Fermentation as a strategy to increase conjugated linoleic acid in dairy products

LINA COSSIGNANI, FRANCESCA BLASI

Department of Pharmaceutical Sciences, Section of Food Science and Nutrition
University of Perugia, Via San Costanzo, 06126 Perugia, Italy

SUMMARY

Conjugated linoleic acids (CLA) are a group of non-methylene interrupted octadecadienoic acids, naturally present in different foods, among which foods derived from ruminants, as milk and dairy products. The rumenic acid (9*cis*, 11*trans*-CLA) is the predominant isomer in milk fat, followed by 10*trans*, 12*cis*-CLA and other isomers in traces. In order to characterize the profile of CLA isomers found in dairy products, the silver-ion high performance liquid chromatography (Ag+-HPLC) equipped with multi-columns provides satisfactory resolution. To evaluate the fatty acid (FA) composition of dairy products, the high resolution gas chromatography (HRGC) analysis of derivatized FA, as fatty acid methyl esters, is generally used. Actually, there is always more interest of researchers in increasing the content of CLA in foods, including milk and dairy products. In fact, a lot of studies, carried out on animals and humans, have suggested that CLA may have important human health benefits, among which antiatherogenic, antiadipogenic, and antidiabetic properties. Among the strategies to increase CLA content during the manufacturing of dairy products, fermentation is one of these. CLA content in fermented dairy products is extremely variable (generally from 3.4 to 8.8 mg/g fat) and strongly linked to the strain type, to the aerobic or anaerobic conditions and to other parameters, as pH, time and temperature of growth culture. Strains of *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Propionibacterium* and *Streptococcus* can be considered potential CLA-producers. The manipulation of the feed and dietary regimen of the animals is an alternative strategy to increase CLA content. Linseed, soybean, olive, canola, sunflower and fish oils, added to feed, can improve the unsaturated FA contents, including CLA, in milk and dairy products. Alternatively, interest in the enzymatic modification of FA composition of dairy products in order to produce healthier fats, is increasing. In this review, an overview of the main strategies to increase CLA content in dairy products, with particular focusing on fermentation, is reported.

KEY WORDS

CLA, nutraceutical, dairy products, milk quality, fermentation.

CHEMISTRY OF CLA

Conjugated linoleic acids (CLA) are a mixture of positional (from 7,9- to 11,13-) and geometric (*cis,cis*; *cis,trans*; *trans,cis*; or *trans,trans*) isomers of linoleic acid (9*cis*, 12*cis*-18:2); differently from linoleic acid, the double bonds in CLA are adjacent, without intermediate methylene groups. Among this type of fatty acids (FA), the 9*cis*, 11*trans* isomer was identified in ruminant milk fat for the first time by Parodi (1977), and then named "rumenic acid". Later, it was confirmed that 9*cis*, 11*trans*-CLA is the predominant isomer in milk fat (75-90% of the total CLA), followed by 10*trans*, 12*cis*-CLA; trace amounts of other isomers (9*trans*, 11*trans*; 7*trans*, 9*cis*) have been also found[1]. Figure 1 shows the two main CLA isomers detected in dairy products.

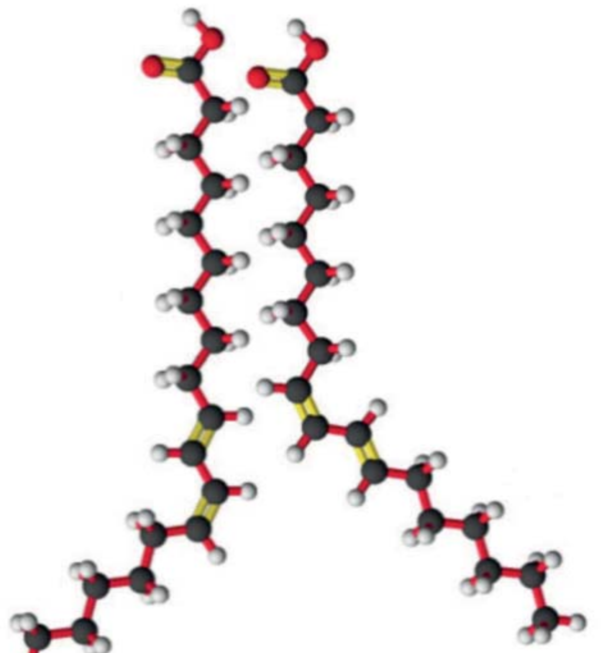

Figure 1 - The predominant isomers in milk fat: 9*cis*, 11*trans*-CLA and 10*trans*, 12*cis*-CLA.

CLA CONTENT OF FERMENTED DAIRY PRODUCTS

Linoleic acid is the key precursor of rumenic acid, which can be synthesized by biohydrogenation of linoleic acid, car-

Corresponding Author:
Francesca Blasi (francesca.blasi@unipg.it).

