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International Dairy Journal 14 (2004) 1–15
INTERNATIONAL DAIRY JOURNAL
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Review

Impact of microbial cultures on conjugated linoleic acid in dairy products—a review

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Received 8 November 2002; accepted 6 July 2003

Abstract

The conjugated linoleic acid (CLA) isomers present in milk fat have a high health amelioration potential. Their high prevalence in fat of ruminants and in milk and dairy products has been described and confirmed over many years. The CLA isomers are formed during biohydrogenation of linoleic acid in the rumen and also through conversion of vaccenic acid in the mammary gland. In addition, several strains of *Lactobacillus*, *Propionibacterium*, *Bifidobacterium* and *Enterococcus* are able to form CLA from linoleic acid and thus could be used to increase the CLA level in fermented dairy products such as yoghurt and cheese. It appears likely that lactic acid bacteria and especially propionibacteria can form CLA during cheese ripening because free linoleic acid is formed in the ripening process. However, for the time being the reviewed data allow no final conclusion on whether these increased levels of CLA are mainly due to formation by microorganisms, or due to cattle feed or breed. Further studies including all these parameters will be necessary to elucidate the potential role of starter cultures to achieve physiologically relevant CLA levels in dairy products. It appears that contribution of presently used dairy starter bacteria to increased CLA content in cheese is relatively minor.

Keywords: Conjugated linoleic acid (CLA); Ruminants; Milk fat; Cheese; Lactic acid bacteria; Bifidobacteria; Propionibacteria

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doi:10.1016/S0958-6946(03)00151-1

1. Introduction

Conjugated linoleic acid (CLA) occurs naturally in a variety of foods as positional and geometric isomers of octadecadienoic acid with conjugated double bonds at 7 and 9, 8 and 10, 9 and 11, 10 and 12, 11 and 13 or 12 and 14 positions. In recent years, the CLA has attracted strong interest in food science and medicine because some of the isomers are believed to have important physiological functionality as demonstrated in various experimental designs with mice, rats or pigs. These include distinct reduction of cancer risk through feeding of a CLA-containing diet; reduction of the low-density lipoprotein concentration and the low-density–high-density lipoprotein ratio; increased body protein; and reduced whole body fat. Other physiological effects discussed in the literature are the normalisation of impaired glucose tolerance and a decreased disposition of platelet aggregation. Biological activity has been attributed to the two major CLA isomers, c9,t11-C18:2 and t10,c12-C18:2 (Banni, Murru, Angioni, Carta, & Melis, 2002; Belury, 2002; MacDonald, 2000; Pariza, Park, & Cook, 2001; Parodi, 1999; Schrezenmeir & Jagla, 2000). An inverse association between dietary and serum CLA concentration and the risk of breast cancer was reported in postmenopausal women (Aro et al., 2000). Recently, CLA was shown to have a growth inhibitory effect on a human hepatoma cell line (Igarashi & Miyazawa, 2001) and the ability to modulate human serum lipids and body fat (Mougios et al., 2001); also, it inhibited experimental atherosclerosis in rabbits (Kritchevsky, Tepper, Wright, & Czarnecki, 2002).

Overall, there are now many indications about the varying physiological functionality of the individual CLA isomers. According to Ip et al. (1999) the reduction of the mammary cancer incidence in rats treated with the carcinogen methylnitrosourea could be mainly attributed to the c9,t11 isomer. This isomer was found to inhibit eicosanoid production (Urquhart, Parkin, Rogers, Bosley, & Nicolaou, 2002); its elongation product, c11,t13-conjugated eicosadienoic acid, reduced the proliferation of three cancer lines in vitro (Palombo, Ganguly, Bistrian, & Menard, 2002). However, the t10,c12 isomer increased fatty acid oxidation in preadipocytes (Evans, Lin, Odle, & McIntosh, 2002), inhibited differentiation of preadipocytes (Kang, Liu, Albright, Park, & Pariza, 2003), stimulated eicosanoid production (Urquhart et al., 2002), had strong inhibitory activity on hepatic stearoyl-CoA desaturase activity (Park et al., 2000) and a potent cytotoxic effect on rat hepatoma dRLh-84 cells (Yamasaki et al., 2002) which was alleviated by oleic or palmitoleic acid (Yamasaki, Chujo, Nou, Tachibana, & Yamada, 2003). This isomer is probably responsible for an improved glucose tolerance (Ryder et al., 2001) and for an antihypertensive effect in rats (Nagao et al., 2003); also, it induced caspase-dependent apoptosis in cells (Palombo et al., 2002), affected lipoprotein lipase activity (Lin, Kreeft, Schuurbiers, & Draijer, 2001) and caused unexpected insulin resistance, increased fasting glucose and lowered HDL cholesterol levels in obese men with the metabolic syndrome (Risérus, 2002).

In this context, the daily intake of CLA is of interest. For Germany, using a national consumption survey, mean daily intake was reported as 360 mg for women and 440 mg for men, nearly two thirds coming from milk and dairy products and one-fourth from meat and meat products (Fritsche et al., 1999). In another study the mean daily intake was estimated to be 246 and 323 mg as measured by a food-frequency questionnaire and a 7-day estimated record, respectively (Fremann, Linseisen, & Wolfram, 2002). For North America, total CLA intake was determined from 3-day food duplicates to be 212 ± 14 and $151 \pm 14\,\mathrm{mg\,d^{-1}}$ (mean ± SEM) for men and women, respectively; the intake of c9,t11-18:2 was estimated to be 193 ± 13 and $140 \pm 14\,\mathrm{mg\,d^{-1}}$ for men and women, respectively (Ritzenthaler et al., 2001). These authors suggest that the daily intake of c9,t11-18:2 must be 620 and 441 mg for men and women to exhibit a cancer protective effect. Ip, Singh, Thompson, and Scimeca (1994) estimated from animal studies a daily intake of $3\,\mathrm{g\,d^{-1}}$ for cancer prevention. However, extrapolation from animal studies should be interpreted cautiously.

The objective of this review is to summarise the knowledge about the formation of CLA through different microbial cultures including those used as starters for fermented dairy foods or cheese, with the aim to find opportunities to increase the CLA concentration in these dairy products. The use of starter or adjunct cultures which may be able to form CLA from linoleic acid can be an opportunity to increase the nutritional value of cheeses (Ross, Stanton, Hill, Fitzgerald, & Coffey, 2000).

2. Formation of CLA in rumen and mammary gland

The formation of the CLA in milk and meat of ruminants can be explained by the conversion of dietary linoleic acid through ruminal bacteria and mainly by conversion of vaccenic acid in the mammary gland. The strict anaerobic bacterium *Butyrivibrio fibrisolvens*, also found in human feces (Brown & Moore, 1960) and in rumen fluid of sheep (Fujimoto, Kimoto, Shishikura, Endo, & Ogimoto, 1993), partially hydrogenates the polyunsaturated fatty acids in feed substrates. Kepler, Hirons, McNeill, and Tove (1966) showed that the biohydrogenation of linoleic acid to stearic and oleic acids in the rumen included several steps. As the first step, *cis*,*trans* and/or *trans*,*cis* conjugated

octadecadienoic acids were formed through the activity of an isomerase from *B. fibrisolvens*. This substance was then identified as c9,11-octadecadienoic acid (Kepler & Tove, 1967). The growth of *B. fibrisolvens* A38 and the subsequent CLA formation were dependent on the linoleic acid concentration in the medium. At 350 μM linoleic acid, the growth was inhibited and CLA accumulated; at 1800 μM linoleic acid, the growth was completely inhibited and no CLA formation from linoleic acid was found (Kim, Liu, Bond, & Russell, 2000). However, other ruminal bacteria such as the *Megasphaera (n.) elsdenii* strains YJ-4 and T81 are able to produce significant amounts of t10,c12-18:2, but not the strains B159, AW106 and JL1 (Kim, Liu, Rychlik, & Russell, 2002).

