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Influence of manufacturing conditions on the conjugated linoleic acid content and the isomer composition in ripened French Emmental cheese

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In a study of the evolution of conjugated linoleic acid (CLA) during cheese production, the influence of Emmental cheese processing on the CLA content and the CLA isomer composition was evaluated. The use of raw and thermised milk, changes of processing temperature and the effect of propionic acid bacteria (PAB) were investigated. The content of CLA in raw milk was 8·6±1·9 mg/g fat and in the ripened cheese at 70 d was 8·6±1·6 mg/g fat, under normal processing conditions. No changes in the CLA content and CLA isomer composition were observed during Emmental cheese manufacturing process. Changes in cooking and moulding temperatures did not influence the CLA content. CLA content of cheese made from microfiltered milk with two different *Propionibacterium freudenreichii* strains was very close to cheeses made without PAB. CLA levels seem to be stable in this type of dairy product under the conditions examined.

Keywords: Emmental cheese, conjugated linoleic acid, propionic acid bacteria.

The term conjugated linoleic acid (CLA) describes a mixture of positional and geometrical isomers containing a conjugated double bond system (Pariza et al. 1985; Ha et al. 1987) CLA are interesting because of their potential anticarcinogenic activity on mammary, skin, colon and forestomach cancers *in-vivo* (in animals) and *in-vitro* (Ip et al. 1994). Other beneficial properties were revealed in various animal experiments. CLA positively influenced atherosclerosis, modulated the immune-response and showed a capacity to change body composition by reducing fat to lean body mass ratio (Nicolosi et al. 1997; Park et al. 1997; Banni et al. 1998; Cook et al. 1998).

The major dietary sources of CLA are foods containing ruminant fat, such as milk and dairy products. About 75–90% CLA in ruminant fat is 9c11t-C18:2, also called rumenic acid by Kramer et al. (1998). It is formed by bioconversion of polyunsaturated fatty acids (PUFA) in the rumen and by Δ9-desaturation of vaccenic acid in the mammary gland of the lactating cow (Griinari et al. 2000).

The relationship between the CLA content and different parameters of food processing in dairy products is still controversial (Shantha et al. 1992, 1995; Shantha & Decker, 1995). Dairy products often undergo a microbial fermentation during processing. The use of different fermentation cultures, or ripening periods could modulate the CLA level in the final foodstuff (Fritsche et al. 1998) A study carried out on different Cheddar cheese varieties revealed minor changes in the CLA contents (Lin et al. 1999) and a Swedish study, testing various hard cheeses showed no differences in the CLA amounts in raw material and in the final product (Jiang et al. 1997).

The present study investigated the influence of manufacturing conditions and the use of different propionic acid bacteria (PAB) on the CLA content in French Emmental cheese. Changes in CLA induced by oxidation or by microbial fermentation processes were studied at all processing stages.

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Material and Methods

Chemicals. All solvents were purchased from SDS (Peypin, France) and hexane was purified by distillation before use. Chemicals were obtained from Sigma-Aldrich (l'Isle d'Abeau, France).

Manufacturing of cheese samples. Manufacturing of French Emmental cheese was carried out in a pilot-scale. Emmental cheese was manufactured using raw (untreated) or thermised milk. Briefly, raw milk, standardized to 30 g fat/kg milk was incubated for 30 min at 32 °C with *Streptococcus thermophilus* and thermophilic lactobacilli (*Lactobacillus helveticus* and *Lb. delbrueckii* subsp. *lactis*). Coagulation was induced by rennet followed by cutting the curd. The mixture of curd particles and whey was stirred for 5 min before cooking to 52 °C in 30 min and then stirred for 20–25 min followed by cooling to 50 °C for moulding. After pressing for 6 h, the cheese was stored in an acidifying room for 18 h at 20 °C. Then the cheese was placed in a cold room for 24 h at 12 °C before salting in saturated brine (48 h at 12 °C). After 10 d in cold room (12 °C, 85% RH) young cheese was ripened in a warm room (21 °C, 80% RH) for 4 to 6 weeks according to visual assessment. Cheeses were then cooled to 8 °C and stored until they were 70 d old. For cheese made from thermised milk, raw milk was thermised at 68 °C for 20 s and *Propionibacterium freudenreichii* was added as starter culture.

The protocol described above was varied to produce cheeses, using raw and thermised milk, at different temperatures (cooking 48 °C/moulding 48 °C and cooking 50 °C/moulding 50 °C) compared with normal cheese (cooking 52 °C/moulding 50 °C). All cheese, were made in triplicate and samples were taken at different steps of the fabrication process to determine the CLA content.