Table 1 - Content (mg/g of fat) of CLA and starter cultures of fermented milks.

FERMENTED MILK / *Microorganism*	CLA
Lb. lactis	1.9[a]-9.2[a]
Lb. buchneri	4.3[a]
Lb. reuteri	4.8[a]
Lb. helveticus	5.4[a]
Lb. brevis	1.7[b]-10.5[b]
Lb. viridescens	1.8[b]-5.7[b]
Lb. casei	1.13[c]-6.3[a]
Lb. plantarum	0.51[c]-4.9[a]
B. animalis ssp. *lactis*	15.5[d]-18.5[d]
B. bifidum	0.46[c]-4.2[e]
B. breve	2.3[e]-4.4[e]
B. pseudolongum ssp. *pseudolongum*	2.7[e]-4.2[e]

[a] Pandit et al., 2012; [b] Puniya et al., 2008; [c] Kuhl et al., 2016; [d] Florence et al., 2009; [e] Gorissen et al., 2012.

ried out by ruminal and lactic acid bacteria. The concentration of CLA in cow milk normally ranges between 2.0 and 6.4 mg/g of fat, and is mainly affected by the lactation stage, rumen microflora, and dietary regime[2]. Milk and dairy products are the main source of CLA in the human diet, providing about 70% of the total CLA daily intake, which varies between 70 and 430 mg/day[3]. Table 1 and 2 show the content, expressed as mg/g of fat, of CLA (total isomers) in fermented milks and yogurts. It can be noted that the CLA content in fermented dairy products is extremely variable (in fermented milk from 1.9 to 18.5 mg/g of fat; in yogurt from 2.1 to 20.8 mg/g of fat) and strongly linked to the strain type and to the conditions of growth culture. In fermented dairy products, the concentration of CLA varies, more commonly, between 3.4 and 8.8 mg/g fat.

STRATEGIES TO INCREASE CLA CONTENT IN DAIRY PRODUCTS

Strategies to increase CLA content are based on the modification of the technological processes used for the manufacturing of dairy products[4]. Fermentation is one of the main approaches employed to increase CLA content in a natural manner; it is strain-dependent, because of the different *linoleate isomerase* activity of the species. Recently, the main bacteria species used for the production of functional dairy products have been summarized[4]. In fact, in addition to the rumen bacteria, strains of *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Propionibacterium*, *Streptococcus* can be considered as potential CLA-producers, because of the presence of the *linoleate isomerase* enzyme. Numerous studies have shown that strains of various bacteria can be used as starter cultures for the development of functional fermented milk and yogurt, having higher contents of CLA[5-12]. Generally, a vegetable oil, a rich source of linoleic acid, is supplemented to the incubation mixture as substrate for improving CLA production. Different types of oils have been used to this aim (sunflower, hydrolyzed soy oil, grape seed oil, castor oil) and different concentration tested. For example, Puniya et al.[5] used sunflower oil at the concentration of 0.25, 0.5 and 1.0% v/v, while Vieira et al.[9] at 1.7% v/v. In addition, the optimal conditions of pH, time and temperature for the production of CLA are very important parameters; values of pH-optimum from 6.5 to 9.5, and temperature from 10 °C to 34 °C are reported[7]. The fermentation time ranges from 12 to 70 hours. Some authors reported that the concentration of the 9-*cis*, 11-*trans* isomer was about three times higher when a mixed rumen bacterial strain was incubated under aerobic conditions rather than under anaerobic conditions. It must be also emphasized that an inhibitory effect of LA on the growth of strains has been reported, suggesting that the conversion of LA to CLA isomers ca be related to a detoxification mechanism. Alternatively, thermal and high pressure processing has been also used[13]. Other strategies, employed to increase CLA content, are based on the manipulation of the feed and dietary regimen of the animals. A lot of researches have shown that the addition to the feed of linoleic acid-rich supplements, such as linseed, soybean, olive, canola, sunflower and fish oils[14-16], can slightly increase both the CLA content and the concentration of unsaturated fatty acids in milk and dairy products. Recently, Ianni et al.[17] reported that cows fed with a diet supplemented with dried grape pomace produced milk with an increased concentration of rumenic acid. Recently, there has been an increasing interest in the modification of fatty acid composition of foods, including dairy products. It is possible to produce healthier fats, for example structured lipids with CLA esterified in *sn*-2 position or medium chain triacylglycerols containing CLA, with nutritional advantages due to their real bioavailability[18-20].

Table 2 - Content (mg/g of fat) of CLA and starter cultures of yogurts.

