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CHANGES IN THE CONTENT OF CLA AND OTHER *TRANS* ISOMERS IN THE KORMORAN CHEESE DURING SIX WEEKS OF RIPENING

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Key words: conjugated linoleic acid (CLA), *trans* fatty acids, cheese, ripening time.

Abstract

The aim of this study was to evaluate the effect of first six weeks of ripening on the content of *cis*9,*trans*11 CLA and *trans* isomers of C18:1 and C18:2 acids in Kormoran cheese. The experimental material included rennet, ripening Swiss – type Kormoran cheeses made of cow's milk. The cheeses were obtained from a dairy plant located in the Warmia and Mazury voivodeship. Analyses were conducted for normalized milk prepared for cheese production, freshly made cheeses and cheeses after 1, 2, 4 and 6 weeks of ripening. Cheeses were ripeing at the manufacturing plant.

The study showed that the time of ripening had a significant impact on the content of conjugated linoleic acid *cis*9,*trans*11 CLA as well as on the content of *trans* C18:2 isomers in cheeses. In the analyzed cheeses, the content of CLA was significantly higher ($P < 0.05$) after 6 weeks of ripening than the content of those isomers in fresh cheeses and those analyzed after 1, 2 and 4 weeks of ripening.

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ZMIANY W ZAWARTOŚCI CLA I INNYCH IZOMERÓW *TRANS* W SERZE KORMORAN PODCZAS SZEŚCIU TYGODNI DOJRZEWANIA

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Słowa kluczowe: sprzężony kwas linolowy (CLA), izomery *trans*, ser, czas dojrzewania.

Abstrakt

Celem pracy była ocena wpływu czasu dojrzewania serów na zawartość kwasu *cis*9,*trans*11 CLA oraz izomerów *trans* kwasu C18:1 i C18:2 w serach Kormoran. Materiałem badanym były sery kwasowo-podpuszczkowe typu szwajcarskiego wyprodukowane z mleka krowiego. Sery pozyskano z zakładu mleczarskiego zlokalizowanego w województwie warmińsko-mazurskim. Analizie poddano mleko znormalizowane przygotowane do produkcji serów, świeże sery oraz sery po 1, 2, 4 i 6 tygodniach dojrzewania. Sery dojrzewały w zakładzie produkcyjnym.

W badaniach wykazano, że czas dojrzewania miał istotny wpływ na zawartość sprzężonego kwasu linolowego *cis*9,*trans*11 CLA, jak również na zawartości izomerów *trans* C18:2 w serach. W objętych badaniem serach Kormoran zawartość CLA była istotnie wyższa ($P < 0,05$) po 6 tygodniach dojrzewania w porównaniu z zawartością tego izomeru w świeżych serach oraz badanych po 1, 2 i 4 tygodniach dojrzewania.

Introduction

The term conjugated linoleic acid (CLA) describes a group of polyunsaturated fatty acids that are isomers of linoleic acid with double conjugated bonds, mainly at carbons 9 and 11 or 10 and 12. For each positional isomer, four pairs of geometric isomers are possible. In fat of the ruminants, the main representative of this group of isomers is *cis*9,*trans*11 CLA which in milk fat constitutes from 76 to over 80% of total isomers of C18:2 acids with conjugated bonds (PRECHT and MOLKENTIN 2000, PARK 2009, KEE et al. 2010). The *cis*9,*trans*11 CLA displays a variety of health-promoting properties, including: antioxidative, anticarcinogenic and antimutagenic ones (KEE et al. 2010, MOLKENTIN 1999, PARODI 2003, PARK 2009, AYDIN 2005).

One of the main sources of CLA for humans is milk and dairy products (butter, fermented milk drinks, soft and hard cheeses). The content of CLA in milk fat fits within a very wide range and depends on the feeding period, lactation period, breed and individual determinants of cows. Out of these

