśniowych pochodzących z różnych krajów UE. Analizie poddano sery wyprodukowane w Polsce (8 rodzajów) oraz sery pochodzące z Danii, Niemiec, Francji i Włoch (po 3 rodzaje serów).

Zawartość kwasu *cis9trans11* C18:2 w tłuszczu badanych serów pleśniowych pochodzących od polskich producentów wynosiła od 0,25 do 0,64% i nie różniła się istotnie ($p > 0,05$) od zawartości tego kwasu w tłuszczu wydzielonym z serów produkowanych w innych krajach UE. Zawartość tego kwasu w serach z Danii wynosiła od 0,50 do 0,54%, w serach z Niemiec od 0,44 do 0,81%, w serach z Włoch od 0,48 do 0,51%, a w serach z Francji od 0,41 do 0,54% ogólnego składu kwasów tłuszczowych.

Zawartość izomerów *trans* kwasu C18:1 w serach z Polski wynosiła od 1,94 do 2,64% ogólnego składu kwasów tłuszczowych. Zbliżoną zawartością tych izomerów charakteryzowały się sery francuskie. W serach pochodzących z Danii, Niemiec i Włoch stwierdzono istotnie wyższą ($p \leq 0,05$) sumaryczną zawartość izomerów *trans* C18:1.

Izomery *trans* kwasu C18:2 w serach z Polski stanowiły od 0,57 do 0,73%. W serach z Danii i Francji były na zbliżonym poziomie. Z kolei w serach z Niemiec i Włoch ich sumaryczna zawartość była istotnie wyższa ($p \leq 0,05$) w porównaniu z zawartością tych izomerów w serach polskich.

W badaniach wykazano, że badane sery pleśniowe pochodzące od różnych producentów i z różnych krajów, znajdujące się na olsztyńskim rynku, charakteryzowały się zbliżoną zawartością sprzężonego kwasu linolowego CLA i zróżnicowaną zawartością izomerów *trans* kwasu C18:1 i kwasu C18:2.

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. Out of that group of isomers, in fat of ruminants the highest content is reported for *cis9trans11* C18:2 acid which in milk fat constitutes from 75 to over 90% of total isomers of C18:2 acid with conjugated bonds (CHIN et al. 1992, PRECHT and MOLKENTIN 2000, PARODI 2003). The *cis9trans11* C18:2 acid exhibits a number of properties being beneficial to health, including anticarcinogenic, antiatherosclerotic, anti-

oxidative and anti-inflammatory ones (PARIZA 1991, PARODI 1994, 1997, MOKKENTIN 1999, CICHOSZ 2007). The main natural source of CLA in a human diet is milk and dairy products. The content of CLA in milk fat fits within a very wide range depending on the feeding period, lactation period, breed and individual determinants of cows. Out of these factors, the greatest significance is attributed to the mode of feeding. Higher contents of this acid occur in fat of milk originating from pasture feeding, whereas lower ones in milk fat from the period of stall feeding (ŻEGARSKA et al. 1996, PRECHT and MOKKENTIN 1997). According to a research by ŻEGARSKA et al. (2006), the mean content of *cis9trans11C 18:2* acid in fat of milk originating from the summer accounted for 1.40% and that in milk fat from the winter period – for 0.40% of the total fatty acid composition. In the case of dairy products (cheeses or fermented drinks), apart from the animal diet, the level of CLA is significantly affected by conditions occurring during technological processing and by the activity of the starter cultures added (SHANTHA et al. 1992, JIANG et al. 1998, LIN et al. 1998, KIM and LIU 2002, BZDUCHA-WRÓBEL and OBIEDZIŃSKI 2009, DOMAGAŁA et al. 2009). As reported by LIN et al. (1995), the content of conjugated linoleic acid in cheeses ranged from 3.59 to 7.96 mg/g fat. The highest contents of CLA were noted in Blu and Brie cheeses (mould cheeses), whereas the lowest ones in processed melted cheeses. In French cheeses analyzed by LAVILLONNIER et al. (1998), the concentration of linoleic acid with conjugated bonds ranged from 5.3 to 15.8 mg/g fat of cheeses. In turn, in German cheeses FRITSHE and STEINHART (1998) reported CLA content at the level of 0.40 to 1.70% of the total fatty acid composition, whereas Turkish cheeses investigated by GÜRISOY et al. (2003) contained from 0.29 to 0.97% CLA in the total fatty acid composition.