The second intermediate of linoleic acid biohydrogenation in the rumen of the lactating dairy cows is the conversion of c9,t11-18:2 to t11-18:1 (vaccenic acid) and its accumulation in the rumen due to a slower conversion rate. In the mammary gland of lactating cows, vaccenic acid is converted to c9,t11-18:2 by the Δ^9-desaturase; this synthesis was estimated to account for 78% of the total c9,t11 CLA in milk fat. The endogenous synthesis in the mammary gland was also the major source of this isomer in milk fat (Corl et al., 2001). An endogenous synthesis of c9,t11-18:2 from vaccenic acid has been shown also in mice (Santora, Palmquist, & Roehrig, 2000; Loor, Lin, & Herbein, 2002), rats (Banni et al., 2001; Ip et al., 1999) and humans (Adlof, Duval, & Emken, 2000; Salminen, Mutanen, Jauhiainen, & Aro, 1998; Turpeinen et al., 2002), but (at least in humans) not after consumption of safflower oil containing triacylglycerol-esterified linoleic acid (Herbel, McGuire, McGuire, & Shultz, 1998).

3. Possibility of a CLA formation through other microorganisms

The fact that CLA is formed in the rumen by the bacteria *B. fibrisolvens* and *M. elsdenii* may lead to a speculation that also other microorganisms may be able to form this important metabolite. The finding that conventional rats show higher levels of CLA than gnotobiotic ones points out to the role of the microorganisms present in the intestine causing the isomerisation of free (but not esterified) linoleic acid to CLA (Chin, Storkson, Liu, Albright, & Pariza, 1994). However, gnotobiotic rats associated with a mixed bacterial culture isolated from a fecal sample of a human volunteer with proven capability of linoleic acid conjugation did not accumulate CLA in various body tissues, when a sunflower seed oil-fortified diet was fed (Kamlage, Hartmann, Gruhl, & Blaut, 1999). This effect can be explained by an inhibition of CLA formation by glucose which is found in higher concentrations in intestinal contents of rats than in rumen fluid from cows consuming a forage-based diet. The exact mechanism is not known, it could be either inhibition of the growth of the linoleic acid-conjugating bacteria or of the expression or of the activity of the enzyme system (Kamlage, Hartmann, Gruhl, & Blaut, 2000). In a study of women with cytological abnormalities, some anaerobic bacteria were associated with c9,t11-18:2 formation; these included *L. brevis*, *P. acnes* and *Corynebacterium* (Fairbank et al., 1988, 1989) and also a few of the 180 strains of common lung bacteria pathogens (Jack, Ridgway, Jackson, & Hind, 1994). Lyophilised cells of five strains of lactobacilli contained a small amount of CLA (32–45 mg total CLA g^{-1} FAME); however, these lyophilised bacteria contained only around 24 mg total fatty acids g^{-1} (Dionisi, Golay, Elli, & Fay, 1999). In analogy, the conversion to CLA could be expected to occur in other microbial systems including bacteria commonly used in the dairy industry.

Ogawa, Matsumura, Kishino, Omura, and Shimizu (2001) postulated from their studies with washed cells of *L. acidophilus* AKU 1137 under microaerophilic conditions that first, 10-hydroxy-*cis*-12- and 10-hydroxy-*trans*-12-octadecenoic acid would be formed from linoleic acid. These two substances were converted in the former case to c9,t11- or t9,c11-octadecadienoic acid and in the latter case to t9,t11-octadecadienoic acid. When 10-hydroxy-*cis*-12-octadecenoic acid was exogenously added to the reaction mixture, it was converted to CLA. As a result, the authors suggested that this hydroxy fatty acid is one of the intermediates of CLA production from linoleic acid. Two strains of *Enterococcus faecalis* isolated from the bovine rumen were able to convert linoleic acid to 10-hydroxy-12- as well as to 13-hydroxy-9-octadecenoic acid; the latter compound was also formed by two ruminal *S. brevis* strains (Hudson, Morvan, & Joblin, 1998). According to Ogawa et al. (2001) the first substance can be the substrate for the CLA formation. Several other microorganisms were shown to produce 10-hydroxy-12-octadecenoic acid from linoleic acid: *Pseudomonas* strain NRRL-B-3266 (Davis, Wallen, Goodwin, Rohwedder, & Rhodes, 1969), *Acetobacterium woodii* (Giesel-Bühler, Bartsch, Kneifel, Sahm, & Schmid, 1987), *Nocardia cholesterolicum* (Koritala, Hosie, Hou, Hesseltine, & Bagby, 1989), a *Flavobacterium* sp. (Hou, 1994), *Lactobacillus* (*L.*) *plantarum* (Yamada et al., 1996) and an extract from *Pseudomonas* strain NRRL-B-2994 (Schroepfer, Niehaus, & McCloskey, 1970).

Lin, Lin, and Wang (2002) studied an enzyme activity from *L. acidophilus* CCRC 14079 and *P. freudenreichii* ssp. *shermanii* CCRC 11076. By determining the amount of CLA produced using free linoleic acid as a substrate, they associated this enzyme activity with linoleic acid isomerase. Reaction results with linoleic acid at 50°C for

Table 1
Possible CLA formation in a specific growth medium or milk by different microorganisms

