

CONJUGATED LINOLEIC ACID-ENRICHED DAIRY PRODUCTS: A REVIEW

Mihaela Ivanova^{*1}

Address(es): Mihaela Ivanova,

¹University of Food Technologies, Technological Faculty, Department of Milk and Dairy Products, 26, Maritsa Blvd., 4002 Plovdiv, Bulgaria, phone number: +35932603783.

*Corresponding author: mihaela_18bg@abv.bg

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ABSTRACT

Conjugated linoleic acid (CLA) is a family of more than 28 isomers of linoleic acid wherein the isomers *cis*-9, *trans*-11 (rumenic acid) and *trans*-10, *cis*-12 are the most abundant. It is associated with a number of potential health benefits for human organism. Many foods are a good source of it but it is mostly found in meat and dairy products, derived from ruminants. Dairy products contain CLA in different amounts. The enrichment of these products with CLA is appropriate given the lower CLA content in these products in comparison with the recommended health intake. Modification of CLA concentration can be done by specific animal feeding and diet modification, by direct CLA supplementation in the form of oils or by addition of specific starter culture. The influence of technological treatments on the stability of the final product during storage and maturation is still not completely elucidated. There is a need for further studies on the physiological effects of CLA isomers on humans. The purpose of this review is to summarize the possibilities for increasing CLA content in milk and dairy products and to determine the possible effects of this enrichment on product stability – sensory, chemical, microbiological profile, shelf life and potential health effects of the obtained products.

Keywords: conjugated linoleic acid, milk, dairy products, functional foods

INTRODUCTION

In recent years, there was an increased discussion about the role of milk fat and its components and their influence on human health. Milk fat is a natural source of high valuable biologically active compounds such as phospholipids, fat-soluble vitamins, short-chain fatty acids and conjugated linoleic acid (CLA) (Bhat and Bhat, 2011). It is believed that the positive effects of these milk fat components are in predominance if compared with the negative influence of saturated fatty acids on human health. Liesbeth *et al.* (2010) demonstrated that *cis*-9, *trans*-11 CLA, presented in significant amounts in the milk of pasture-grazed cows, might offset the adverse effect of the saturated fat content of dairy products. Further research on this dependence can be made in order to determine even more precisely the predominance of the positive health effects of the fatty acid profile of dairy products over the often wrongly alleged unhealthy ones.

CLA is naturally formed in the rumen as an intermediate product in the digestion of dietary fat (Song and Kennelly, 2002). According to Meraz-Torres and Hernandez-Sanchez (2012), it is synthesized by two ways – the first is by incomplete biohydrogenation of linoleic and linolenic acids in the rumen by the endogenous bacteria and the second one is the conversion of transvaccenic acid to CLA in the animal tissues which represents 60-95% of the total CLA in the foods produced from animals.

CLAs are conjugated dienes of linoleic acid (Fig.1). This name refers to a group of positional and geometric isomers of linoleic acid, characterised by a conjugated system of double bonds, separated by one single bond (National Centre for Biotechnology Information, 2020).

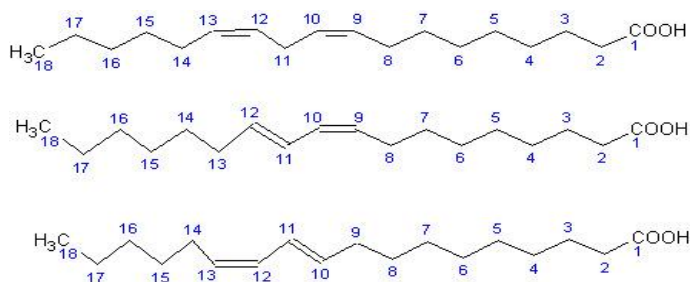


Figure 1 Chemical formulas of CLA isomers

There are 28 potential CLA isomers. The most common isomers are *cis*-9, *trans*-11, and *trans*-10, *cis*-12 of which rumenic acid (C18:2 *cis*-9, *trans*-11) is dominant in milk fat (Virsaingbhai *et al.*, 2020).

Natural CLA content in milk and dairy products is presented in Table 1. Markiewicz-Kęszycka *et al.* (2013) reviewed the fatty acid profile of milk and found that sheep milk was the richest source of conjugated linoleic acid in comparison with cow and goat milk. This fact was confirmed by Prandini *et al.* (2011). Different factors, such as diet, production system, breed, or stage of lactation could influence on the CLA content in milk and dairy products respectively. Recent review (Ahmad *et al.*, 2019) declared dairy products with natural CLA concentration ranging from 3.4 to 10.7 mg/g fat in milk.

