

Changes in Conjugated Linoleic Acid Content in Emmental-Type Cheese during Manufacturing

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Abstract

DOMAGAŁA J., PLUTA-KUBICA A., PUSTKOWIAK H. (2013): **Changes in conjugated linoleic acid content in Emmental-type cheese during manufacturing.** Czech J. Food Sci., **31**: 432–437.

The fatty acids composition including the conjugated linoleic acid content in the milk and in the samples of Emmental-type cheese during the manufacturing and ripening period was determined. The highest amount of volatile and the lowest amount of saturated fatty acids were observed at the end of ripening. In turn, the highest content of monounsaturated fatty acids was found in the curd, however, it declined during processing. The richest in polyunsaturated fatty acids were the cheese samples after the warm room stage, however, the amount of these fatty acids became highly significantly lower at the end of ripening. The level of conjugated linoleic acid increased during manufacturing. Its content in the milk and the curd was highly significantly lower than at the other stages of production.

Key words: fatty acids; hard cheese manufacture; industrial conditions

Emmental represents one of the Swiss-type cheeses – hard, long ripened and manufactured from highly heated curd. It is produced in many European countries – Switzerland, where it originates from, Germany, Finland, France, Austria, and Poland. Emmental cheese is manufactured from raw bovine milk (in Switzerland, Finland, France, and usually in Germany) but from pasteurised milk as well (also in France) (MAIR-WALDBURG 1974). In Poland, Emmental-type cheese is manufactured from pasteurised milk of the highest quality. As a dairy product, it is considered to be a good source of unsaturated fatty acids, in particular vaccenic and conjugated linoleic acid (CLA) (GÜRSOY *et al.* 2003).

Vaccenic acid is a monounsaturated *cis*- or *trans*-11-octadecenoic acid. The evidence obtained so far shows that both isomers inhibit the growth of cancer cells *in vitro* (PRZYBOJEWSKA & RAFALSKI 2003). Another fatty acid with beneficial health

effects is conjugated linoleic acid, which represents a mixture of geometrical and positional isomers of octadecadienoic acid including conjugated double bonds. Two of them – *cis*-9,*trans*-11 and *trans*-10,*cis*-12 – have so far revealed beneficial biological effects in animal models. The isomer *cis*-9,*trans*-11 represents over 80% of CLA which naturally occurs in food and 80–90% of CLA in milk fat (SIEBER *et al.* 2004; FILIP *et al.* 2010; VAN WIJLEN & COLOMBANI 2010; PRANDINI *et al.* 2011).

CLA has many health-promoting properties. It helps to provide oxidant-antioxidant homeostasis, whose disturbance can result in atherosclerosis, diabetes, neurodegenerative diseases, and cancer (CICHOSZ *et al.* 2011). Hence, CLA consequently prevents the occurrence of these illnesses. Previous studies also imply that CLA modulates human serum lipids and body fat, has an anti-obesity effect and is capable of immune system enhancement (SIEBER *et al.* 2004; HOU *et al.* 2011).

Supported by the Ministry of Science and Higher Education, Poland, Project No. DS-3700/WTŻ/.

Ruminant dairy products, especially cheeses, are the richest dietary sources of CLA (GÜRSOY *et al.* 2003). CLA is synthesised by anaerobic bacteria in the rumen as an intermediate during the biohydrogenation of polyunsaturated fatty acids, mainly linoleic and α -linoleic, as well as formed in desaturation of vaccenic acid (the isomer *trans*) in the mammary gland via Δ^9 -desaturase activity, which is the predominant source of the isomer *cis*-9,*trans*-11 in milk fat (PRANDINI *et al.* 2007; FILIP *et al.* 2010). Moreover, several strains of bacteria used as starter cultures in dairy products and some probiotic ones (such as bifidobacteria, lactobacilli, propionibacteria, and enterococci) are capable of producing CLA *in vitro* (TELANG *et al.* 2005).