YOGURT	CLA
S. thermophilus + L. delbrueckii ssp. bulgaricus	2.1[a]-16.5[b]
S. thermophilus + L. delbrueckii ssp. bulgaricus + B. bifidum	2.7[c]-4.2[c]
S. thermophilus + L. delbrueckii ssp. bulgaricus + B. breve	2.3[c]-4.4[c]
S. thermophilus + L. delbrueckii ssp. bulgaricus + B. pseudolongum ssp. pseudolongum	3.0[c]-4.4[c]
S. thermophilus + L. delbrueckii ssp. bulgaricus + L. acidophilus L10	4.7[d]-11.0[d]
S. thermophilus + L. delbrueckii ssp. bulgaricus + B. animalis	3.6[a]-9.6[d]
S. thermophilus + L. delbrueckii ssp. bulgaricus + L. acidophilus	3.3[a]-20.8[e]

[a] Akalin et al., 2007; [b] Florence et al., 2009; [c] Gorissen et al., 2012; [d] Santo et al., 2012; [e] Dave et al., 2002.

BIOLOGICAL ACTIVITIES AND POTENTIAL ADVERSE EFFECTS OF CLA

CLA can be considered a multifunctional natural nutraceutical from the rumen, since it shows numerous potential health properties. For this reason, in the last two decades, there was an explosion of interest into deepening the knowledge on biological activity of dairy and fermented products. A lot of recent papers, carried out on animals and humans, confirmed that CLA isomers possess antiatherogenic[21], antiadipogenic[22], anticancerogenic[23], antinflammatory[24] and antidiabetic[25] properties. Moreover, CLA isomers have effect on weight loss[26]. As regards fermented dairy products, milk fermented with *Lactobacillus casei* NCDC19 decreases epididymal fat mass and adipocyte size in obese mice[27].
In any case, it must be underlined that generally the bioactivity is linked to a specific isomer, and that these properties can be additive, antagonistic, or independent. According to experimental trials on animals, a 70-kg individual would benefit from a daily intake of 3.5 g of CLA[2]. Human studies on CLA supplementation in doses of up to 7 g/day have shown no adverse effects. Some studies focused on genotoxicity/antigenotoxicity of CLA by using test *in vitro* as comet test[28,29]. It has been reported that CLA possess antigenotoxic properties, evaluated using HepG2 cells, mainly acting as desmutagenic agents, and that qualitative/quantitative effects strongly depend on CLA isomers. The main CLA isomers (9*cis*, 11*trans* and 10*trans*, 12*cis*) of fermented dairy products were effective against 4-nitroquinoline N-oxide-induced DNA damage. At the same time, Aydin et al.[30] suggested that CLA (200 mg/kg/day) and whey protein (5 mg/kg/day) can improve the antioxidant defenses of rats, against acrolein-induced toxicity.
The potential adverse effects associated with CLA intake, such as its role in oxidative stress, glucose metabolism, milk fat depression, and liver functions, have been reported[3,31].

ANALYSIS OF CLA IN FERMENTED DAIRY PRODUCTS

In recent years, there has been an increasing interest in analytical methods, reliable and precise, useful for identification and quantification of CLA isomers in dairy products, nutraceuticals, and nutritional supplements. The technique of silver ion (or "argentation") chromatography was often used for CLA analysis, in particular the silver-ion high performance liquid chromatography (Ag+-HPLC) equipped with single column[32] or multi-columns[33]. This last approach led to a more satisfactory chromatographic resolution of the CLA isomers found in dairy products. However, a combination of high resolution gas chromatography (HRGC) and Ag+-HPLC was found to be necessary to resolve all CLA isomers[34]. Because of the multiplicity of geometrical and positional CLA isomers, complete separation and accurate analysis of complex mixtures are needed. To evaluate the FA composition of fermented dairy products, the HRGC analysis of derivatized FA has been generally used[35].
Lipases have been widely used to separate the two most common CLA isomers (9*cis*, 11*trans*-CLA and 10*trans*, 12*cis*-CLA)[36]. The single most comprehensive method for CLA analysis is the high resolution NMR spectroscopy[37], which permitted the identification and quantification of all the positional (7,9- to 11,13-) and geometrical CLA isomers.
Since dairy products are subjected to heat treatments to elongate their shelf-life, it is important to evaluate the oxidative modifications of CLA[38] and the productions of volatile compounds[39,40] during thermal processes. Another interesting aspect is the determination of the position esterified by CLA in the glycerol backbone, important aspect for physiological/nutritional reasons[41] and for detection of adulterations[42-44].

CONCLUSIONS

Despite the interesting biological activities of CLA isomers, dietary CLA intake is relatively low to promote the desired physiological effects. Therefore, the improved production of CLA by bacteria in foods is an important technological challenge. In this perspective, more research is needed in order to exploit the potential of milk fermentation as a valid approach for manufacturing dairy products with high CLA content. In this context, the optimization and standardization of fermentation conditions are necessary to obtain reproducible concentrations of CLA isomers in functional foods and nutraceuticals. Moreover, the development of simple and fast analytical methods is desirable for quality control procedures.

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