The objective of this study was to determine the content of *cis9trans11C18:2* (CLA) acid and of *trans* isomers of C18:1 and C18:2 acids in fat of mould cheeses originating from various producers (from different countries) available on the market in the city of Olsztyn.

Materials and Methods

Material to be analysed were 20 types of mould cheeses from different manufactures from different EU countries are available on the market in Olsztyn. Cheeses produced in Poland (8 types) and cheese from Denmark, Germany, France and Italy (after the 3 types of cheese) were analysed. The study included cheeses on the market in winter (January – February). All products were tested during their shelf life.

Fat from the analysed products was extracted according to the Folch's method (CHRISTIE 1973).

Fatty acid methyl esters of the fat were determined with the IDF method using a methanolic KOH solution (*IDF standard*. 182:1999).

Separation of methyl esters of fatty acids of the isolated fat was conducted with the gas chromatography (GC) method using a Hewlett Packard 6890 chromatograph with a flame-ionization detector (FID).

Determinations were carried out under the following conditions: capillary column – 100 m x 0.25 mm i.d. (Chromopack), film thickness – 0.20 µm, stationary phase – CP Sil 88, column temperature: 60°C (1 min) – 180°C, $\Delta t = 5^\circ\text{C}/\text{min}$; the injector and detector temperatures: 225 and 250°C, respectively; carrier gas: helium, flow rate: 0.8 cm³/min, split 100:1.

The identification of CLA and *trans* isomers was based on retention times of standards (Sigma and Supelco) and literature data (LUND and JENSEN 1983, HENNINGER and ULBERTH 1994, WOLFF 1994, PRECHT and MOLKENTIN 1995, 1996, 1997).

The results for the contents of CLA and *trans* isomers were given in reference to the total fatty acid composition (weight percentage). All samples were analysed in duplicate and mean values reported.

Statistical calculations were made based on the program STATISTICA PL (6.0).

Results and Discussion

The contribution of *cis9trans11* C18:2 acid (CLA) and the sum of *trans* isomers of C18:1 and C18:2 acids in the total fatty acid composition of fat extracted from the investigated mould cheeses was presented in Table 1. The exemplary chromatogram separation of *trans* and *cis* isomers C18:1 and *cis,trans/trans,cis* C18:2 acids is presented in Figure 1.

Data presented in Table 1 demonstrate that the content of *cis9trans11* C18:2 acid (CLA) in the total fatty acid composition of fat extracted from the investigated mould cheeses produced in Poland ranged from 0.25% (Hochland a la Brie) to 0.64% (Valbon fit jogurtowy). The mean content of CLA in fat extracted from the mould cheeses originating from Polish producers reached 0.43% and did not differ significantly from the respective values assayed in the cheeses produced in the other EU Member States. In fat of Danish cheeses, the mean content of CLA accounted for 0.53%, in that of German cheeses – for 0.58%, in that of Italian cheeses – for 0.50%, and in that of French cheeses – for 0.46% of the total fatty acid composition.

A similar CLA content of the total fatty acid composition in fat of mould cheeses available on the Olsztyn market in the winter period (February and March) was reported by ŻEGARSKA et al. (2008), i.e. from 0.42 to 0.54% (mean:

Table 1
 The content of *cis9trans11* C18:2 acid (CLA) and the sum of *trans* isomers of C18:1 and C18:2 acids in the total fatty acid composition of fat extracted from the investigated mould cheeses

Isomery <i>trans</i>	Country of origin																			
	Poland (n=8)			Denmark (n=3)			Germany (n=3)			Italy (n=3)			France (n=3)							
	min.	max.	\bar{x}	s	min.	max.	\bar{x}	s	min.	max.	\bar{x}	s	min.	max.	\bar{x}	s				
<i>Cis9trans11</i> C18:2 (CLA)	0.25	0.64	0.43^a	0.12	0.50	0.54	0.53^a	0.02	0.44	0.81	0.58^a	0.16	0.48	0.51	0.50^a	0.01	0.41	0.54	0.46^a	0.06
Σ <i>trans</i> C18:1	1.94	2.64	2.34^a	0.22	2.58	3.63	3.18^b	0.44	2.59	3.08	2.77^b	0.22	3.51	4.46	3.79^b	0.48	2.29	3.01	2.67^a	0.29
Σ <i>trans</i> C18:2	0.57	0.73	0.63^a	0.05	0.65	0.67	0.66^a	0.01	0.71	0.86	0.78^b	0.06	0.75	0.85	0.80^b	0.04	0.60	0.71	0.65^a	0.05