Dairy products originating from various producers and from various countries were characterized by similar contents of CLA and diversified contents of trans isomers of C18:1 and C18:2 acids (Paszczyk *et al.*, 2012a) which impose the need of a broader analysis of similar products prepared under different conditions. It can be seen that in some types of dairy products, such as yoghurt, butter, ice cream, fresh cheeses and white pickled cheeses, large deviations in the reported natural CLA content were found. This imposes on researchers the need to optimize methods of analysis, to seek comparability of results and is an opportunity for future research. The effect of initial fat content on the CLA content of the finished product must be investigated in more depth. The highest CLA content in all analysed cheeses was found in hard cheeses with long ageing time while white pickled cheeses, which were produced without ageing or with short ageing time, contained only low amounts of CLA. From the presented data it is evident that there is a need to study the influence of various factors on the natural content of CLA in dairy products.

Collomb *et al.* (2006) summarized CLA variation and physiological effects on milk fat quality and quantity. According to Kirchnerová *et al.* (2012) it is not possible to influence some groups of desirable fatty acid, e.g. CLA, without the influence on other fatty acids. In this view, it is recommended that studies dedicated on the current topic provide an overall assessment of the fatty acid profile of the analysed products. Natural CLA concentrations are not sufficient to demonstrate specific health promoting effects. CLA enrichment of foods can cause a number of beneficial health effects on animal and on human organism. In recent scientific research, authors suggested that enrichment of dairy products with CLA may positively influence on human immune system stimulation, may

present antihypertensive, anticarcinogenic, and antiatherogenic effects and promotes the health benefits by body weight loss (Yang *et al.*, 2015).

Table 1 Natural CLA content in milk and dairy products

Type of product	CLA concentration, mg/g fat	Source	Type of product	CLA concentration, mg/g fat	Source
<i>Fresh dairy products</i>			<i>Cheeses</i>		
Cow milk	4.5-5.7	Markiewicz-Kęszycka <i>et al.</i> (2013)	Mozzarella	4.9	Chin <i>et al.</i> (1992)
Sheep milk	7.6	Markiewicz-Kęszycka <i>et al.</i> (2013)	Mold cheeses	2.5-8.1	Paszczyk <i>et al.</i> (2012a)
Goat milk	4	Cossignani <i>et al.</i> (2014)	Bloomy mould cheese	5.4-8.8	Paszczyk and Rafałowski (2015)
Buffalo milk	4.2	Van Nieuwenhove <i>et al.</i> (2007b)	Blue-veined cheeses	3.5-11	Paszczyk and Rafałowski (2015)
Homogenized milk	5.5	Chin <i>et al.</i> (1992)	Feta and Greek cheeses	8	Zlatanoz <i>et al.</i> (2002)
Yoghurt	0.9-4.8	Chin <i>et al.</i> (1992) Paszczyk and Rafałowski (2015) Trigueros and Sendra (2015)	Tulum	3.4-4.5	Gürsoy <i>et al.</i> (2003)
Keffir	4.6	Paszczyk and Rafałowski (2015)	Teneke Tulum	2.7-5.4	Gürsoy <i>et al.</i> (2003)
Kaymak	0.3	Okur and Guzel-Seydim (2012)	Fresh Kashar	0.01-5.4	Gürsoy <i>et al.</i> (2003)
<i>Long-life milks</i>			Aged Kashar	2.1-12.6	Gürsoy <i>et al.</i> (2003) Okur and Guzel-Seydim (2012)
UHT milk	3.3	Paszczyk and Łuczyńska (2020)	White pickled cheeses	0.0-5.5	Gürsoy <i>et al.</i> (2003)
<i>High fat dairy products</i>			Mild Cheddar	1.9	Mushtaq <i>et al.</i> (2010)
Double cream	3.7	Mushtaq <i>et al.</i> (2010) Chin <i>et al.</i> (1992)	Processed cheese	5.7-7.3	Zhang <i>et al.</i> (2007) Mushtaq <i>et al.</i> (2010)
Butter	0.9-11.3	Okur and Guzel-Seydim (2012) Paszczyk <i>et al.</i> (2012b)	Fresh goat cheese	6.0	Cossignani <i>et al.</i> (2014)
<i>Frozen dairy products</i>			Semi hard goat cheese	8.0	Cossignani <i>et al.</i> (2014)
Ice cream	0.9-4.6	Chin <i>et al.</i> (1992) Mushtaq <i>et al.</i> (2010)			