CLA content of milk fat depends on some environmental factors (the most significant is the type of fodder, however breed and lactation number or age also contribute to a lesser extent), as well as technological ones (temperature during manufacturing, time of storing) (COLLOMB *et al.* 2006; CICHOSZ *et al.* 2011). Moreover, the CLA content in the raw milk determines its quantity in the cheese. Also some technological factors during cheese manufacturing are of importance: the temperature, type of the starter culture applied, and access to air (DOMAGAŁA *et al.* 2010).

The aim of this study was to evaluate the fatty acids composition including the conjugated linoleic acid content in raw milk and Emmental-type cheese at different stages of production in industrial conditions in Poland.

MATERIAL AND METHODS

The investigated Emmental-type cheese was produced in the industrial conditions in three subsequent production cycles. The raw material was obtained in one season (summer). The fatty acids profile, including the CLA (the isomer *cis*-9,*trans*-11) content, was determined in the raw milk and the samples collected at the selected stages of the manufacturing process (the curd after cooking, the cheese samples after warm room, three and six months of ripening). The production process was as follows: pasteurisation (72°C, 15 s), addition of starter cultures (propionibacteria, mesophilic and thermophilic lactic acid bacteria), calcium chloride and rennet, coagulation (32°C), cutting, stirring and cooking (54°C), pressing, brining (20%

NaCl, 12–13°C, 4 days), dripping, ripening in the first cold room (12–13°C, 2 weeks), ripening in the warm room (19–21°C, 3–4 weeks), ripening in the second cold room (6–8°C, 4–5 months).

Extraction of fat. The extraction of total lipids was performed according to the FOLCH *et al.* (1957) method with modifications. The cheese sample was homogenised with a 17-fold higher amount of chloroform-methanol (v/v 2:1) (POCH S.A., Gliwice, Poland) mixture at 5000 rpm for 10 min using MPW-120 (Mechanika Precyzyjna, Warszawa, Poland). After a 5-min pause it was homogenised again at 1000 rpm for 5 minutes. Then the mixture was transferred quantitatively to a regular cylinder and made up with the extraction mixture to a volume that was 20-fold higher than the sample weight. Next, it was filtered through a filter paper and its volume was measured. Then 0.58% NaCl (POCH S.A., Gliwice, Poland) solution was added in the amount of 20% of the filtrate volume. After shaking and separation of the phases, the alcohol-water phase was removed, and the chloroform phase was washed 3 times using 1–2 ml of the mixture of the solutions (chloroform-methanol-0.58% NaCl, v/v 3:48:47). Afterwards the chloroform phase was recovered, the extract contained approximately 0.05 g of fat in 1 ml.

Esterification and determination of fatty acids composition. The esterification and determination of total fatty acids composition were performed according to the DE MAN (1964) method. 0.1 ml of the extracted fat was placed in a glass test tube and 0.5 ml of 0.025M methanolic solution of the catalyst – sodium methylate (POCH S.A., Gliwice, Poland) was added. Then the mixture was heated at 60°C until it became clear. The fatty acids analysis was performed using a gas chromatograph Trace GC Ultra (Thermo Electron Corp., Waltham, USA) equipped with a Supelcowax 10 column (with dimensions 30 m × 0.25 mm × 0.23 µm). As gaseous phase helium 5.0 (Linde Gaz Polska Sp.z o.o., Kraków, Poland) was applied with the flow rate of 5 ml/min. The feeder and the detector had the temperature of 220 and 250°C, respectively. The temperature of the column was kept at 60°C for 3 min, then was raised at a rate of 7°C/min up to 200°C and was held at this temperature for 20 minutes. The obtained peaks on chromatograms were compared with an internal standard and the percentages of particular fatty acids were calculated based on their areas. The results were expressed in % of total peaks area. A typical chromatogram is presented in Figure 1.