Values in rows marked by the same letter are not significantly different ($p>0.05$)

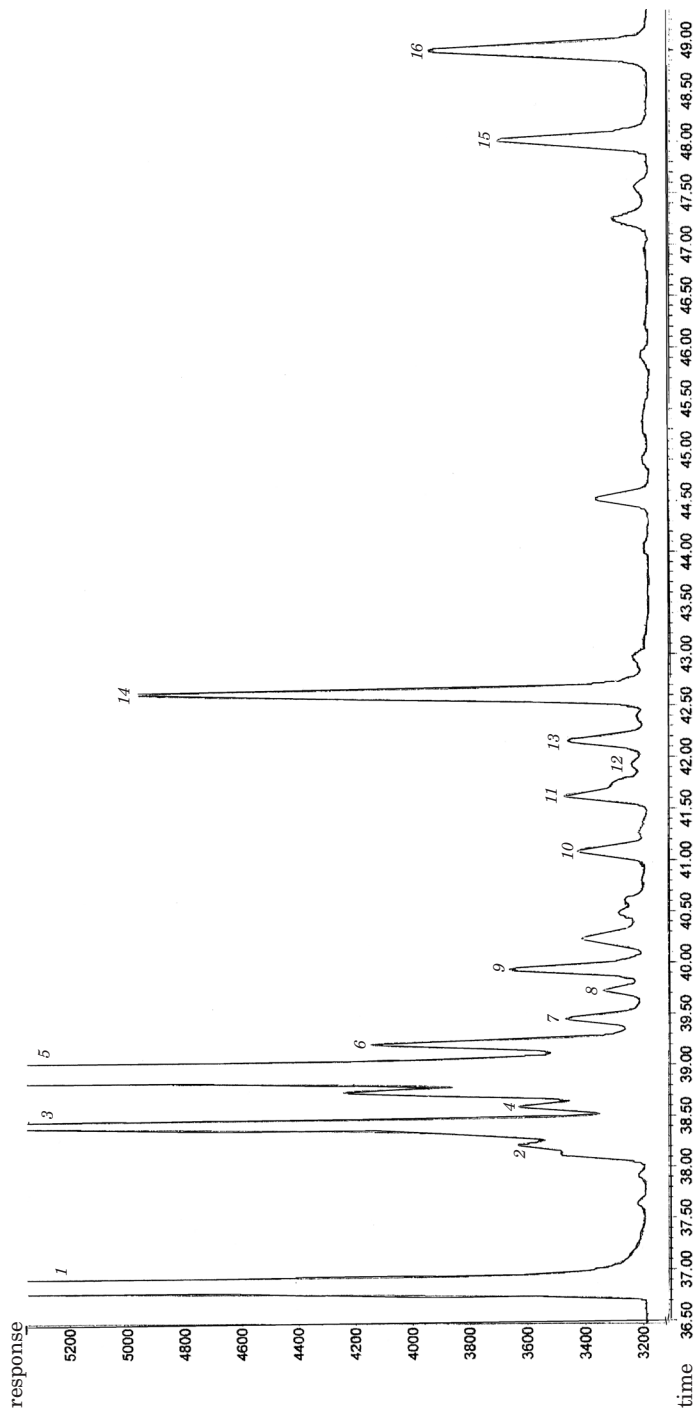


Fig. 1. Separation of *trans* and *cis* isomers of C18:1 and *cis*, *trans/trans*, *cis* of C18:2 fatty acids of mould cheese (produced in Poland) by GC. Identification: 1. C18:0; 2. *trans*6-9 C18:1; 3. *trans*10+11 C18:1; 4. *trans*12 C18:1; 5. *cis*9 C18:1; 6. *cis*11 C18:1; 7. *cis*12 C18:1; 8. *cis*13 C18:1; 9. *trans*16 C18:1; 10. *cis*9*trans*13 C18:2; 11. *cis*9*trans*12 C18:2; 12. *trans*9*cis*12 C18:2; 13. *trans*11*cis*15 C18:2; 14. *cis*9*cis*12 C18:2; 15. *cis*9*cis*12*cis*15 C18:3; 16. *cis*9*trans*11 C18:2 (CLA).

0.48%). A significantly higher content of that acid, ranging from 0.59 to 1.24%, was noted by those authors in fat of mould cheeses available on the market in November. The content of CLA in German cheeses investigated by FRITCHE and STEINHART (1998), ranged from 0.49% in Brie cheese to 0.69% in Gorgonzola cheese.

