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Conjugated Linoleic Acid in Dairy Products: A Review

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ABSTRACT

Conjugated Linoleic Acid (CLA) is a group of octadecadienoic acids that are naturally present in foods derived from ruminant animals such as meat and dairy products. Many bacteria from cheese starters and some probiotics are able to produce CLA from the linoleic acid present in milk, increasing the content of this compound. Some *in vitro* and animal studies have suggested that CLA may have important health benefits for humans including cancer prevention, immune response modulation and weight loss. More research is necessary before a definitive recommendation can be issued.

Key words: Conjugated linoleic acid, dairy products, lactic acid bacteria, probiotics, antimicrobial activity

INTRODUCTION

Conjugated Linoleic Acid (CLA) is the name of a group of isomers of the 18 carbon dienoic acid known as linoleic acid (9,12-cis-octadecadienoic acid). These compounds are then linoleic acid derivatives with cis-9, trans-11-; trans-9, cis-11-; trans-9, trans-11-; trans-10, trans-12 and trans-10, cis-12-octadecadienoic acids as the main isomers and cis-9, cis-11-; cis-l0, cis12-; cis-l0, trans-12 and cis-11, cis-13-octadecadienoic acids as the minor isomers (Lin *et al.*, 1995). However, only the isomers that contain trans double bonds are biologically active. Linoleic acid is transformed into several CLA isomers in ruminants via the action of many bacteria and enzymes in the rumen and other tissues. The consumption of CLA by humans has associated many possible health benefits and some of the major sources of this group of compounds are dairy products such as milk, cream, yogurt, cheese and butter (Singh and Sachan, 2011). Milk and dairy products have always been considered as very important functional foods (Bhat and Bhat, 2011). Some of these benefits include cancer prevention, antioxidant activity, immune response and lipid metabolism modulation (Crumb, 2011).

CLA CONTENT OF DAIRY PRODUCTS

Dairy products have been identified as good sources of CLA and this has increased the positive nutritional image of these foods. This fact has led to a series of studies with the sole purpose of determining the CLA content of dairy products. The most common analysis involves HPLC. Usually, 4 mL of extract are evaporated and methylated using sulphuric acid in mild conditions (Aldai *et al.*, 2005) to obtain the fatty acids methyl esters extract. Separation of the CLA methyl esters is carried out using a HPLC system equipped with UV detector operated at 233 nm. An additional and very useful methodology involves the use of gas chromatography and covalent adduct chemical ionization tandem mass spectrometry (CACI-MS/MS). This has been shown to be a powerful tool for identifying positional and geometric isomers of CLA methyl esters (Gomez-Cortes *et al*., 2009). CLA is synthesized by two main pathways in ruminant animals. The first involves the incomplete biohydrogenation of linoleic and linolenic acids in the rumen by the endogenous bacteria. The second is the conversion of transvaccenic acid to CLA in the tissues of the ruminants. This second conversion accounts for 60-90% of the total CLA in food products from ruminants (Khanal and Dhiman, 2004). The CLA content in milk and dairy products is affected by several factors, such as animal's breed, age and diet, being this last factor the most important and also the easiest to manipulate for the purpose of enhancing the final CLA content of dairy products (Khanal and Olson, 2004). Milk produced by grass-fed cows has a higher content of CLA than the milk of cows fed low forage diets (Singh and Sachan, 2011). CLA in milk has been shown to be stable under normal cooking and storage conditions however, a significant reduction (ca. 21.80%) between raw and UHT milk samples (from 10.18 to 7.96 mg g^{-1}) has been detected in some studies (Costa *et al*., 2011). According to Herzallah *et al*. (2005), this reduction of CLA could be due to an oxidation process which results in the formation of hydroperoxides that could cause the conversion or degradation of CLA. In the case of refrigerated milk, the cis-9/trans-11 isomer concentration has been shown to remain stable after 2 weeks of refrigerated storage. However, a significant loss of both the cis-9/trans-11 and the cis-10/trans-12 isomers occurred at the end of 3 weeks of refrigerated storage. This loss could be due to lipase activity derived from microbial growth (Campbell *et al*., 2003). The CLA content of cheese heated in a microwave oven for 5 min decreased by 21% and further heating for 10 min caused a decrease of 53% compared with that of fresh cheese Herzallah *et al*. (2005). Total CLA content in milk or dairy products ranges from 3.4 (whole milk) to 10.7 mg g^{-1} (processed cheese) of total fat (Dhiman *et al*., 2005). The CLA content of cheeses ranged from 3.6 to 8 mg g^{-1} of total fat. Blue, Brie, Edam and Swiss cheeses had significantly higher CLA content than other cheeses and yogurt has a content similar to that of whole milk (Lin *et al*., 1995). In the case of sheep milk, the CLA content in individual samples varies from 17.8 to 56.5 mg g^{-1} fat. The highest CLA concentrations in sheep milk products are found in white brined cheese (35.6 mg g^{-1}), followed by yogurt (29.5 mg g^{-1}) and the lowest in yellow cheeses (21.8 mg g^{-1}). Differences in CLA contents are attributed, as in the case of cows, to the sheep breeds and to the diet (Mihailova and Odjakova, 2011). In the case of goats, the range of CLA content in milk goes from 6.4 to 7.9 mg g^{-1} (Nudda *et al*., 2003). There are also reports of dairy products to which CLA has been added to increase their original content (Lopes *et al*., 2009).