It was found that consumption of CLA-enriched food products may produce a modest loss in body fat in humans when given at a dose of 3.2 g/day (Whigham *et al.*, 2007). De Almeida *et al.* (2014) proved that butter naturally enriched in *cis*-9, *trans*-11 CLA prevented hyperinsulinemia and increased both serum HDL cholesterol and triacylglycerol levels in rats. Davoodi *et al.* (2013) reviewed many reports over the years which suggested that CLA, as a component of milk fat exhibited some anticarcinogenic effects – against colorectal cancer (Liew *et al.*, 1995), breast cancer (Zlatanoz *et al.* 2002; Keley *et al.*, 2007), prostate cancer (Parodi, 2009). Virsangbhai *et al.* (2020) suggested that CLA is a potent fat-soluble antioxidant. Recommendations for CLA daily intake levels have to be established in the EU. However, recommended 3-6 g/day level intake could be suggested on the basis of potential health benefits (MacDonald, 2000).

The aim of the present paper was to provide an overview of the CLA-enriched dairy products, to discuss potential health benefits of CLA-enriched dairy products and to identify future directions for research as well as applications.

FACTORS AFFECTING CLA-CONTENT IN MILK AND DAIRY PRODUCTS

Fig. 2 represents the pathway of CLA from raw milk to dairy products in accordance with the applied process technology.

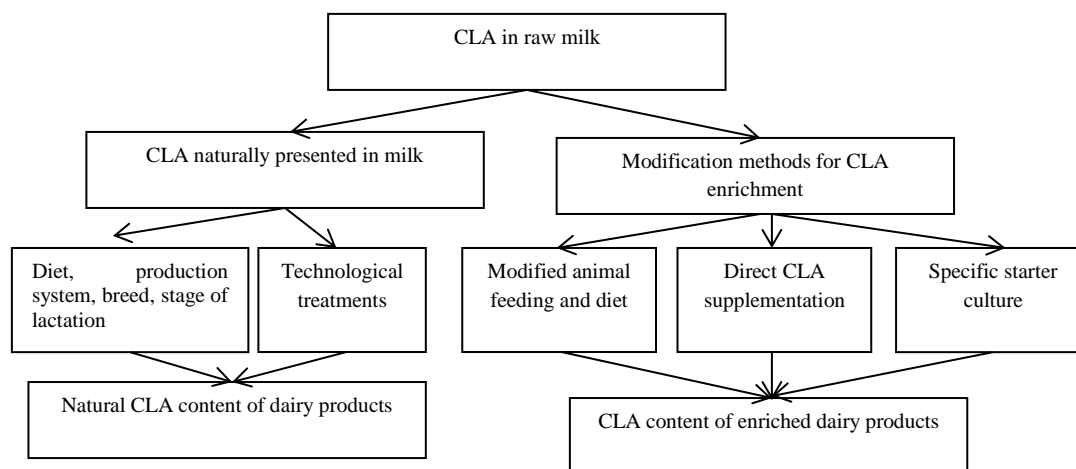


Figure 2 Factors affecting CLA-content in milk and dairy products

CLA NATURALLY PRESENTED IN MILK

Diet, production system, breed, stage of lactation

Pasture based feeding diet had a beneficial effect on milk fatty acid profile (Stanton *et al.*, 2018) in comparison to indoor feeding. The same tendency was confirmed by Ruiz *et al.* (2016). Kim *et al.* (2009) found 8.12 mg CLA/g fat for cows fed on pastures and 6.76 mg CLA/g fat for cows fed indoor. Cabiddu *et al.* (2003) studied the influence of different pastures on milk composition, with particular reference to CLA and its precursors. Higher amount of CLA and its precursors in milk and cheese were found in the group *Lolium rigidum* + *Medicago polymorpha* + *Chrysanthemum coronarium*.