No CLA formation by	Ref.	CLA formation proved	Ref.
L. asodolphous	9	*L. acidophilus* CCRC14079	7
L. acidophilus ATCC 4356	2,4a,5ab	AKU 1137	6,8
L. acidophilus 56, ATCC 43121	5a	IAM 10074, AKU 1122	6
L. brevis	9	*L. acidophilus* 96	5ab
L. bulgaricus	4a,9	*L. acidophilus* 56, ATCC 43121	5b
L. bulgaricus sp.	5ab	*L. acidophilus* L1, O16	1
L. delbrueckii ssp. *lactis* NCIMB 8117, 8118	2	*L. brevis* IAM 1082	6
L. casei, L. casei F-19	4a	*L. casei* E5, E10	1
L. fermentum	4a	*L. delbrueckii* ssp. *bulgaricus* CCRC14009	7
L. fermentum ATCC 14931, 23271	9	*L. delbrueckii* ssp. *lactis* CCRC14078	7
L. helveticus	9	*L. fermentum*	3
L. helveticus ATCC 15009,	2,4a,5ab,	*L. paracasei* ssp. *paracasei* IFO 12004,	6
NCIMB 700257, 701244	2	JCM 1109, AKU 1142, IFO 3533	
L. johnsonii 88	5ab	*L. pentosus* AKU 1142, IFO 12011	6
L. murinus ATCC 35020	9	*L. plantarum* 4191	5ab
L. paracasei UCC 43338, 43364, 42319,	2	*L. plantarum* AKU 1009, 1124, JCM 8341, 1551	6
43348, DPC 5336		*L. rhamnosus* AKU 1124	6
L. plantarum	9	*L. reuteri* PYR8 (ATCC 55739)	9
L. reuteri ATCC 23272, NCIMB 11951, 701359,	4a,9,2	*Lc. casei* Y2, 210, IO-1	5ab
701089, 102655, 702656		*Lc. lactis* M23, 400	5b
L. salivarius UCC 43310	2	*Lc. lactis* ssp. *cremoris* CCRC12586	7
Lc. lactis DPC 3147	2	*Lc. lactis* ssp. *lactis* CCRC10791	7
Lc. lactis M23, 400	5a	*S. thermophilus* CCRC12257	7
Lc. lactis ssp. *cremoris* ATCC 19257, NCFB 924	4a	*B. adolescentis* NCFB 2204, 2231,	2
Lc. lactis ssp. *lactis* NCFB 176, ATCC 19435	4a	*B. angulatum* NCFB 2236, *B. bifidum* NCFB, 795,	
S. thermophilus, S. thermophilus ATCC 19285	4a	*B. breve* NCFB 2257, 2258, 11815, 8815, 8807,	
B. adolescentis NCFB 2230	2	*B. dentium* NCFB 2243,	
P. freudenreichii ssp. *shermanii* PS-1,	4a	*B. infantis* NCFB 2205, 2256, *B. lactis* Bb12,	
P. jensenii ATCC 4867, *P. thoenii* ATCC 4874		*B. pseudocatenulatum* NCIMB 8811	
P. avidum VPI 575, 576, 598, 668, 671,	10	*P. shermanii* AKU 1254	6
ATCC 25557, CN 6976, 5888, 6278,		*P. freudenreichii* ssp. *freudenreichii* ATCC 6207,	4ab
P. granulosum VPI 4977, 5621, 6500,		Propioni-6, *P. freudenreichii* ssp. *shermanii* 9093	10
ATCC 25564, *P. jensenii* NCIB 5960, 5967, 5962,		*P. freudenreichii* ssp. *freudenreichii* NCIB 8896, 5959,	
P. thoenii NCIB 8072, 5966,		*P. fr.* ssp. *shermanii* NCIB 10585, 5964, 8099,	
P. lymphophilum CN 5936		*P. acidi-propionici* NCIB 8070, 5958,	
Pediococcus pentosaceus	2	*P. technicum* NCIB 5965, *P. acnes* ATCC 6919,	
		6921, VPI 162, 163, 164, 174, 186, 199	
		Pediococcus acidilactici AKU 1059	6
		Enterococcus faecium AKU 1021	6

References: (1) Alonso et al. (2003): MRS medium with 50, 100, 200, 500 $\mu g\,mL^{-1}$ linoleic acid and skim milk with 200 $\mu g\,mL^{-1}$ linoleic acid (see Table 2). (2) Coakley et al. (2003): MRS medium with 550 $\mu g\,mL^{-1}$ linoleic acid; for bifidobacteria (see Table 4). (3) Ham et al. (2002): MRS medium with 5000 $\mu g\,mL^{-1}$ linoleic acid. (4) Jiang et al. (1998): (a) MRS medium with 25 $\mu g\,mL^{-1}$ linoleic acid; (b) MRS medium with 10–1500 $\mu g\,mL^{-1}$ linoleic acid. (5) Kim and Liu (2002): (a) MRS medium with 100 $\mu g\,mL^{-1}$ linoleic acid for 12 h at 37°C anaerobically; (b) whole milk with 100 $\mu g\,mL^{-1}$ linoleic acid for 12 h at 37°C anaerobically. (6) Kishino et al. (2002b): MRS medium (see Table 3). (7) Lin et al. (1999): medium containing 120 $g\,L^{-1}$ skim milk powder with 1000 or 5000 $\mu g\,mL^{-1}$ linoleic acid. (8) Ogawa et al. (2001): MRS medium with 0.1% linoleic acid. (9) Pariza and Yang (1999, 2000). (10) Verhulst et al. (1987): Brain Heart Infusion medium with 20 $\mu g\,L^{-1}$ linoleic acid.

10 min at pH 5, 6, 7, and 8 showed that pH 5 was optimal for the activity of linoleic acid isomerase extracted from *L. acidophilus* but pH 7 for that extracted from *P. freudenreichii* ssp. *shermanii*. The amounts of the total CLA as well as that of t10,c12-, c9,t11- and c11,t13-18:2 isomers were greater using *L. acidophilus* rather than *P. freudenreichii* ssp. *shermanii*. These authors explained the wide ranges of raw data by the presence of impurities in the retentates during partial purification. In a further study the same authors investigated the reaction using 50 or 75 mg linoleic acid and 25, 50 or 75 mg crude enzyme extract of the aforementioned *L. acidophilus* strain at 50°C for 10 min at pH 5. The total CLA concentration increased from 8 to 350 μg in 50 mg linoleic acid treatment and from 116 to 439 μg in 75 mg linoleic acid treatment. Eight CLA isomers (t8,t10-, t9,t11-, t10,t12-, t11,t13-, t8,c10-, c9,t11-, t10,c12-, c11,t13-CLA) were found; 48% of

these were in c,t/t,c form and the percentage of c9,t11-CLA was 14% (Lin, Lin, & Wang, 2003). Thus, from all these results, it is to be expected that strains of various traditional dairy cultures may possess greatly different abilities to produce CLA during fermentation of milk and other dairy substrates.

4. CLA formation by lactobacilli, lactococci and streptococci

Investigations on the formation of CLA through common lactic acid bacteria were carried out by Jiang, Björck, and Fondén (1998), Lin, Lin, and Lee (1999), Pariza and Yang (1999, 2000), Ogawa et al. (2001), Ham et al. (2002), Kim and Liu (2002), Kishino, Ogawa, Omura, Matsumura, and Shimizu (2002b), Coakley et al. (2003) and Alonso, Cuesta, and Gilliland (2003). Many strains of lactobacilli, lactococci and streptococci are able to produce CLA from linoleic acid in a special growth medium or in skim or whole milk; however, many others show no such activity (Table 1). Jiang et al. (1998) incubated different lactobacilli in MRS (de Man-Rogosa-Sharpe) medium with a linoleic acid concentration of 25 μg mL^{-1}. None of the used lactobacilli, lactococci or streptococci formed CLA (Table 1), but the growth of some of these strains was inhibited by linoleic acid (25 μg mL^{-1}) in the medium. Unfortunately these experiments were not extended to other culture media combined with higher concentrations of linoleic acid. Also, Coakley et al. (2003) found no CLA formation in a MRS medium with 550 μg mL^{-1} linoleic acid by the different strains of lactobacilli, lactococci and pediococci (Table 1).