factors, the greatest significance is attributed to the period of feeding. Higher contents of this acid occur in milk fat originating from pasture feeding, whereas lower ones in milk fat from the period of stall feeding (PRECHT and MOLKENTIN 1997, KELSEY et al. 2003, ŻEGARSKA et al. 2006, ZUNONG et al. 2008, FRELICH et al. 2012). In the case of dairy products (cheeses or fermented drinks), apart from the animal diet, the level of CLA was shown to be significantly affected by conditions occurring during technological processing and by the activity of the starter cultures added (SIEBER et al. 2004, SHANTHA et al. 1992, JIANG et al. 1998, LIN et al. 1998, LIN 2000, KIM and LIU 2002, BZDUCHA and OBIEDZIŃSKI 2007, DOMAGAŁA et al. 2009, MURTAZA et al. 2014). KIM et al. (2009) demonstrated that after aging for 4 months, the cheese made of milk obtained from pasture-raised cows contained relatively higher levels of CLA compared to the cheese made of milk obtained from indoor-raised cows (8.12 mg g^{-1} fat vs 6.76 mg g^{-1} fat, respectively), but the difference was not observed in 7-month-aged cheeses. However, both from pasture and indoor feeding, the 7-month-aged cheeses had a higher CLA content than the 4-month-aged ones, which indicates that the time of ripening is another important factor affecting CLA content in cheeses.

The aim of this study was to evaluate the effect of first six weeks of ripening on the content of *cis*9, *trans*11 CLA and *trans* isomers of C18:1 and C18:2 acids in rennet, ripening Swiss – type Kormoran cheese.

Materials and Methods

Experimental material

The experimental material was rennet, ripening Swiss-type Kormoran cheese, made of cow's milk. The cheese was obtained from a dairy plant located in the Warmia and Mazury voivodeship. The production process was as follows: pasteurization (72°C, 15s), normalization of milk, addition of sourdough of pure cultures (mesophilic, thermophilic and propionibacteria) and calcium chloride, mixing and heated to 32–34°C, addition of rennet, coagulation and cutting a clot, reheating (53–54°C), forming and pressing cheese, salting in brine (18–22% NaCl, 12-14 °C, 8–10 days), dripping, ripening: first two weeks (13–14°C), next two weeks (19–21°C), further ripening period (8–10°C). The content of *cis*9, *trans*11 CLA and *trans* isomers of C18:1 and C18:2 acids was determined in normalized milk used for

the cheese production, freshly made cheese and the cheese after 1, 2, 4 and 6 weeks of ripening. The cheeses were ripening at the manufacturing plant. Three samples of the cheese were taken for analyses each week. All determinations were conducted in two parallel replications.

Analytical Methods

Fat content

Fat content in milk was determined with the Roese-Gottlieb's method according to the Polish norm *Milk-Determination*… PN-EN ISO 1211:2011. Fat content in cheese was determined with the Schmidt-Bondzyński-Ratzlaff's method according to the Polish norm *Cheese and processed*… PN-EN ISO 1735:2006.

Lipid extraction

To determine CLA and *trans* C18:1 and C18:2 isomers, fat was isolated from normalized milk and chesses with the Folch method (CHRISTIE 1973).

Preparation of fatty acid methyl esters

Methyl esters of fatty acids were prepared according to the International Dairy Federation (IDF) method, using a methanol solution of KOH (according to *Milkfat. Preparation*… ISO 15884:2002).

Gas chromatography (GC) analysis

Determinations of CLA and *trans* isomers of unsaturated fatty acids were carried out with a gas chromatography with flame ionization detector (GC-FID) method using the HP 6890 chromatograph (Palo Alto, CA, USA). 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 (Varian, USA). Separation conditions were as follows: column temperature: 60°C (1 min) – 180°C, $\Delta t = 5°C\ min^{-1}$; detector temperature: 250°C; injector temperature: 225°C; carrier gas: helium, flow rate: 1.5 ml/min, and injector: split 50:1.

The *cis*-9,*trans*-11 CLA isomer was identified using a mixture of CLA methyl esters (Sigma-Aldrich). The positional *trans* isomers of C18:1 were identified using the following methyl ester standards of these isomers:

*trans*6 (Supelco, Bellefonte, USA; *trans*9 and *trans*11(Sigma-Aldrich, St. Louis, USA) and literature data (CONTARINI et al., 2013). The *trans* isomers of C18:2 acid (*cis,trans* and *trans,cis*) were identified with the use of a standard mixture of these isomers (Supelco, Bellefonte, USA).

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 analysis

The statistical analysis was carried out using STATISTICA software ver.10. One-way analysis of variance (ANOVA) was used to determine differences among time periods of ripening at a significance level of $\alpha = 0.05$. Differences between each mean values were evaluated with the Duncan's *post hoc* test. The Pearson`s correlation coefficients between the content of *cis*9,*trans*11 CLA, *trans* C18:1 and *trans* C18:2 isomers were evaluated using a STATISTICA software ver.10.