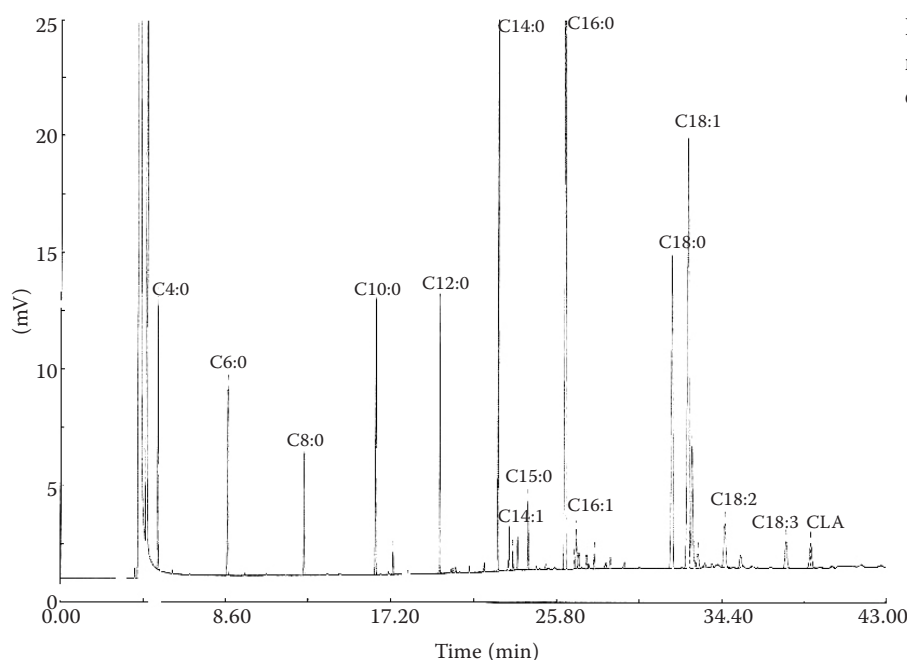


Figure 1. A representative chromatogram of fatty acids methyl esters of the examined cheese

Statistical analysis. The analyses were carried out in three independent tests. The results were statistically analysed using Statistica 6.0 (StatSoft, Inc., Tulsa, USA). A one-way ANOVA was employed and the significance of the differences between the means was established using the Duncan's test.

RESULTS AND DISCUSSION

Fatty acids profile

Significant differences occurred in the individual stages of production (Table 1). The highest amount of volatile fatty acids (C4:0, C6:0, C8:0, and C10:0) and the lowest amount of saturated fatty acids (C12:0, C14:0, C15:0, C16:0, C17:0, and C18:0) were found in the cheese samples after 6 months

of ripening and these amounts were highly significantly different from those occurring in the other stages of production. The highest content of monounsaturated fatty acids was observed in the curd but it decreased during manufacturing and was the lowest after 6 months of ripening, although not significantly lower than that in the milk. In turn, the richest in polyunsaturated fatty acids were the cheese samples obtained after the warm room stage. However, the level of these fatty acids declined after this stage of production and it became highly significantly lower at the end of the manufacturing process. Nevertheless, the amount of polyunsaturated fatty acids was highly significantly higher at the end of the cheese manufacturing than that in the milk.

According to Hou *et al.* (2011) volatile, saturated, monounsaturated and polyunsaturated fatty acids represent, respectively: 0.89–13.01, 29.09–88.06,

Table 1. Fatty acids composition of Emmental-type cheese at different stages of production (mean values \pm standard deviation)

Stage of production	Fatty acids composition (%)				Total
	volatile	saturated	mono-unsaturated	poly-unsaturated	
Milk	12.26 ^A \pm 0.32	62.48 ^{A,B,a} \pm 0.74	20.95 ^{a,b} \pm 0.85	2.07 ^{A,a} \pm 0.13	97.75 ^a \pm 0.44
Curd	10.59 ^B \pm 0.08	62.06 ^{A,B,a,c} \pm 0.17	21.81 ^a \pm 0.11	2.27 ^{A,C,b} \pm 0.03	96.73 ^{b,c} \pm 0.30
After warm room	9.75 ^C \pm 0.11	62.93 ^{A,a} \pm 0.27	21.79 ^a \pm 0.07	2.71 ^B \pm 0.08	97.18 ^{a,c} \pm 0.33
After 3 months of ripening	11.92 ^A \pm 0.24	61.40 ^{B,b,c} \pm 0.66	21.15 ^{a,b} \pm 0.76	2.31 ^{A,C,b} \pm 0.14	96.78 ^{b,c} \pm 0.01
After 6 months of ripening	14.36 ^D \pm 0.06	59.28 ^C \pm 0.24	20.61 ^{b,c} \pm 0.60	2.42 ^{C,b} \pm 0.10	96.67 ^{b,c} \pm 0.86