In order to examine the effect of PAB, cheeses were made with or without PAB. Two stains of *Propionibacterium freudenreichii* which have shown low (ITG P18) or high (ITG P14) lipolytic activity in previous experiments were used (Chamba et al. 2002). Milk was microfiltered before utilisation to eliminate >85% of the natural bacteria, especially PAB. Before microfiltering, raw milk was entirely skimmed, the cream was pasteurized (78 °C, 30 s) and then added back to microfiltered skimmed milk to standardise the fat content at 30 g/kg. The microfilter device used was MFS7 (TetraPak, Aarhus, Denmark) with ceramic membrane 1·4 µ Menhralox (Société de Céramiques, Tarbes, France). All cheeses were made in duplicate.

Four bulk milk samples, collected during the last two weeks of April, were used to produce the different cheeses, corresponding to eight days of manufacturing. During the first six days of manufacturing, each series of three cheeses were made alternatively from raw or thermised milk at different cooking and moulding temperatures. Then in the last two days, cheeses were made with or without PAB (in duplicate) and the standard cooking (52 °C) and moulding temperature (50 °C) was applied. The same lactic acid bacteria starters were used during all experiments.

Lipid extraction and methylation. Liquid samples were lyophilized before extraction. The other samples were minced. Lipids were extracted after dispersion with 5 ml hexane/diethyl ether (50 : 50 v/v) according to Sehat et al. (1998). The extracted lipids were stored at −80 °C. The CLA content was determined using C23 : 0 methyl ester as internal standard. A defined aliquot (containing about 12–15 mg lipids) of extracted lipids and internal standard solution were mixed. Methyl esters were obtained according to Carreau et al. (1978).

GC-Analyses. FAME were analysed using a Hewlett Packard HP 5890 Series II (Hewlett Packard Ltd, Wokingham, UK) equipped with a split/splitless injector (using split mode – split ratio 1 : 20) and a flame ionization detector. The temperature of both the injector and detector was 250 °C. Hydrogen was used as the carrier gas. The analyses were performed using a CPSil88-fused silica capillary column (100 m × 0·25 mm I.D., 0·25 µm film thickness Chrompack, Middleburg, Netherlands) using a temperature program as described by Sehat et al. [1998, 70 °C (4 min), 13 deg C/min to 175 °C (27 min), 4 deg C/min to 215 °C (31 min)]. Data were collected using a Borwin workstation (JMBS Developments) including an acquisition interface, software and a computer.

Analysis of the CLA isomer composition by Ag^+-HPLC. FAME were analysed by Ag^+-HPLC using three columns in series according to Rickert et al. (1999). The analysis was performed on a Merck/Hitachi 655A-12 LC (L-5000 controller) equipped with a Photodiode array detector (Waters PDA 996) using 3 ChromSpher 5 Lipids columns in series (stainless steel, 250 mm × 4.6 mm I.D., 5 µm particle size, Chrompack). 0·1% acetonitrile in hexane (flow rate 1 ml/min) was used as mobile phase. Results were computed using the Millennium Software (Waters).

Statistics. Results were expressed as mean ± SD. Statistical analysis was carried out using the SAS-software. A multifactorial analysis of variance was used to determine differences between the groups. A value less than 0·05 was considered as statistically significant.

Results and Discussion

Effect of the manufacturing process

The CLA content remained unchanged during manufacturing using raw or thermised milk (Table 1), ranging from 8·6 ± 1·9 mg/g fat in milk to 8·6 ± 1·6 and 8·4 ± 1·5 mg/g fat in the ripened cheese prepared from raw and thermised milk respectively.

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Table 1. CLA content at different steps of cheese manufacturing

Values are means ± SD for $n=3$

	CLA content in mg/g fat					
	Raw milk cheese			Thermised milk cheese		
Raw Milk	8·6 ± 1·9			—		
Thermised Milk	—			8·5 ± 1·9		
Coagulated Milk	8·4 ± 1·2			8·6 ± 1·6		
	A	B	C	A	B	C
Fresh Curd†	8·5 ± 1·3	8·6 ± 1·4	8·6 ± 1·5	8·7 ± 1·5	8·4 ± 1·3	8·5 ± 1·4
Salted Curd‡	8·2 ± 1·9	8·4 ± 1·8	8·1 ± 1·6	8·6 ± 1·4	8·5 ± 1·3	8·5 ± 2·4
Cheese§	8·7 ± 1·7	9·0 ± 1·7	8·9 ± 1·6	8·5 ± 1·5	6·9 ± 3·0	8·5 ± 1·9
Ripened cheese (70 d)	8·6 ± 1·6	8·9 ± 1·8	8·6 ± 1·7	8·4 ± 1·5	9·0 ± 2·6	8·4 ± 1·8