Results and discussion

The content of fat in milk intended for cheese production was 3.10%. Freshly produced cheese contained 26.4% of fat.

The content of *cis*9,*trans*11 CLA and C18:1 and C18:2 *trans* isomers in milk intended for cheese production, as well as in fresh cheese and in cheeses after 1, 2, 4 and 6 weeks of ripening, is presented in Table 1. The average content of *cis*9,*trans*11 CLA was 3.50 mg g^{-1} fat. The content of this acid in fresh cheese was similar (3.65 mg g^{-1} fat, $P > 0.05$). The study showed that CLA content in the cheese analyzed after 1, 2 and 4 weeks of ripening did not differ significantly from that found in normalized milk and fresh cheeses ($P > 0.05$). A significantly higher content of *cis*9*trans*11 CLA was found in the cheese analyzed after 6 weeks of ripening (4.17 mg g^{-1} fat, $P < 0.05$). BIAŁEK and TOKARZ (2009) analysed 44 different kinds of cheeses available on polish market, which included: soft-ripened cheeses (10) – camembert (7) and brie (3), blue cheeses (10), yellow cheese (17), goats cheeses (5), oscypek and home-made cottage cheese. The study has shown that blue cheeses were characterized by the lowest content of CLA (1.66 to 2.39 mg g^{-1} of fat), oscypek cheese contained CLA on the level goats cheeses ranged from 1.96 to 2.5 mg g^{-1} of fat. The most amounts as well as the biggest variety of the content of *cis9trans11* C18:2 acid was of 1.7 mg g^{-1} of fat and cottage cheese 3.0 mg g^{-1} of fat. In analysed soft-ripened cheeses the content of CLA ranged from 1.74 to 3.07 mg g^{-1} of fat and

Table 1

Changes in the content of CLA and C18:1 and C18:2 *trans* isomers in the Kormoran cheese during ripening [mg g^{-1} fat]

Isomers	Normalized milk $\overline{x}$ ± s /SD	Ripening time [weeks]				
		fresh $\overline{x}$ ± s /SD	1 week $\overline{x}$ ± s /SD	2 weeks $\overline{x}$ ± s /SD	4 weeks $\overline{x}$ ± s /SD	6 weeks $\overline{x}$ ± s /SD
***cis*9,trans11 CLA**	**3.50^{b} ± 0.06**	**3.65^{b} ± 0.05**	**3.79^{b} ± 0.22**	**3.45^{b} ± 0.21**	**3.60^{b} ± 0.18**	**4.17^{a} ± 0.03**
Σ *trans* C18:1	**17.13^{b} ± 0.62**	**19.10^{a} ± 0.07**	**19.63^{a} ± 0.62**	**$18.19^{a,b}$ ± 0.93**	**$18.43^{a,b}$ ± 1.05**	**19.54^{a} ± 0.46**
t6 - t9	3.26^{b} ± 0.15	3.74^{a} ± 0.05	3.86^{a} ± 0.14	3.60^{a} ± 0.19	3.60^{a} ± 0.23	3.82^{a} ± 0.08
*t*10 + *t*11	9.55^{c} ± 0.33	$10.27^{a,b,c}$ ± 0.05	$10.67^{a,b}$ ± 0.30	$9.82^{b,c}$ ± 0.56	$10.05^{a,b,c}$ ± 0.54	10.78^{a} ± 0.39
t12	2.01^{b} ± 0.05	2.41^{a} ± 0.03	2.39^{a} ± 0.10	2.23^{a} ± 0.13	2.30^{a} ± 0.15	2.38^{a} ± 0.06
t16	2.32 **^{b}** ± 0.09	2.67 **^{a,b}** ± 0.10	2.77 **^{a}** ± 0.16	2.55 **^{a,b}** ± 0.09	2.48 **^{a}** ± 0.13	2.56 **^{a,b}** ± 0.07
Σ *trans* C18:2	**4.67^{c} ± 0.28**	**5.14^{b} ± 0.07**	**4.25^{d} ± 0.06**	**5.14^{b} ± 0.17**	**5.08^{b} ± 0.29**	**5.65^{a} ± 0.24**
c9 t13	1.55^{a} ± 0.30	1.78^{a} ± 0.02	1.10^{b} ± 0.11	1.75^{a} ± 0.10	1.68^{a} ± 0.24	1.62^{a} ± 0.03
c9 t12	$2.15^{b,c}$ ± 0.04	$2.32^{a,b}$ ± 0.02	1.85^{c} ± 0.15	$2.28^{a,b}$ ± 0.13	$2.34^{a,b}$ ± 0.16	2.42^{a} ± 0.12
t11 c15	0.97^{d} ± 0.01	$1.04^{c,d}$ ± 0.03	1.20^{b} ± 0.10	1.11^{c} ± 0.05	$1.06^{c,d}$ ± 0.04	1.61^{a} ± 0.13