O'Callaghan *et al.* (2016b) investigated the effects of different feeding systems on milk quality and composition. They have found that the pasture feeding systems induced significantly higher concentrations of CLA content of milk compared with that of the total mixed ration diet (TMR) feeding system. This was confirmed by other authors who established that milk obtained from cows fed in the traditional system, especially in the summer season, contained 1.42% more CLA (Barłowska *et al.*, 2012). Ruiz *et al.* (2016) compared animal feeding system utilized by the dairy farm and found higher CLA levels in raw milk based on grazing systems than housing systems using TMR diets. Prandini *et al.* (2004) stated that *cis*-9, *trans*-11 CLA content was negatively correlated with the concentration of crude fiber.

The impact of lactation stage on milk fat fatty acid profile of grazing dairy cows was studied (Kirchnerová *et al.*, 2013). They found that with the number of days of lactation a significant decrease in milk CLA content was established in relation to the amount of already produced milk, fat and protein. The rumenic acid content of Emmental cheese varied between 0.6% and 1.5% of total fatty acids according to the season and region of production, and appeared homogeneous through the cheese wheel (Chamba *et al.*, 2006). According to Ruiz *et al.* (2016) average values of CLA were 1.72 g/100g of total fatty acids, in spring milk and 0.42 g/100 g in summer milk and according to Kim *et al.* (2009) about 6.8 mg CLA/g fat in spring and relatively low in midsummer and winter (about 4.3 mg CLA/g fat). Seasonal variation was observed by Rutkowska and Adamska (2011) in the content of conjugated linoleic acid C18:2 *cis*-9, *trans*-11 (CLA) with significant content of 580 mg CLA/100g of product on average (determined in butter samples produced in the summer and autumn season). Prandini *et al.* (2004) found higher *cis*-9, *trans*-11 CLA concentration in spring milks (14.53 mg/g fat) and gradually decreased from summer to winter, when the lowest values were found (9.20 mg/g fat). In recent studies, mathematical models were created based on data of the CLA and fatty acid composition of dairy products in the purpose to predict the CLA content of other dairy products (Nunes and Torres, 2012).

Bonczar *et al.* (2016) studied the influence of three breeds on sweet cream. CLA prevailed in the milk fat produced by the cows of the Polish Holstein-Friesian breed, red-white variety. Mountain Grana Padano cheese contained more CLA than low-land hill Grana Padano and this fact was more pronounced when cheese was produced from summer milk in comparison with spring milk (Prandini *et al.*, 2009b).

Several authors have found that organic milk has a higher CLA content (Prandini *et al.*, 2009a; Tudisco *et al.*, 2010). Butler *et al.* (2011) suggested that organic or low-input dairy management was more likely to result in milk higher in beneficial isomers of CLA. This tendency was even more remarkable when producing fermented organic milks with significantly higher amounts of CLA than the same milk before fermentation (Florence *et al.*, 2009). Grana Padano cheese was characterized by a higher CLA content when produced by organic farming system in comparison with the control sample produced by conventional system (Prandini *et al.*, 2009a). The fat from the bio-yoghurts had the highest mean content of CLA (7.62 mg/g) (Paszczuk and Luczyńska, 2020). The analysis of the considered data showed that the natural content of CLA was influenced by a number of factors. Further investigations could be done in order to predict more precisely the estimated natural CLA content according to the diet, applied production system, breed type and stage of lactation.

Technological treatments

Heat treatments and homogenization of milk caused oxidation of valuable anticancer CLA through exposure to high temperatures, high pressures, and reduction of fat globule size (Norgauer, 2005). Application of high temperature short time pasteurisation (HTST) resulted in a significant decrease of the *cis*-9, *trans*-11 isomer and other minor CLA isomers (Campbell *et al.*, 2003). These results were found in milk that was fortified with CLA at very high doses (30%), which resulted in a significant, but rather moderate loss of CLA (c.a. 10% loss). Refrigerated storage of HTST milk decreased the *cis*-9, *trans*-11 isomer concentration after 3 weeks (Campbell *et al.*, 2003). Campbell *et al.* (2003) found no differences in lipid oxidation between milks with or without added CLA even the fact that fortified HTST milks were supplemented with vitamin E and rosemary extract to prevent lipid oxidation. Ruiz *et al.* (2016) found that milk processing significantly affected the transferring of CLA from raw milk into dairy products (sweetened condensed milk and powdered milk). Shantha *et al.*