^{A–D}statistically highly significant differences between means ($P \leq 0.01$) marked with different letters in the columns; ^{a–c}statistically significant differences between means ($P \leq 0.05$) marked with different letters in the columns; “total” refers to identified fatty acids; the 100% base was the total peaks area

21.89–37.31, and 1.61–12.73% of the total bovine milk fatty acids.

PRANDINI *et al.* (2007), who investigated 8 kinds of Swiss Emmental, showed that monounsaturated and polyunsaturated fatty acids represent 29.85% and 3.48% of the total fatty acids, respectively. The results obtained are slightly lower. However, the contents of volatile and saturated fatty acids reported before for two kinds of Emmentaler (DOMAGAŁA *et al.* 2010) were much lower than in the investigated cheese after 3 and 6 months of ripening. These differences can be explained by the fact that the fatty acids profile of cheeses strongly depends on the source of lipids in the animals' diet (PRANDINI *et al.* 2007).

The observed highly significant rise of the amount of volatile fatty acids during cheese manufacturing may have been caused by proteolytic changes. During cheese ripening, rennet, milk proteinase and starter proteinase degrade casein to peptides and then to amino acids, from which volatile fatty acids can be formed (TUCKER & WOODS 1995). Proteolysis, as well as lipolysis, is an important phenomenon in cheese ripening. However, the latter is generally low in Emmental cheese. It leads to the release of free fatty acids which can be transformed by β -oxydation and esterification (CHAMBA & PERREARD 2002). VAN NIEUWENHOVE *et al.* (2007) analysed the influence of some bacteria used as adjunct cultures on the fatty acid composition in buffalo cheese. They observed a slightly higher amount of saturated fatty acids and a lower content of polyunsaturated fatty acids in the raw milk compared to the cheese after 15 days of ripening. It seems to be possible that some bacteria exhibit the ability to catabolise saturated fatty acids and form polyunsaturated ones.

CLA concentration

The lowest amount of this fatty acid was found in the milk and it increased during manufacturing. The CLA contents in the milk and the curd were highly significantly lower ($P \leq 0.01$) than in the cheese samples after the warm room stage and the ripening periods (Figure 2).

The amount of CLA in bovine milk is variable, ranging from 2.9 to 11.3 mg/g fat or even from 2 to 53.7 mg/g fat (SIEBER *et al.* 2004; COLLOMB *et al.* 2006). This large range in CLA content can be caused by a number of factors. The most sig-

nificant is the animal's diet. High values usually occur in the case of fresh pasture. However, a total mixed ration (TMR) feeding system including fish or sunflower oil has induced much higher levels of CLA in milk (COLLOMB *et al.* 2006). It is also worth pointing out that cows can be characterised by a large variation in CLA level in milk among individuals. According to HOU *et al.* (2011), who has analysed milk samples from 235 lactating Holstein dairy cows being on the same diet, CLA represented 0.15–1.05% of the total milk fatty acids. The obtained results are in agreement with this data.

CLA represents 7.66 mg/g fat in Swiss Emmental (PRANDINI *et al.* 2007) and 0.74 or 1.26% of the total fatty acids in Emmentaler (DOMAGAŁA *et al.* 2010). Although the obtained results were slightly lower, they are not surprising because the CLA content in the milk used for the cheese manufacturing was relatively low and, as stated before, the CLA concentration in the milk determines its quantity in the cheese (COLLOMB *et al.* 2006).