The mean content of *trans* isomers of C18:1 acid in the total fatty acid composition of fat of the examined mould cheeses produced in Poland ranged from 1.94 to 2.64%, with the mean value accounting for 2.34%. Those values are similar to the ones reported for milk fat originating from the intermediate feeding period (May, October and November) (ŻEGARSKA et al. 2006). Alike total contents of *trans* isomers of C18:1 acid in mould cheeses were determined by ŻEGARSKA et al. (2008). According to those authors, in cheeses purchased in February-March, the total content of C18:1 *trans* isomers constituted from 1.40 to 2.47%, and in cheeses purchased in November it was higher and ranged from 2.52 to 3.91% of the total fatty acid composition.

Cheeses originating from France and available on the Olsztyn market in January and February were characterized by a similar content of those isomers. The mean total content of C18:1 *trans* isomers in fat of the investigated French cheeses reached 2.67% and did not differ significantly ($p>0.05$) from the mean content of those isomers noted in the Polish cheeses. A significantly ($p\leq 0.05$) higher total content of *trans* isomers was assayed in Danish, German and Italian cheeses (Table 1). The total content of *trans* isomers in the analyzed cheese ranged from 2.58 to 3.63% in cheeses from Denmark, from 2.59 to 3.08% in cheeses from Germany, and from 3.51 to 4.46% in cheeses from Italy. German cheeses investigated by FRITSCHE and STEINHART (1997) contained from 1.23 to 2.30% of *trans* isomers of C18:1 acid.

The content of *trans* isomers of C18:2 acid in fat of the analyzed mould cheeses originating from Polish producers ranged from 0.57 to 0.73%, with the mean value accounting for 0.63% of the total fatty acid composition (Table 1). The cheeses originating from Denmark and France were characterized by a similar mean total content of *trans* isomers of C18:2 acid. In contrast, a significantly ($p\leq 0.05$) higher mean content of those isomers was assayed in fat extracted from the German and Italian cheeses, i.e. 0.78% and 0.80% of the total fatty acids, respectively.

In mould cheeses purchased in February and March and examined by ŻEGARSKA et al. (2008), the total content of *trans* isomers of C18:2 acid was in the range of 0.39 to 0.69% of the total fatty acid composition. In cheeses purchased in November, it was significantly higher.

Conclusions

The study demonstrated that the investigated mould cheeses originating from various producers and from various countries, available on the Olsztyn market, were characterized by similar contents of *cis9trans11* C18:2 acid (CLA). The mean total content of *trans* isomers of C18:1 acid in fat of mould cheeses originating from Poland reached 2.34%. Alike content of those isomers was assayed in French cheeses. In turn, significantly higher contents of those isomers were reported in cheeses originating from Denmark, Germany and Italy. The mean total content of *trans* isomers of C18:2 was similar in cheeses originating from Poland, Denmark and France, whereas contents of those isomers assayed in German and Italian cheeses were significantly higher.