Lin et al. (1999) used a medium containing 120 g L^{-1} skim milk powder. Firstly, all six strains (see Table 1) were activated in MRS medium for 12 h at 37°C (or at 26°C for *Lc. lactis* ssp. *cremoris*) and then incubated in the skim milk powder medium[1] containing 0, 1000 or 5000 μg mL^{-1} linoleic acid for 0, 24 or 48 h. In the first 24 h a growth inhibition of these strains by linoleic acid was not seen. Without linoleic acid in the medium, a low level of CLA formation was noted (Fig. 1), but with an addition of 1000 μg mL^{-1} linoleic acid, the amount of CLA increased three to four times. Increasing the amount of the linoleic acid to 5000 μg mL^{-1} or extending the incubation time 24–48 h resulted in no additional CLA formation. Two strains each of *L. acidophilus* (L1, O16) and *L. casei* (E5, E10) were able to produce CLA (c9t11, t10c12, t9c11) in MRS media as well as in skim milk supplemented with linoleic acid (Table 2). In this study, free CLA was assayed in the spent growth media after removing the cells. The stage of growth at which most CLA was formed was the stationary phase (Alonso et al., 2003).

More than 250 strains from the genera of *Lactobacillus*, *Streptococcus* (*S.*), *Pediococcus*, *Leuconostoc*, *Propionibacterium*, *Bifidobacterium* (*B.*), *Weissella*, *Aquaspirillum*, *Enterococcus*, *Tetragenococcus*, *Aerococcus*, *Butyrivibrio* and *Lactococcus* (*Lc.*) were tested by Kishino et al. (2002b) for their CLA-producing ability. Of these, some strains belonging to the genera *Lactobacillus*, *Propionibacterium*, *Enterococcus* and *Pediococcus* (Table 1) produced more than 70 μg total CLA mL^{-1} reaction mixture. In the produced CLA mixture the two c9,t11 or t9,c11 and t9,t11 isomers were present, mostly with the latter substance predominating (Table 3). Washed cells of *L. plantarum* AKU 1009a with high levels of CLA production were then used for optimisation of reaction conditions: 0.1 M potassium phosphate buffer, pH 6.5, temperature 37°C, time 72 h, free form of linoleic acid. No effect of oxygen on CLA production was observed. In *L. plantarum* CLA was found in the cells or associated with the cells. Various compounds such as L-serine, glucose, NaCl or $AgNO_3$ can influence the formation of c9,t11- and t9,t11-18:2 from linoleic acid by this strain (Kishino et al., 2003). The same group (Ogawa et al., 2001) found earlier a strain of *L. acidophilus* AKU 1137 with a high CLA production during microaerophilic transformation of linoleic acid. However, the presence of oxygen resulted in lower CLA production by promoting oxidative metabolism. According to Pariza and Yang (1999, 2000) the strain *L. reuteri* PYR8 (ATCC 55739) was able to convert linoleic acid to c9,t11-CLA (about 98%, minor products were c9,c11- and t9,t11-18:2), while other species of lactobacilli were ineffective (Table 1). This *L. reuteri* PYR8 strain, isolated with three other CLA-forming strains from whole intestinal tract of two conventional rats under anaerobic conditions, possesses a characteristic linoleate isomerase activity. Similarly, Ham et al. (2002) isolated 34 lactic acid bacteria from 19 feces samples of healthy babies. Only one strain showed a CLA forming ability and was identified as *L. fermentum*.

Kim and Liu (2002) studied thirteen lactic acid bacteria, some of which are commonly used as starter cultures for yoghurt. Among these (Table 1), five were able to produce CLA in a MRS medium as well as in fermented whole milk (from 2 mg CLA g^{-1} fat or less to 4 mg CLA g^{-1} or more), four were effective only in fermented whole milk, while the remaining four strains showed no effect in either the MRS medium or the fermented whole milk. The strain *Lc. lactis* IO-1 showed the highest CLA production in fermented whole milk. In MRS medium, growth of these strains was completely inhibited at a dose higher than 500 μg mL^{-1} linoleic acid.

Lin (2000) studied the effect of saccharose, lactose, fructose and sodium chloride on the CLA formation

[1] Sterilised skim milk contained 7.2 μg mL^{-1} CLA and 23.4 μg mL^{-1} linoleic acid.

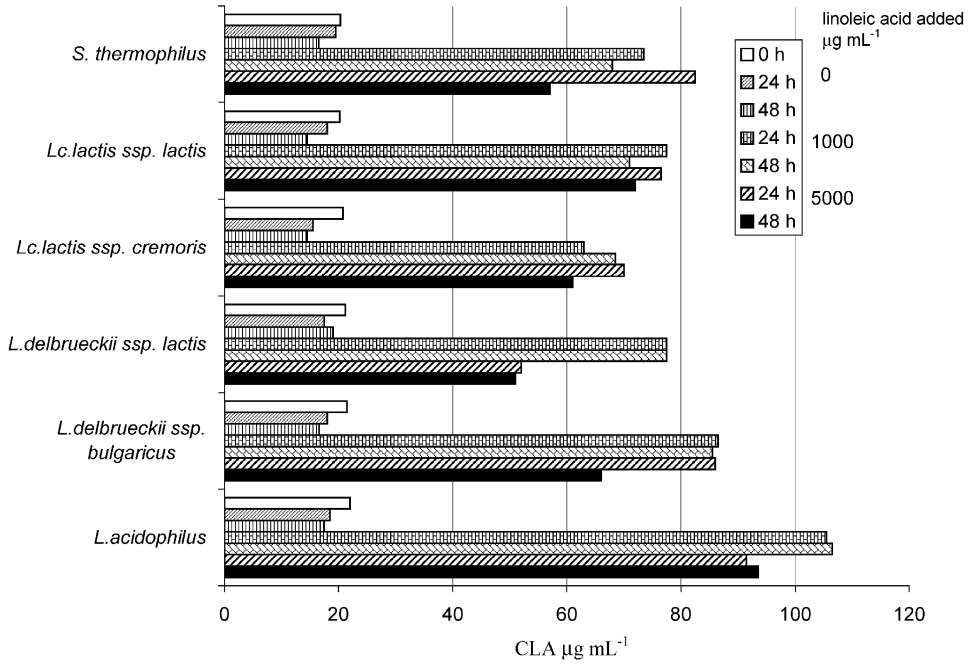

Fig. 1. Formation of CLA by six strains of lactic acid bacteria in a sterilised skim milk powder medium with added free linoleic acid at different incubation times (Lin et al., 1999).

Table 2
Production of free c9,t11- and total CLA by *L. acidophilus* and *L. casei* of human intestinal origin in MRS broth and skim milk (Alonso et al., 2003)

	MRS broth c9,t11-CLA ($\mu g\,mL^{-1}$)	Total CLA	Skim milk c9,t11-CLA ($\mu g\,mL^{-1}$)	Total CLA
L. acidophilus L1	115.1	131.6	100.3	116.5
L. acidophilus O16	54.8	60.7	45.3	54.3
L. casei E5	93.9	111.2	85.0	99.6
L. casei E10	70.7	80.1	61.0	71.4

MRS broth and skim milk were supplemented with $200\,\mu g\,mL^{-1}$ linoleic acid and incubated for 24 h at 37°C.