† at moulding
‡ after brining
§ at 20 d in warm room
A = Cooking 52 °C-Moulding 50 °C
B = Cooking 48 °C-Moulding 48 °C
C = Cooking 50 °C-Moulding 50 °C

CLA contents in the final ripened Emmental cheeses were comparable to those published in previous studies, where the CLA content in different cheeses varied from 2·8 ± 0·1 mg/g fat in Cheddar cheese (Lin et al. 1998) to 20·8 ± 4·0 mg/g fat in French hard cheese (Lavillonnière et al. 1998). For Swiss cheeses, produced by fermentation with PAB, and similar to the investigated French Emmental cheese, CLA contents of 6·7 ± 0·6 and 5·5 ± 0·6 mg/g fat were found (Chin et al. 1992; Lin et al. 1995). Swedish hard cheeses using PAB for fermentation contained 7·1 ± 0·3 mg/g fat (Jiang et al. 1997).

Lin et al. (1999), studying Cheddar cheese, described a slight increase in CLA content at the fresh curd stage and after 3 months aging compared with the initial raw milk. This was attributed to enzymic isomerization reactions and an interaction of proteins as hydrogen donors, with linoleic acid oxidation products, during heating under anaerobic conditions (Lin et al. 1999). However the CLA content varied by only about 0·4 mg CLA/g fat between milk and the final ripened cheese which seems negligible, when compared with the variation between different types of cheese described above. Moreover the authors used strong methylation conditions (hydrolysis by alkali at 100 °C and methylation with BF_3/MeOH) which can lead to isomerization reactions of CLA and errors in CLA content determination (Christie et al. 2001).

Furthermore manufacture of Cheddar and Emmental is very different, which may influence the CLA. For example, different starter cultures are used and Cheddaring is an additional processing step, where the Cheddar curd is exposed to oxygen for much longer than the curd of Emmental.

Effect of cooking temperatures on the CLA content

No changes in the CLA content of final ripened cheese were observed as a result of using different temperatures for cooking and moulding (Table 1). The results indicate that moderate changes in processing temperatures did not change the CLA content.

Table 2. Effect of propionic acid bacteria on CLA content in ripened cheese

Values are means ± SD for $n=2$

	CLA content in mg/g fat
Milk	10·0 ± 0·5
Cheese, without PAB	9·5 ± 0·3
Cheese, with high lipolytic PAB strain (ITG p14)	10·0 ± 0·7
Cheese, with low lipolytic PAB strain (ITG P18)	9·9 ± 0·7

Effect of PAB on the CLA content

Addition of two different strains of *Propionibacterium freudenreichii* with high or low lypolytic activity failed to induce any variations in CLA content of the ripened cheese (Table 2). This indicates that neither strain was able to convert linoleic acid to CLA at detectable levels. The ability of different bacteria, in culture, to convert linoleic acid into CLA (9c11t-C18 : 2), was recently reported. Two PAB strains (Jiang et al. 1998) and six *Lactobacillus* spp. were shown to have this ability (Lin et al. 1999; Lin, 2000). It was suggested that the same effects could be induced during fermentation of dairy products and specific starter cultures could be used to increase the CLA content in the final foodstuff.

Influence on the CLA isomer composition

The major CLA isomer was 9c11t-C18 : 2 at about 85%. Other important isomers were 7t9c-C18 : 2, 11t13c-C18 : 2,

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Table 3. CLA isomer composition of raw milk cheese during manufacturing

Values are means ± SD for $n=2$

	% of total CLA			
	Raw milk	Fresh curd†	Cheese‡	Ripened cheese§
12t14t	0·63 ± 0·08	0·71 ± 0·22	0·72 ± 0·11	0·74 ± 0·16
11t13t	1·64 ± 0·08	1·79 ± 0·14	1·82 ± 0·12	1·78 ± 0·05
10t12t	0·26 ± 0·08	0·45 ± 0·25	0·55 ± 0·08	0·69 ± 0·03
9t11t	0·73 ± 0·12	0·70 ± 0·04	0·88 ± 0·09	0·85 ± 0·04
8t10t	0·22 ± 0·08	0·40 ± 0·01	0·28 ± 0·11	0·37 ± 0·03
7t9t	0·52 ± 0·01	0·45 ± 0·02	0·54 ± 0·11	0·58 ± 0·17
6t8t	0·20 ± 0·03	0·20+0·02	0·22 ± 0·02	0·29 ± 0·11
12c,14t+12t,14c	0·47 ± 0·01	0·30 ± 0·11	0·42 ± 0·07	0·37 ± 0·03
11t13c	2·58 ± 0·08	3·00 ± 0·52	2·91 ± 0·51	2·96 ± 0·50
11c13t	0·17 ± 0·03	0·31 ± 0·02	0·26 ± 0·11	0·31 ± 0·04
10t12c	0·47 ± 0·17	1·27 ± 0·54	0·80 ± 0·28	0·82 ± 0·12
9c11t	86·79 ± 0·19	83·59 ± 2·08	85·26 ± 0·52	84·77 ± 1·07
8t10c	1·62 ± 0·20	1·38 ± 0·34	1·21 ± 0·30	1·24 ± 0·31
7t9c	3·71 ± 0·14	5·45 ± 1·46	4·15 ± 0·41	4·24 ± 0·33
Σ t/t	4·20 ± 0·16	4·70 ± 0·65	5·00 ± 0·38	5·28 ± 0·05
Σ c/t+t/c	95·80 ± 0·15	95·30 ± 0·65	95·00 ± 0·38	94·72 ± 0·05