(1995) found increased CLA concentration in salted butter (1.32 fold) in comparison with unsalted butter (1.27). They stated no changes in CLA content in processed dairy products such as low-fat yogurt, regular yogurt, low-fat and regular ice cream, sour cream or cheeses such as Mozzarella, Gouda and Cheddar. Storage did not affect CLA concentration in any products, suggesting that CLA is a stable component (Shantha *et al.*, 1995). Dave *et al.* (2002) established no significant influence of milk processing (incorporation of milk powder and heat treatment at 85°C for 30 min, fermentation with yogurt and probiotic bacteria and storage) on CLA-concentrations of probiotic CLA-enriched yoghurts. Garcia-Lopez *et al.* (1994) analysed changes of CLA content in processed cheese during processing and found CLA to increase from 9.5 mg/g of fat in the raw ingredients to 10.7 mg/g of fat in the finished product without apparent changes in the isomer distribution. The culinary utilization and processing did not change the rumenic acid content of Emmental cheese (Chamba *et al.*, 2006). Avilez and Meyer (2014) assessed the effect of processing fresh milk into milk powder and condensed milk. They found 1.35 g CLA/100 g of fatty acids in fresh milk and 1.45 and 0.93 g/100 g fatty acids transferred to the milk powder and condensed respectively. As isomers, the *cis*-9, *trans*-11 had higher levels in the powdered milk than fresh milk in 7 months of the 10 months studied with values from 0.50 to 0.84 g/100 g fatty acids. These data were confirmed by Ruiz *et al.* (2016) who found that sweetened condensed milk presented lower CLA values than raw and powdered milk. No differences were found in the profiles of fatty acids in the fats of milk, sweet cream, and cream, which confirmed their stability during the centrifugation and pasteurisation of milk as well as during the acidification with starter cultures (Bonczar *et al.*, 2016). 7-month cheese aging showed higher CLA content than 4 months-aged cheeses, independently of the type of feeding (Kim *et al.*, 2009). The same tendency was established for Swiss - type Kormoran cheeses which accumulated more CLA over 6-weeks of ripening (Paszczuk and Luczyńska, 2018). It was established that thermal processing applied in the manufacture of fermented milk on the stability of CLA diminished the quantity of polyunsaturated CLA in the product (Liutkevičius *et al.*, 2009). It was concluded that aging is an essential factor to obtain higher amounts of conjugated linoleic acid (Okur and Guzel-Seydim, 2012). Zhang *et al.* (2007) observed no effect on CLA content in processed cheese food and processed cheese spread caused by mixing (reducing), homogenization and storage period, but heating at 90°C relative to 75°C significantly increased total CLA content in both cheeses. Zengin *et al.* (2011) studied the influence of milk pasteurization (between 70 and 90°C for 5 min) on its CLA-content in order to produce white pickled cheese and found no effect on CLA concentrations. CLA content in ghee prepared using indigenous method was higher as compared to that of a creamery method (Tyagi *et al.*, 2015). Romno *et al.* (2008) found that ripening grade did not influence on the CLA concentration (the highest content was of *cis*-9, *trans*-11 isomer) of Provolone del Monaco cheese. The same fact was observed by Renes *et al.* (2019) in CLA-enriched sheep cheese. Prandini *et al.* (2011) suggested that the factors involved in the cheese making process generally did not affect the CLA content in milk fat because they found no statistically significant differences in CLA concentration when cheeses were produced from the same ruminant species but through different production technologies. Milk processing and cheese aging had a negative effect on CLA concentration in Pecorino cheese (Prandini *et al.*, 2004). The obtained results demonstrated that the applied technological treatment influenced CLA content in milk and dairy products.