Table 3
CLA production by various strains of lactobacilli and other bacteria (Kishino et al., 2002b)

Strain	c9,t11- or t9,c11-18:2[a] ($\mu g\,mL^{-1}$) reaction mixture	t9,t11-18:2	Total CLA
L. acidophilus AKU 1137	850	650	1500
L. acidophilus IAM 10074	180	420	600
L. acidophilus AKU 1122	20	100	120
L. paracasei ssp. *paracasei* IFO 12004	50	150	200
L. paracasei ssp. *paracasei* JCM 1109	20	50	70
L. paracasei ssp. *paracasei* AKU 1142	40	30	70
L. paracasei ssp. *paracasei* IFO 3533	50	40	90
L. rhamnosus AKU 1124	690	720	1410
L. brevis IAM 1082	230	320	550
P. shermanii AKU 1254	90	20	110
Enterococcus faecium AKU 1021	40	60	100

Strains cultivated in MRS medium supplemented with 0.06% (w/v) linoleic acid for 72 h at 28°C.
[a] Recently, this mixture was identified as c9,t11-18:2 (Kishino et al., 2003).

with the six strains from the above-mentioned study (Lin et al., 1999; Fig. 1). All strains were activated in lactobacilli MRS broth and then transferred to the medium containing $150\,g\,L^{-1}$ skim milk powder. In addition to $1000\,\mu g\,mL^{-1}$ linoleic acid, the effects of additions of $60\,mg\,mL^{-1}$ saccharose, lactose, fructose

and 10 mg mL^{-1} sodium chloride were tested. With the exception of *L. delbrueckii* ssp. *lactis* 12586 where the addition of fructose caused an increase, the c9,t11-18:2 formation was inhibited by these additives to a different extent. Reductions of 28% and 33% in relation to the control were observed by the addition of sucrose to the medium inoculated with *L. acidophilus* or *Lc. lactis* ssp. *cremoris,* respectively, whereas the addition of lactose did not inhibit the CLA formation of the latter strain. Further, the addition of 10 g L^{-1} sodium chloride to the skim milk media inoculated with the six lactic cultures reduced the c9,t11-18:2 concentration by 13% (*Lc. lactis* ssp. *lactis*) to 32% (*S. thermophilus*).

5. CLA formation by bifidobacteria

Bifidobacteria are normal inhabitants of the human digestion tract and are now being used as probiotics in the production of fermented dairy products. Coakley et al. (2003) assessed the possible role of these bacteria in CLA formation from linoleic acid. Different strains of bifidobacteria were incubated at 37°C for 48 h in a MRS medium containing L-cysteine hydrochloride and 550 μg mL^{-1} linoleic acid. The CLA isomers were mainly extracted from the cell supernatant fluid and determined by GLC. *B. breve*, *B. dentium* and *B. lactis* formed relatively high amounts of CLA, whereas the other bifidobacteria did not convert linoleic acid to CLA at any significant level (Table 1). The predominant isomer formed was the c9,t11 and, in lower concentrations, also the t9,t11 (Table 4). The formation of c9,t11 isomer ceased when the culture entered stationary phase, but this isomer was further converted to the t9,t11 isomer.

6. CLA formation by propionibacteria

According to Jiang et al. (1998) three *Propionibacterium* (*P.*) strains were able to form CLA in the MRS medium, with the CLA mainly found in the extracellular phase: *P. freudenreichii* ssp. *freudenreichii* ATCC 6207 (PFF); *P. freudenreichii* ssp. *freudenreichii* Propioni-6 (PFF6); and *P. freudenreichii* ssp. *shermanii* 9093 (PFS). In contrast, *P. freudenreichii* ssp. *shermanii* PS-1, *P. jensenii* ATCC 4867 and *P. thoenii* ATCC 4874 showed no such activity. With the three former strains, the CLA formation was studied at different concentrations of free linoleic acid and in two additional culture media. As the free linoleic acid concentration in the MRS medium was increased from 10 to 1500 μg mL^{-1}, the growth of these strains was inhibited (the strain PFF being most sensitive) and a negative correlation was found between the total bacteria count and the linoleic acid concentration in MRS medium. At a free linoleic acid level of 500 μg mL^{-1} the strain PFF did not form any CLA whereas the strain PFS reached the highest CLA formation at this level. The strain PFF6 formed the most CLA at a linoleic acid concentration of 750 μg mL^{-1} (Table 5). At the same linoleic acid concentration (100 μg mL^{-1}) only the strain PFF6 produced more CLA in the sodium lactate medium, however, all three strains were effective using sterilised skim milk. In this medium about 60–90% of the free linoleic acid was converted to CLA (Table 5). Using the data of Lin et al. (1999) who found that sterilised skim milk contained a CLA content of 7.2 μg mL^{-1} and a linoleic acid content of 23.4 μg mL^{-1} (provided that it is free linoleic acid), it can be assumed that by a similar occurrence the established percentage conversion of

Table 4
Formation of CLA by selected bifidobacteria (Coakley et al., 2003)

Species	Strain	Source	Conversion in %		
			c9,t11	t10,t12	t9,t11
B. adolescentis	NCFB 230	Adult intestine	—	—	—
	NCFB 2204	Adult intestine	0.3	0.2	0.1
	NCFB 2231	Adult intestine	0.1	0.3	0.1
B. angulatum	NCFB 2236	Human feces	0.1	0.1	0
B. bifidum	NCFB 795	Unknown	0.2	0	0
B. breve	NCFB 2257	Infant intestine	48.5	0.2	1.3
	NCFB 2258	Infant intestine	66.2	0	6.2
	NCTC 11815	Infant intestine	30.6	0.5	2.5
	NCIMB 8815	Nursling stools	40.7	0.5	2.9
	NCIMB 8807	Nursling stools	27.7	0.1	0.9
B. dentium	NCFB 2243	Dental caries	22.8	0.3	8.0
B. infantis	NCFB 2205	Infant intestine	0.2	0.3	0.2
	NCVB 2256	Infant intestine	3.3	0.3	0.8
B. lactis	Bb12	Chr.Hansen	28.0	0.5	2.4
B. pseudocatenulatum	NCIMB 8811	Nursling stools	3.1	0.4	0.8

Conditions: medium supplemented with 550 μg mL^{-1} linoleic acid.

linoleic acid to CLA could be similar in the skim milk used by Jiang et al. (1998).

In skim milk the inhibiting effect of linoleic acid on the growth of bacteria was smaller than in two other culture media. This was explained by Jiang et al. (1998) as being due to the presence of proteins (Boyaval, Corre, Dupuis, & Roussel, 1995). In the above-mentioned study, Kishino et al. (2002b) also found a CLA-producing strain of *Propionibacterium* (Table 3). Verhulst, Janssen, Parmentier, and Eyssen (1987) tested 36 strains of several species of the genus *Propionibacterium* (Table 1). Some strains produced 9c,11t-18:2 and 11t-18:1, whereas strains of *P. acnes* (ATCC 6919 and 6921, VPI 162, 163, 164, 174, 186, 199) formed t10,c12-18:2 and t10-18:1 in presence of linoleic acid.

As showed by Jiang et al. (1998), higher concentrations of linoleic acid can inhibit the growth of propionibacteria. For reduction of this effect, Rainio, Vahvaselkä, Suomalainen, and Laakso (2001, 2002b) dispersed linoleic acid in a polyoxyetheylene sorbitan monooleate detergent (Tween-80). In a whey permeate medium *P. freudenreichii* ssp. *shermanii* converted 57–87% of the linoleic acid (up to 2000 $\mu g\,mL^{-1}$ in the medium) into CLA, mainly forming the c9,t11 isomer. Fed-batch fermentations in which the linoleic acid and the detergent were added at different time lead to varying CLA formation (Table 6).