Some researchers, who investigated Cheddar, Swedish hard cheeses, Edam or Emmental, concluded that ripening does not affect the CLA content in cheese (LUNA *et al.* 2007). However, other scientists stated that several strains of bacteria are able to convert linoleic acid to CLA *in vitro*. LIN *et al.* (1999) investigated six lactic cultures in a medium with the addition of free linoleic acid at different incubation times. All of the examined strains exhibited the ability to convert linoleic acid to CLA (when 1000 $\mu\text{g/ml}$ was added) within the first 24 hours. There are also non-starter lactic acid bacteria (NSLAB) typical for ripened semi-

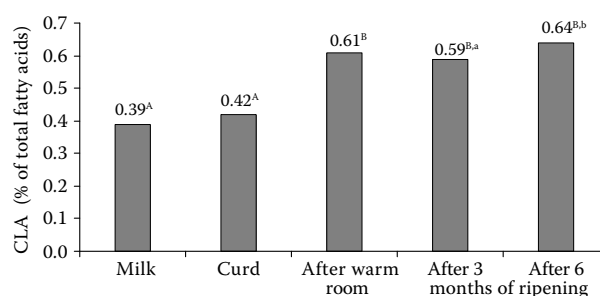


Figure 2. Conjugated linoleic acid (CLA) content in Emmental-type cheese at different stages of production

^{A–B}statistically highly significant differences between means ($P \leq 0.01$) marked with different letters; ^{a–b}statistically significant differences between means ($P \leq 0.05$) marked with different letters

hard cheeses that are able to produce CLA, e.g. *Lb. paracasei* and *Lb. plantarum* (KISHINO *et al.* 2002; LAHT *et al.* 2002). Propionibacteria were found also to possess this ability – two strains of *P. freudenreichii* ssp. *freudenreichii* and two strains of *P. freudenreichii* ssp. *shermanii* (JIANG *et al.* 1998; RAINIO *et al.* 2002). Some researchers claim that cheeses with a long ripening time contain more CLA than cheeses with a short one. GÜRSOY *et al.* (2003) showed that fresh Turkish hard cheese Kashar contained 0.61% CLA and 1.39% after ripening. The long aging Greek hard cheeses examined by ZLATANOS *et al.* (2002) contained more CLA than the shortly aging ones. Also LOBOS-ORTEGA *et al.* (2012) concluded that ripening has significant effects on the CLA content in cheeses made of cow's, ewe's, and goat's milks. VAN NIEUWENHOVE *et al.* (2007) found that buffalo cheeses manufactured with *Lb. casei*, *B. bifidum*, and *S. thermophilus* showed significantly higher CLA levels after a 15-day ripening period in comparison with the raw milk ($P < 0.05$). An increase in the content of CLA *cis*-9, *trans*-11 isomer was reported in the probiotic cheeses inoculated with *B. lactis* or *Lb. casei* manufactured with pasteurised cow's milk after 60 days of ripening (RODRIGUES *et al.* 2012).

Hence, the highly significant difference between the contents of *cis*-9, *trans*-11-octadecadienoic acid in the milk and the mature Emmental-type cheese after 6 months of ripening observed in this study confirms the assumption that lactic acid bacteria (LAB) and propionibacteria, used for Emmental-type cheese manufacturing, as well as NSLAB can exhibit the ability to produce CLA during cheese ripening (KISHINO *et al.* 2002; SIEBER *et al.* 2004; PRANDINI *et al.* 2007).

CONCLUSIONS

The highly significant rise of the amount of volatile fatty acids, the decrease of saturated fatty acids content and the increase of the polyunsaturated fatty acids amount was observed during the cheese manufacturing.

The highly significant difference between the CLA content in the milk and the Emmental-type cheese after 6 months of ripening was observed.

The study shows that the ripening process of the Emmental-type cheese results in *cis*-9, *trans*-11-octadecadienoic acid formation, mainly during the warm room stage. The obtained results confirm

that this particular type of cheese is a great source of this bioactive fatty acid.

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Received for publication October 9, 2012

Accepted after corrections April 22, 2013

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