Translated by JOANNA MOLGA

Accepted for print 10.12.2011

References

- BZDUCHA-WRÓBEL A., OBIEDZIŃSKI M. 2009. Zmiany zawartości CLA w układzie serów modelowych z dodatkiem *Bifidobacterium Animalis* subsp. *Lactis* i *Lactobacillus Acidophilus*. *Bromat. Chem. Toksykol.*, XLII, 3: 241–246.
- CICHOSZ G. 2007. Prozdrowotne właściwości tłuszczu mlekowego. *Przeg. Mlecz.*, 5: 4–8.
- CHIN S.F., LIU W., STORKSON J.M., HA Y.L., PARIZA M.W. 1992. *Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens*. *J. Food Compos. Anal.*, 5: 185–197.
- CHRISTIE W.W. 1973. *Lipid analysis. Isolation, separation, identification and structural analysis of lipids*. Pergamon Press, Oxford: 39–40.
- DOMAGAŁA J., SADY M., NAJGEBAUER-LEJKO D., CZERNICKA M., WIETESKA I. 2009. *The content of conjugated linoleic acid (CLA) in cream fermented using different starter cultures*. *Biotechnology in Animal Husbandry*, 25(5–6): 745–751.
- FRITSCHÉ J., STEINHART H. 1997. *Contents of trans fatty acids (TFA) in German foods and estimation of daily intake*. *Fett/Lipid*, 99(9): 314–318.
- FRITSCHÉ J., STEINHART H. 1998. *Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake*. *Z Lebensm. Unters. Forsch., A.*, 206: 77–82.
- GÜRSOY O., SECKIN A.K., KINIK O., METIN M. 2003. *Conjugated linoleic acid (CLA) content of most popular Turkish hard and soft cheeses*. *Milchwissenschaft*, 58(11/12): 622–623.
- HENNINGER M., ULBERTH F. 1994. *Trans fatty acid content of bovine milk fat*. *Milchwissenschaft*, 49(10): 555–558.
- IDF standard 182:1999. Milkfat. Preparation of fatty acid methyl esters.*
- JIANG J., BJÖRCK L., FONDÉN R. 1998. *Production of conjugated linoleic acid by dairy starter cultures*. *J. Appl. Microbiol.*, 85: 98–102.
- KIM Y.J., LIU R.H. 2002. *Increase of conjugated linoleic acid content in milk by fermentation with lactic acid bacteria*. *J. Food Sci.*, 67(5): 1731–1737.
- LAVILLONNIERE F., MARTIN J.C., BOUGNOUX P., SEBEDIO J.L. 1998. *Analysis of conjugated linoleic acid isomers and content in French cheeses*. *J. Am. Oil Chem. Soc.*, 75(3): 343–352.
- LIN H., BOYLSTON T.D., CHANG M.J., LUEDECKE L.D. 1995. *Survey of the conjugated linoleic acid contents of dairy products*. *J. Dairy Sci.*, 78: 2358–2365.
- LIN H., BOYLSTON T.D., LUEDECKE L.D., SHULTZ T.D. 1998. *Factors affecting the conjugated linoleic acid content of Cheddar cheese*. *J. Agric. Food Chem.*, 46(3): 801–807.

- LUND P., JENSEN F. 1983. *Isomeric fatty acids in milk fat*. *Milchwissenschaft*, 38: 193–196.
- MOLKENTIN J. 1999. *Bioactive lipids naturally occurring in bovine milk*. *Nahrung*, 43(3): 185–189.
- PARIZA M.W. 1991. *CLA, a new cancer inhibitor in dairy products*. *Bull. IDF.*, 257: 29–30.
- PARODI P. 2003. *Anti-cancer agents in milkfat*. *Aust. J. Dairy. Technol.*, 58(2): 114–118.
- PARODI P.W. 1994. *Conjugated linoleic acid: an anticarcinogenic fatty acid present in milk fat*. *Aust. J. Dairy Technol.*, 49: 93–97.
- PARODI P.W. 1997. *Cow's milk fat components as potential anticarcinogenic agents*. *J. Nutr.*, 1055–1059.
- PRECHT D., MOLKENTIN J. 1995. *Trans fatty acids: Implications for health, analytical methods, incidence in edible fats and intake*. *Die Nahrung*, 39(5/6): 343–374.
- PRECHT D., MOLKENTIN J. 1996. *Rapid analysis of the isomers of trans-octadecenoic acid in milk fat*. *Int. Dairy J.*, 6: 791–809.
- PRECHT D., MOLKENTIN J. 1997. *Effect of feeding on trans positional isomers of octadecenoic acid in milk fats*. *Milchwissenschaft*, 52(10): 564–568.
- PRECHT D., MOLKENTIN J. 2000. *Frequency distributions of conjugated linoleic acid and trans fatty acid contents in European bovine milk fats*. *Milchwissenschaft*, 55(12): 687–691.
- SHANTHA N.C., DECKER E.A., USTUNOL Z. 1992. *Conjugated linoleic acid concentration in processed cheese*. *J. Am. Oil Chem. Soc.*, 69(5): 425–428.
- WOLFF R.L. 1994. *Contribution of trans-18:1 acids from dairy fat to European diets*. *J. Am. Oil Chem. Soc.*, 71(3): 277–283.
- ŻEGARSKA Z., PASZCZYK B., BOREJSZO Z. 1996. *Trans fatty acids in milk fat*. *Pol. J. Food Nutr. Sci.*, 5/46(3): 89–97.
- ŻEGARSKA Z., PASZCZYK B., BOREJSZO Z. 2008. *Conjugated linoleic acid (CLA) and trans C18:1 and C18:2 isomers in fat of some commercial dairy products*. *Pol. J. Natur. Sc.*, 23(1): 248–256.
- ŻEGARSKA Z., PASZCZYK B., RAFAŁOWSKI R., BOREJSZO Z. 2006. *Annual changes in the content of unsaturated fatty acids with 18 carbon atoms, including cis9trans11 C18:2 (CLA) acid, in milk fat*. *Pol. J. Food Nutr. Sci.*, 15/56(4): 41–46.