In a resting cell experiment Rainio, Vahvaselkä, and Laakso (2002a) achieved a maximum yield of CLA of 90% at an initial linoleic acid level of 510 $\mu g\,mL^{-1}$. Again mainly c9,t11 isomer was formed (94%). At this final stage of the isomerisation 1 g cell mass contained 31 mg of CLA, which meant that 46% of all the CLA formed was in the cell material. Adding more sorbitan monooleate than required for the maintenance of linoleic acid in the micellar phase did not improve the isomerisation rate nor the yield of CLA. Of course, the use of a detergent is possible, e.g. in a MRS medium to study physiological effects, but not in cheese.

Table 5
Formation of CLA by three strains of propionibacteria in different media supplemented with different amounts of free linoleic acid under anaerobic conditions at 20°C for 72 h (Jiang et al., 1998)

Strain	Total CLA ($\mu g\,mL^{-1}$)							
Medium	MRS				SLM		SM	
Linoleic acid ($\mu g\,mL^{-1}$)	0	100	500	750	0	100	0	100
PFF	6.4	23.2			0	0	2.1	62.5
PFF6	5.6			265.3	1.2	42.0	2.1	92.3
PFS	9.1		111.8		3.1	3.6	2.7	84.5

PFF = *P. freudenreichii* ssp. *freudenreichii* ATCC 6207, Reading.
PFF 6 = *P. freudenreichii* ssp. *freudenreichii* Propioni-6, Wiesby.
PFS = *P. freudenreichii* ssp. *shermanii* 9093, Kemikalia.
MRS = De Man-Rogosa-Sharpe medium.
SLM = sodium lactate medium.
SM = medium with sterilised skim milk.
Total CLA means c9,t11-/t9,c11-18:2, t10,c12-18:2 and t9,t11-/t10,t12-18:2.

Table 6
Formation of CLA in batch and fed-batch fermentations of *P. freudenreichii* ssp. *shermanii* JS in whey permeate medium supplemented with polyoxyethylene sorbitan monooleate (SO) (Rainio et al., 2002b)

Linoleic acid added ($\mu g\,mL^{-1}$)	SO added ($\mu g\,mL^{-1}$)	Reaction time (h)	CLA formed ($\mu g\,mL^{-1}$)	c9,t11 isomer (%)
Batch fermentation				
0	0		0	
600 ± 13	9000		490±21	85±3
930 ± 38	15000		812±7	93±1
1400 ± 46	15000		1140±51	95±1
2000 ± 28	15000		1600±87	95±1
Fed-batch fermentation A[a]				
600	15000	3	58±5	
		6	170±15	
		28	518±60	
Fed-batch fermentation B[b]				
600	15000	0.5	6±1	
		3	246±25	
		6	322±33	
		22	338±52	

[a] Linoleic acid was added into culture containing polyoxyetheylene sorbitan monooleate after the growth phase.
[b] Linoleic acid and polyoxyetheylene sorbitan monooleate were added in the late exponential phase.

7. Formation of CLA in fermented dairy foods and cheese by microbial dairy cultures

7.1. Fermented milk

Several studies reported elevated CLA levels in fermented milk products: in dahi, 26.5 mg g^{-1} fat compared to the 5.5 mg g^{-1} fat in the raw material (Aneja & Murthy, 1990); in yoghurt with 0.05% fat, 5.25 mg g^{-1} fat compared to unprocessed milk content of 4.40 mg g^{-1} fat (Shantha, Ram, O'Leary, Hicks, & Decker, 1995), the latter confirmed by Jiang, Björck, and Fondén (1997). However, Lin, Boylston, Chang, Luedecke, and Shultz (1995) and Shantha et al. (1995) found no statistically significant difference between milk and yoghurt on fat basis (Table 7) and between milk and yoghurt with 1.0% and 3.25% fat. In the work of Boylston and Beitz (2002), processing of milk into yoghurt and storage for 7 days did not change significantly the CLA levels which were 7.2, 7.5, 6.6 in the neutral lipid fraction and 9.5, 8.6, 10.1 mg g^{-1} fat in the polar lipid fraction for milk, yoghurt stored for 1 day or 7 days.

Investigations on the increase of the CLA content in milk by fermentation with *Lc. lactis* I-01 were carried out by Kim and Liu (2002). The CLA production by this strain was influenced by the substrate concentration, incubation time, culture conditions as well as pH. As a substrate, sunflower oil (concentration of linoleic acid 66%) at concentrations of 0–800 μg mL^{-1} in whole milk was used. The CLA production was maximal for cells in the growing (8 h) as well as in the stationary (12 h) phase at a concentration of 100 μg mL^{-1} sunflower oil added initially; at all concentrations the growing cells produced more CLA than the stationary cells. However, when sunflower oil was added only 10 or 60 min before the end of incubations, the cells in the stationary phase formed more CLA, and its level was the highest at a sunflower oil concentration of 200 μg mL^{-1} added 10 min before the end of the incubations. When cells were grown in whole milk with additions of sunflower oil (200 μg mL^{-1}), dry milk powder (6%) and glucose (0.3%) for 24 h, the CLA production was maximal (8.5 mg g^{-1} fat) after 12 h of incubation at a pH above of 5.5 and decreased until the end of the incubation when the pH reached 4.6.

Recently, Lin (2003) studied the production of 9c,11t-18:2 in non-fat set yoghurt using 0.1% linoleic acid, 5% fructooligosaccharides, *L. acidophilus* CCRC 14079 and yoghurt bacteria (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*). Only the use of mixed cultures increased significantly the content of this isomer to 2.95 μg g^{-1}

Table 7
Concentration of CLA in commercial samples of fermented dairy products and cheeses

Study of Lin et al. (1995)			Study of Jiang et al. (1997)		
Sample	c9,t11 CLA		Sample	Starter	c9,t11 CLA
	(mg g^{-1} fat)	(mg g^{-1} sample)			(mg g^{-1} fat)
Fluid milk products			*Fluid milk products*		
Whole milk 3.2% fat	4.49 ± 0.64	0.14	Pasteurised milk 3% fat		5.88 ± 0.08
2% milk 1.9% fat	4.14 ± 0.37	0.08	Pasteurised milk 1.5% fat		5.83 ± 0.06
Fermented dairy products			*Fermented dairy products*		
Yoghurt 1.9% fat	3.82 ± 0.13	0.07	Yoghurt 3% fat	S. + L.b.	6.15 ± 0.02
Buttermilk 1.2% fat	4.66 ± 0.18	0.06	Yoghurt mild 0.5% fat	S. + L.b.	6.22 ± 0.14
Sour cream 18.4% fat	4.13 ± 0.17	0.76	Fjällfil	Lc.	6.12 ± 0.62
			Mellanfil	Lc.	6.07 ± 0.35
Cheeses			Bifilus	Lc. + S.	4.47 ± 0.29
Blue 1	4.87 ± 0.10	1.48	Dofilus	L.a.	5.16 ± 0.22
Blue 2	7.96 ± 0.12	2.29	Hälsofil	Lc.	5.24 ± 0.38
Brie	4.75 ± 0.28	1.29			
Cheddar medium	4.02 ± 0.29	1.40	*Cheeses*		
Cheddar sharp	4.59 ± 0.30	1.61	Blue	Lc. + Pen.	6.20 ± 0.27
Cougar Gold (Cheddar)	3.72 ± 0.17	1.30	Cheddar	Lc.	5.86 ± 0.89
Cream	4.30 ± 0.42	1.43	Prätost	Lc.	5.01 ± 0.16
Cottage	4.80 ± 0.38	0.20	Herrgårdsost	Lc.	5.45 ± 0.27
Edamer	5.38 ± 0.90	1.42	Västerbottenost	Lc.	6.02 ± 0.17
Monterey Jack	4.80 ± 0.38	1.43	Grevé	Lc. + P.	7.06 ± 0.33
Mozzarella	4.31 ± 0.21	0.91			
Parmesan	4.00 ± 0.53	0.90			
Swiss	5.45 ± 0.59	1.61			
Viking	3.59 ± 0.01	1.20			