CLA AND PROBIOTICS

The processes of isomerization and biohydrogenation of linoleic and linolenic acid, to produce CLA that take place in the rumen are performed, as described before, by ruminal bacteria, such as *Butyrivibrio fibrisolvens* and *Megasphaera elsdenni*. These processes and the fact that numerous fermented dairy products contain higher levels of CLA than their non-fermented counterparts indicated the possibility of elaborating fermented dairy products with high levels of CLA by using *Bifidobacterium* and Lactic Acid Bacteria (LAB). The identification of LAB able to produce CLA from a source of linoleic acid would then be of great significance to produce functional dairy product with high CLA content for human consumption (Rodriguez-Alcala *et al*., 2011). Probiotic strains of *Lactococcus lactis*, *Lactobacillus acidophilus*, *L. plantarum* and *Bifidobacterium animalis* have been shown to produce CLA in the range of 40 to 50 μg mL^{-1} of skim milk using linoleic acid as a precursor (Rodriguez-Alcala *et al*., 2011). The ability of cultures of *L. acidophilus* and *L. casei* of human intestinal origin to produce CLA in skim milk supplemented with 0.02% free linoleic acid has also been studied. In this medium, the total amounts of free CLA after 24 h of incubation ranged from 54.31 to 116.53 μg mL^{-1} (Alonso *et al*., 2003). In the case of the incubation of washed nongrowing cells of a probiotic strain of *L. acidophilus* (La-5), total CLA accumulation reached an average concentration of 388 μg g^{-1} (CLA/dry biomass) after 72 h (Macouzet *et al*., 2009). In other study, *L. rhamnosus*, in coculture with yogurt culture, produced a high content of CLA. Similarly, growth and CLA formation by propionibacteria were enhanced in the presence of yogurt cultures (Xu *et al*., 2005). This mutualistic effect deserves to be studied further. In addition, several strains of *Propionibacterium* and *Enterococcus* are able to form CLA from linoleic acid. However, several investigations on yogurt and cheese have not shown particularly high CLA levels, possibly because these dairy products were not elaborated with specific CLA-producing LAB strains (Sieber *et al*., 2004). Thus, the addition of this kind of strains could be used to increase the CLA level in fermented dairy products such as yogurt, kefir and cheese. Furthermore, if these strains are also probiotics, an exceptional functional dairy product could be obtained.

ANTIMICROBIAL ACTIVITY OF CLA

The potassium salt of CLA (CLA-K) has been tested against Gram-positive (*Bacillus cereus*, *Staphylococcus aureus* and *Streptococcus mutans*) and Gram-negative (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Vibrio parahemolyticus*, *Klebsiella pneumoniae* and *Proteus mirabilis*) bacteria showing clear growth inhibition for all tested strains, particularly the Gram-positive strains. The CLA-K at lower concentrations, slowed down the growth of all the tested strains and completely inhibited the growth at higher concentrations. All the microorganisms grown in the CLA-K-containing culture medium had CLA in their membranes and exhibited irregular cell surface and disruption. It is possible that the growth inhibition by CLA was mediated through the lipid peroxidation of CLA in the membranes and in the medium (Byeon *et al*., 2009). This antimicrobial activity is an added value for any CLA-containing functional dairy product.

CONCLUSION

All dairy products contain CLA in different amounts, so in a certain sense, all of them can be considered as functional foods. The CLA content of milk can be increased by modifying the feed and the milk production conditions. Also, this content can be modified by direct addition of this nutraceutical compound or by the addition of a precursor such as linoleic or linoleic acid and a CLA-producing bacterial strain. An added value could be obtained if the producer strain is a probiotic microorganism.

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