† at moulding
‡ at 20 d in warm room
§ at 70 d

Table 4. CLA isomer composition in cheese prepared of raw milk using different temperatures for cooking and moulding

Values are means ± SD for $n=2$

	% of total CLA			
	Milk	Cooking 52 °C Moulding 50 °C	Cooking 48 °C Moulding 48 °C	Cooking 50 °C Moulding 50 °C
12t14t	0·63 ± 0·08	0·74 ± 0·16	0·81 ± 0·09	0·87 ± 0·04
11t13t	1·64 ± 0·08	1·78 ± 0·05	1·72 ± 0·01	1·94 ± 0·21
10t12t	0·26 ± 0·08	0·69 ± 0·03	0·59 ± 0·02	0·86 ± 0·62
9t11t	0·73 ± 0·12	0·85 ± 0·04	0·98 ± 0·14	0·93 ± 0·40
8t10t	0·22 ± 0·08	0·37 ± 0·03	0·34 ± 0·01	0·42 ± 0·11
7t9t	0·52 ± 0·01	0·58 ± 0·17	0·49 ± 0·16	0·47 ± 0·11
6t8t	0·20 ± 0·03	0·29 ± 0·11	0·21 ± 0·06	0·22 ± 0·06
12c,14t+12t,14c	0·47 ± 0·01	0·37 ± 0·03	0·34 ± 0·08	0·33 ± 0·04
11t13c	2·58 ± 0·08	2·96 ± 0·50	2·97 ± 0·37	2·64 ± 0·01
11c13t	0·17 ± 0·03	0·31 ± 0·04	0·42 ± 0·16	0·48 ± 0·33
10t12c	0·47 ± 0·17	0·82 ± 0·12	1·03 ± 0·22	1·27 ± 0·27
9c11t	86·79 ± 0·19	84·77 ± 1·07	84·43 ± 1·53	84·90 ± 1·82
8t10c	1·62 ± 0·20	1·24 ± 0·31	1·29 ± 0·63	0·96 ± 0·31
7t9c	3·71 ± 0·14	4·24 ± 0·33	4·36 ± 0·74	3·72 ± 0·61
Σ t/t	4·20 ± 0·16	5·28 ± 0·05	5·14 ± 0·26	5·71 ± 1·55
Σ c/t+t/c	95·80 ± 0·15	94·72 ± 0·05	94·86 ± 0·26	94·29 ± 1·55

11t13t-C18 : 2 and 8t10c-C18 : 2 (Table 3). No large effect on the CLA isomer distribution was observed during cheese production. The composition of raw milk, fresh curd, cheese after 20 d ripening in warm room and final ripened cheese are presented in Table 3. Small changes in the amount of *trans-trans* isomers were observed as production progressed (from 4·2 ± 0·2 to 5·3 ± 0·1 %). Modifications of the cooking and moulding temperatures led to slight variations in the CLA isomer composition for cheese samples prepared from raw milk only (Table 4), whereas samples prepared from thermised milk showed no alteration (results not shown). The results showed a small increase in the content of the total *trans-trans* CLA isomers which increased further as treatment temperatures

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increased. However, these small changes were not significant (Table 4). The use of two different strains of *Propionibacterium freudenreichii* had no influence on CLA isomer composition of the cheese samples.

Only one comparable study was carried out to investigate manufacturing dependent changes in the CLA isomer using three types of Cheddar cheese, and no difference was reported (Werner et al. 1992). As Cheddar cheese uses pasteurized milk and a specific starter culture, the observed changes on the CLA isomer composition in this work seem to be related to the natural bacteria found in raw milk. It can only be hypothesized that bacteria of the natural flora are able to alter the CLA isomer composition.

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