Explanation: $\overline{x}$ ± s /SD – mean value ± standard deviation; n = 3, *a*, *b*, *c*, *d* – values in the rows denoted by different letters differ statistically significantly ($P < 0.05$)

in yellow cheeses – from 0.5 to 6.26 mg g^{-1} of fat. CLA content in Canadian chesses analysed by MA et al. (1999) ranged from 2.7 mg g^{-1} fat in goat cheese to 4.7 mg g^{-1} in Imperial Cheddar cheese and farmer cheese. As reported by LIN et at. (1995), the content of conjugated linoleic acid in 15 tested cheeses ranged from 3.59 to 7.96 mg g^{-1} fat. Blue, Cottage, Monterey Jack, Brie, Edam and Swiss cheeses had a higher CLA content than the other cheeses: Cheddar-medium, Cheddar-sharp, Cougar Gold, Cream, Mozzarella, Proccesed cheese, Proccesed American, Parmesan and Viking. The content of CLA in Italian commercial cheeses from cow's milk: Alpine cheeses (9 samples), Swiss Emmental (8 samples) Fontina Valdostans (8 samples) and Grana/Parmigiano (5 samples) ranged from 3.85 to 8.11 mg g^{-1} fat (PRANDINII et al. 2007). CLA contents of most popular Turkish hard cheeses, that is Tulum, Teneke Tulum, Aged Kashar and Fresh Kashar studied by GÜRSOY et al. (2003) were in the range of 3.38–4.88 mg g^{-1} fat, 2.700–5.390 mg g^{-1} fat, 3.120–12.610 mg g^{-1} fat and 0.082–5.390 mg g^{-1} fat, respectively. The same authors found that the content of CLA in Turkish White Pickled cheeses was in the range of 0.010 to 5.470 mg g^{-1} fat. In Italian and French cow cheeses studied by PRANDINI et al. (2011), the average content of CLA was 5.66 mg g^{-1} fat. In fresh cheeses made of cow's milk studied by these authors, the CLA content was 6.10 mg g^{-1} fat. According to CICOGNINI et al. (2014), the content of CLA in Italian cheeses made of cow`s milk that were studied from January to December 2011 ranged from 4.32 mg g^{-1} fat in Gouda cheeses to 10.21 mg g^{-1} fat in Gruyere cheeses. In fact, the CLA content may increase in some dairy products subjected to processing, such as heat treatment (GARCIA-LOPEZ et al. 1994), aeration (LIN et al. 1995) and ripening. Many authors reported an increase in the CLA levels in cheeses as the ripening progressed (LAVILLONNIERE et al. 1998, LIN et al. 1999, TALPUR et al. 2008, KIM et al. 2009, LOBOS-ORTEGA et al. 2012, DOMAGAŁA et. al. 2013, CICOGNINI et al. 2014). According to LAVILLONNIERE et al. (1998), the content of CLA in French cheeses with a long ripening time (>4 month) ranged from 9.9 to 15.8 mg g^{-1} fat, whereas in cheeses ripening for a shorter period of time (<1 month) it ranged from 5.3 to 6.0 mg g^{-1} fat. The studies of CICOGNINI et al. (2014) showed that the highest content of CLA (8.45 mg g^{-1} fat) was found in long-ripening cheeses (>180 days). The average content of CLA in fresh cheeses (<45 days of ripening) was 5.70 mg g^{-1} fat, in cheeses ripened for >45 and <90 days, it was 4.85 mg g^{-1} fat and in cheeses ripening for 90 to 180 days, it was 5.45 mg g^{-1} fat. There are also authors who indicated that the ripening time of cheeses did not significantly affect the content of CLA (LIN et al. 1998, MURTAZA et al. 2014, LUNA et al. 2007). On the one hand, a longer exposure to bacterial action cause an increase in CLA contents,

but on the other hand, oxidation reactions could lead to the destruction of the double bonds, thus decreasing CLA levels (LIN et al. 1995, SHANTHA 1995, YANG et al. 2000).