Abbreviations of starter cultures: S. = *Streptococcus thermophilus*; L.a. = *Lactobacillus acidophilus*; L.b. = *Lactobacillus bulgaricus*; Lc. = *Lactococcus* spp.; Pen. = *Penicillium roqueforti*; P. = *Propionibacterium* spp.

yoghurt with linoleic acid alone and to 2.33 μg g^{-1} yoghurt with linoleic acid and fructooligosaccharides, compared to 0.93 μg g^{-1} in the control yoghurt and to 1.18 μg g^{-1} yoghurt with fructooligosaccharides only. These results were higher than the 0.71 μg g^{-1} found by Chin, Liu, Storkson, Ha, and Pariza (1992) or the 1.87 μg g^{-1} reported by Shantha et al. (1995) for non-fat yoghurt. It seems that only a small amount of the added linoleic acid was converted to CLA. Another explanation of this surprising result is the fact that, according to the author, the method used determined only esterified CLA and not free CLA. The 9c,11t-18:2 content was influenced by the addition of fructooligosaccharides to a small extent. In the presence of linoleic acid, yoghurt bacteria caused an insignificant increase of the content of this isomer (1.63 versus 1.10 μg g^{-1} yoghurt) compared to unsupplemented yoghurt; the effect of *L. acidophilus* (0.63 versus 0.48 μg g^{-1} yoghurt) was also insignificant. The product acceptability by the sensory panel was not affected (Lin, 2003). However, strains of *L. acidophilus* (L1 and O16) incubated in reconstituted nonfat milk at 37°C for 24 h and supplemented with 200 μg mL^{-1} linoleic acid produced between 54 und 117 μg mL^{-1} total free CLA (Table 2, Alonso et al., 2003).

7.2. Cheese

The linoleic acid content of milk is somewhat more than 10 mg g^{-1} fat, with the free linoleic acid concentration more than 0.1 mg g^{-1} fat (Collomb, Eyer, & Sieber, 2002b). In the conversion of the milk into cheese, lactic acid bacteria and in some cases propionibacteria are used as starter cultures. The judicious use of suitable strains of lactobacilli, lactococci and streptococci can increase the CLA content of cheese. Indeed, esterified as well as free linoleic acid can serve as a substrate for CLA production by *Lc. lactis* I-01 (Kim & Liu, 2002). However, castor oil was not converted to CLA by *L. plantarum* AKU1009a, only when this oil was treated with a lipase (Kishino, Ogawa, Ando, Omura, & Shimizu, 2002a). Although fatty acids such as linoleic, lauric, myristic as well as oleic acid in a concentration of 10 mg L^{-1} in a lactate-yeast extract (YEL) based medium had an inhibitory effect on the growth of *P. freudenreichii* ssp. *shermanii* LRTL 30, this effect was not confirmed in milk, retentate of milk ultrafiltration (UF) or cheese curd made from microfiltered milk (Boyaval et al., 1995). For the manufacture of Emmental cheese, lactic acid bacteria and propionibacteria are used. Therefore, due to the potential ability of some strains of propionibacteria to produce CLA from free linoleic acid, it may be expected that increased amounts of CLA might be formed in this cheese.

Results of investigations of the occurrence of free fatty acids in different Swiss cheeses were reported recently (Collomb, Malke, Spahni, Sieber, & Bütikofer, 2003). The following cheeses (in each case $n = 10$) were included: Emmental (age of the ripened cheese 6 months); Gruyère (6 m); Sbrinz (12 m); Appenzell (3 m); Tilsit (2.5 m); Vacherin fribourgeois (3 m). Emmental contained between 106 and 190 μg of free linoleic acid g^{-1} cheese whereas the other cheeses showed between 18 and 73 μg g^{-1}. The results for Emmental tend to confirm the trend reported by Chamba and Perreard (2002) who found a linoleic acid content of Emmental cheese to be 37 after 7 d, 43 after 50 d and 55 μg g^{-1} cheese after 90 d of ripening.

Similarly, Roquefort and Blue cheese belong to the cheeses with a high content of free fatty acids. In these two cheeses, more than 17 mg g^{-1} C18-congeners were found (Woo, Kollodge, & Lindsay, 1984), whereas Italian cheeses contained distinctly less (Woo & Lindsay, 1984).

These results appear to indicate that during ripening, mainly of Emmental and Blue cheese, CLA can be formed from linoleic acid through the action of the primary or secondary cultures including the propionibacteria in Emmental. Using a skim milk powder-containing medium, lactobacilli, lactococci and streptococci converted up to 10% (Fig. 1) and propionibacteria up to 90% (Table 5) of the free linoleic acid into CLA. From the above-mentioned content of free linoleic acid in a 6 month old Emmental, additional formation of 90–170 μg CLA g^{-1} cheese could be calculated. This approach ignores the continous release of free linoleic acid during cheese ripening and, as a consequence, the possible formation of yet additional CLA. It is also possible that the released linoleic acid could be converted to CLA very quickly without inhibiting the growth of propionibacteria. The pH of the ripening hard cheeses lies in the 5.6–5.8 range (Steffen et al., 1980, 1981; Steiger & Flückiger, 1979) which is suitable for a high CLA production (Kim & Liu, 2002). At first sight the CLA content of different cheeses (Table 7) can suggest that different starter cultures contribute to the CLA content of cheeses differently (Jiang et al., 1997; Lin et al., 1995). According to Werner, Luedecke, and Shultz (1992) who found a difference in some individual CLA isomers of aged Cheddar, Viking and Cougar Gold cheeses, this difference can be explained by the use of different starter cultures as well as by different manufacturing conditions. French Emmental cheese (age: 70 days) produced with high or low lipolytic strains of *Propionibacterium* sp. contained a CLA content of 9.98 or 9.87 mg g^{-1} fat compared to 9.54 mg g^{-1} fat in a cheese with a normal *Propionibacterium* strain (Gnädig, 2002). Matured Comté cheeses manufactured with milk of the same season contained slightly more CLA (17.2 mg g^{-1} fat) after 1 year than after 5 months of ripening (16.1 mg g^{-1} fat) (Lavillonnière, Martin, Bougnoux, & Sébédio, 1998). Also,

Zlatanos, Laskaridis, Feist, and Sagredos (2002) found that hard cheeses long ageing time has higher CLA content (9.4, 4.9–19.0 mg g^{-1}) than hard cheeses short ageing time (7.4, 5.1–11.0). However, Lin et al. (1995) failed to find differences in the CLA content of the same Cheddar cheeses, manufactured with different starters (Table 7). Jiang et al. (1997) produced Harrgårdsost and Grevé cheese with different starter cultures (*Lactococcus* spp. or *Lactococcus* spp. and *Propionibacterium* spp.) but under identical conditions. These cheeses showed slight variations in CLA content (between 5.45 and 7.06 mg CLA g^{-1} fat, Table 7). After 24 weeks of ripening the CLA content of both cheeses did not change. These authors concluded that the activity of starter cultures did not influence the CLA content of cheese and that the higher CLA content of the commercial Grevé can be explained by a higher CLA content of milk. Mozzarella cheese manufactured from milk of cows fed diets containing full fat extruded soybeans and cottonseed showed a CLA level of milk of 6.9 and 6.0 and of cheese of 7.3 and 6.0 mg g^{-1} FAME (Dhiman, Helmink, McMahon, Fife, & Pariza, 1999b).

8. Other possibilities for increasing CLA in dairy products

The CLA content of raw milk itself can be affected by feeding and these changes can be significant. The CLA levels can be influenced by several measures: feeding of fresh pasture (Kelly, Kolver, Bauman, van Amburgh, & Muller, 1998b); addition of oils such as rapeseed, linseed, sunflower or soybean (Collomb et al., submitted; Dhiman, Arnand, Satter, & Pariza, 1999a; Dhiman et al., 1999b; Dhiman et al., 2000; Kelly et al., 1998a; Lawless, Murphy, Harrington, Devery, & Stanton, 1998; Whitlock et al., 2002); feeding fish meal or oil (Abu-Ghazaleh, Schingoethe, & Hippen, 2001; Abu-Ghazaleh, Schingoethe, Hippen, & Whitlock, 2002; Baer et al., 2001; Whitlock et al., 2002) or a high fat diet (Bell & Kennelly, 2002). Using a permanent pasture increased CLA level of milk to 22.1 compared to 3.8 mg g^{-1} FAME with diet containing normal corn (Dhiman et al., 1999a) or to 10.9 versus 4.6 mg g^{-1} milk fat (Kelly et al., 1998a). Extruded soybeans and fish oil fed alone or in combination increased total CLA levels to 11.8, 20.7 and 18.6 compared to control with 6.0 mg g^{-1} fatty acid butyl esters (Whitlock et al., 2002). Feeding of a high fat diets to lactating Holstein cows (details of the diets were not given due to patent confidentiality) increased the FAME of c9,t11-18:2 to 5.63 compared to 0.49 in the control, to 0.56 in low fat diet and to 3.7% in another high fat diet. The CLA yield also increased to 45.8 compared to 5.1, 5.4 and 28.5 g d^{-1}. The levels of t10,c12-18:2 were small. Another interesting fact was a reduced milk fat content and a reduced milk yield (Bell & Kennelly, 2002). Also feeding of cows on high Alpine meadows increased the total CLA levels of milk to 23.6 compared to milk from mountains with 16.1 and lowlands with 8.7 mg g^{-1} fat (Collomb, Bütikofer, Sieber, Bosset, & Jeangros, 2001); the presence of fatty acids could be correlated with some plants consumed (Collomb, Bütikofer, Sieber, Jeangros, & Bosset, 2002a). However, Comté cheeses (unripened 20 h) produced from milk of cows grazing on the high plateau of the Jura mountains contained less CLA (15.0 mg g^{-1} fat) than cheeses originating from milk of cows grazing in the plains (20.8 mg g^{-1} fat) or at medium altitude (20.6 mg g^{-1} fat) and matured cheese 16.1 (5 months) and 17.2 (1 year) mg g^{-1} fat (Lavillonnière et al., 1998). Additional factors determining the CLA levels of milk are the breed (Lawless et al., 1999), the age of animals (Stanton et al., 1997a), individual variation of animals (Peterson, Kelsey, & Bauman, 2002) and the season (Collomb & Bühler, 2000; Parodi, 1977; Stanton, Lawless, Murphy, & Connolly, 1997b). The CLA level of milk changed throughout the year being significantly higher in May, June and July than in all other months (15 versus 7.7 mg g^{-1} fatty acid methyl esters (Lock & Garnsworthy, 2003) or during pasture feeding (Jahreis, Fritsche, & Steinhart, 1997; Precht & Molkentin, 1999). A further opportunity to increase CLA content in milk is the fortification, but to increase consumer acceptability, chocolate or strawberry flavors had to be added (Campbell, Drake, & Larick, 2003). Based on the data available so far, it appears that the increase of CLA by bacterial fermentations in cheese and other fermented dairy foods is relatively minor in comparison to these other approaches.

9. Conclusions and further prospects

The investigations of Jiang et al. (1998), Lin et al. (1999), Kim and Liu (2002), Kishino et al. (2002b), Coakley et al. (2003) and Alonso et al. (2003) showed that there exist strains of lactobacilli, bifidobacteria and propionibacteria which are able to convert efficiently linoleic acid to CLA. However, several investigations on yoghurt and cheese did not show elevated CLA levels, possibly because these dairy products were not manufactured with specific CLA-producing lactic acid bacteria strains. Also, the origin of the milk, the seasonal variation and the resulting cheese may often be different and some conditions of processing and ripening could influence the CLA content of cheeses.

For the human nutrition, other CLA-rich sources besides milk and dairy products are ruminant meats and to a lesser extent non-ruminant meats, while vegetable products contain only small amounts (Dufey, 1999; Fritsche & Steinhart, 1998; Fritsche et al., 1999, 2000; Sieber, 1995). CLA has many nutritionally important

biological properties so that an increasing intake of CLA is recommendable for humans. As one of the most accessible avenues, the CLA levels of milk and dairy products can be raised by manipulation of the diet of ruminants and, to a lesser extent, by manufacturing fermented dairy products or cheese with starter cultures selected for a high CLA-producing potential. More extensive investigations have to show if, during yoghurt manufacturing and cheese ripening, lactic acid bacteria and (in the case of cheese) also propionibacteria are able to produce CLA in sufficient amounts to be of physiological importance for human nutrition. This should be studied especially in cheeses which show high lipolytic activity (e.g. Blue cheese varieties) and, as a result, a high content of free linoleic acid. A second group of cheeses offering worthwhile target of studies are long ripened cheeses in which propionibacteria are used (e.g. Emmental). Another opportunity to increase CLA level is the use of a small amount of a high linoleic acid-containing oils or linoleic acid for manufacturing yoghurt as illustrated by the fermentation of milk with *Lc. lactis* I-01 (Kim & Liu, 2002) and by the production of non-fat set yoghurt with *L. acidophilus* (Lin, 2003). Use of lactic acid bacteria with high CLA-producing abilities could further increase the possibilities for an additional formation of the CLA content in common fermented dairy foods or cheese varieties without major changes in processing regimes.

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