LIN et al. (1999) evaluated the content of CLA in Cheddar, Cougar Gold and Viking cheeses after 1, 3 and 6 months of ripening. The highest content of CLA was found in all cheeses after 3 months of ripening. The content of this isomer was 3.76 mg g^{-1} fat in Cheddar cheese, 3.44 mg g^{-1} fat in Cougar Gold cheese and 3.47 mg g^{-1} fat in Viking cheese. All cheeses analyzed after 6 months of ripening were characterized by a lower content of CLA than those analyzed after 3 months of ripening. According to KUMAR et al. (2006), the content of *cis*9,*trans*11 CLA in buffalo Cheddar cheeses analyzed after 3 and 6 months of ripening was lower than in the fresh cheeses.

The total content of *trans* C18:1 isomers in milk was 17.13 mg g^{-1} fat. The total content of these isomers in fresh cheeses was significantly higher than in the milk they were produced from ($P < 0.05$). Only slight changes were observed in total content of C18:1 acid *trans* isomers during 6 weeks of ripening (Table 1). Conducted studies have shown that the content of each marked positional *trans* isomers of C18:1 acid in milk, freshly produced cheeses and cheeses analysed during 6 weeks of ripening have changed. The content of *trans* 6–9 isomers as well as *trans* 12 isomer was significantly higher in freshly produced cheeses than in milk prepared for its production. During ripening period the content of those isomers did not change significantly (Table 1). Fluctuations in the content of 10+11 *trans* isomers and *trans* 16 isomer were observed during ripening period.

The total content of *trans* C18:2 isomers in fresh cheeses was 5.14 mg g^{-1} fat and was significantly higher than the total content of those isomers in milk they were produced from ($P < 0.05$; Table 1). The total content of *trans* isomers of C18:2 acid in cheeses analyzed during the six weeks of ripening differed significantly, except for cheeses after 2 and 4 weeks (Table 1). The lowest total content of C18:2 acid *trans* isomers was after 1 week of ripening (4.25 mg/g fat) and the highest content was after 6 weeks of ripening (5.65 mg g^{-1} fat). Results presented in Table 1 indicate that the contents of each marked positional *trans* isomers of C18:2 acid in freshly produced cheeses did not differ significantly from the content of those isomers in milk used for production. During ripening period, the content of those isomers did change. Significantly lower ($P < 0.05$) contents of marked *trans* isomers of C18:2 acid were found in cheeses analysed after 1 week of ripening than in freshly produced cheeses. In cheeses analysed after 2, 4 and 6 weeks of ripening the content of *cis*9*trans*11 and *cis*9*trans*12 isomers fluctuated slighlty. The content of *trans*11*cis*15 was

significantly higher ($P < 0.05$) in cheeses analysed after 6 weeks of ripening than in chesses analysed after 2 and 4 weeks of ripening (Table 1).

The calculated linear Pearson's correlation coefficients indicate that the content of CLA in fat of cheeses was strongly, positively correlated with the content of *trans* C18:1 isomers (Table 2). These results are consistent with the findings of FRITSCHE and STEINHART (1998) who obtained a high, positive correlation ($r = 0.806$) between the content of CLA and the sum of *trans* C18:1 isomers in the fat of milk and dairy products. ŻEGARSKA et al. (2008) demonstrated that the CLA content in the fat from hard cheeses was highly positively correlated with the content of *trans* C18:1 isomers ($r = 0.828$, $P \leq 0.01$).

Table 2

The correlation coefficients between the content of *cis*9,*trans*11 CLA , *trans* C18:1 and *trans* C18:2 isomers

Specification	∑ *trans* C18:1	∑ *trans* C18:2
*cis*9,*trans*11 CLA	0.836 *	0.345
∑ *trans* C18:2	0.074	–

* – values are significantly different at $P \leq 0.001$

Conclusions

The study demonstrated that the time of ripening had a significant impact on the content of *cis*9,*trans*11 CLA as well as on the content of *trans* C18:2 isomers in the cheese. Only a slight change was observed in the content of *trans* C18:1 isomers during 6 weeks of ripening. In the analyzed Kormoran cheese, the content of *cis*9,*trans*11 CLA was significantly higher after 6 weeks of ripening compared to the content of this isomer in fresh cheese and in the cheese after 1, 2 and 4 weeks of ripening.

Comparing obtained results of the content of CLA in the analysed cheese with data obtained by other authors on the contents of this acid in a variety of cheeses, it can be concluded that Kormoran cheese is a good source of CLA in our diet.

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