MODIFICATION METHODS ENHANCING CLA-CONTENT IN DAIRY PRODUCTS

Diet modification

Another possibility to enhance milk CLA content was to modify the animal's diet. Peşmen (2016) summarized the factors affecting CLA content enhancement of milk and dairy products and concluded that fish, sunflower, corn and canola oil had positive effects for this purpose. Oils were added into the diet in the form of protected oils, free oils, processed oilseeds or whole oilseeds (extruded, crushed, roasted or ground) (Virasbhai *et al.*, 2020). Gonzalez *et al.* (2003) altered the degree of unsaturation in milk fat by modifying the diets of Holstein cows in order to produce high-oleic and high-linoleic butter and ice cream. Cow and buffalo fed roughage based diet type one berseem (*Trifolium alexandrinum*) fodder along with wheat straw and type two concentrate mixtures and wheat straw (Tyagi *et al.*, 2015). It was found that total CLA content in milk was higher in berseem fed groups of both the species. Jones *et al.* (2005) fed early-lactation Holstein British Friesian cows with a mixture (45 g/kg on dry matter basis) of fish oil and sunflower oil (1:2 wt/wt) during two consecutive weeks. Control sample milk produced contained 0.54 in total CLA/100 g fat and milk enriched in CLA 4.68 g, respectively. Dave *et al.* (2002) collected milk from cows fed four diets consisting of a control (C), C with 2% fish oil (FO), C with 1% each of fish oils and extruded soybeans (FOES), and C with 2% extruded soybeans (ES) in order to enhance milk CLA-content. They found that milk, yogurt mix and yogurt from cows fed with FO or FOES diets showed a 4-fold increase in the concentration of CLA. Dos Santos *et al.* (2020) analysed the fatty acid profile in butter from cows fed with sunflower seeds with or without

lignosulfonate and proved that the use of diets with pelleted sunflower seeds was possible to obtain butter with a desirable nutritional value in dairy products with 23.74mg/g total lipid rumenic acid content. Dairy cows were fed sunflower seeds and pasture in order to increase of conjugated linoleic acid and vaccenic acid in anhydrous milk fat using dry fractionation (Herrera-Meza *et al.*, 2012). The use of linseeds was also applied in order to enrich in CLA ewe's milk (Luna *et al.*, 2005). The supplementation of elephant grass-based-diets with soybean oil increased CLA content linearly as the level of soybean oil in the diet increased (Lopes *et al.*, 2019). The butter produced from afternoon milk had a lower content of C16:0 and a higher content of *cis*-9 C18:1 ($P < 0.05$). Soybean oil and, to a small extent, the selective segregation of milk obtained from afternoon milking sessions are strategies that can be used to improve the fatty acid composition of butterfat. Different approaches can be applied in order to increase CLA in milk and dairy products. New sources can be used to achieve even higher CLA content.

Direct supplementation

Campbell *et al.* (2003) enhanced CLA in dairy products by direct addition of triglyceride oil (ClarinoTM G-80 1 and 2%) into skim milk followed by HTST pasteurization and homogenization. Rodríguez-Alcalá and Fontecha (2007) added CLA oil supplement (Tonalin-80TM, with 80% CLA content) to skim milk such that the consumer received 2.4 g/d of CLA by consuming 2 servings of fortified fresh dairy products with predominant isomers C18:2 *cis*-9, *trans*-11 CLA and C18:2 *cis*-10, *trans*-12 CLA, in a similar ratio, which ranged from 0.97 to 1.05. The same supplement (microcapsules powder - Tonalin® 60 WDP, and oily preparation - Tonalin®TG 80) was applied by Liutkevičius *et al.* (2009) in order to enrich in CLA fermented milks. Virsangbhai *et al.* (2020) reported the use of SafflorinTM (a mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers of octadecadienoic acid in a 40:60 ratio) for CLA enrichment. Zhuang *et al.* (2018) prepared milk protein-based conjugated linoleic acid microcapsules and investigated the effect of casein micellar structure on their stability. The authors found adverse effects on the casein micellar structure which suggested a modification of casein micelles in order to improve the efficiency and chemical stability of such microcapsules.

Addition of specific starter culture

The addition of specific starter culture with or without specific substrate addition can be considered as a promising possibility to increase CLA levels in dairy products. The fermentation using microorganisms having linoleate isomerase activity was employed as a promising technological alternative for the manufacture of fermented milk products with high content of CLA (Gutiérrez, 2016). Van Nieuwenhove *et al.* (2007a) investigated the influence of bacteria used as adjunct culture (*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*) and the sunflower oil addition (to a final concentration of 200 µg/ml of linoleic acid) on the CLA content in buffalo cheese. They have found that only *Streptococcus thermophilus* strain did not increase the CLA content in fresh cheese. Bzducha and Obiedziński (2007) determined the influence of two probiotic *Lactobacillus* strains on the CLA content in model ripening cheeses. They found no significant changes in CLA content during the eight weeks of ripening at the storage temperature of 6°C. Das *et al.* (2005) investigated the effect of yeast and bacterial and yeast adjuncts (three strains of *Propionibacterium freudenreichii* ssp. *shermanii*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Geotrichum candidum* and *Yarrowia lipolytica*) on the CLA content and flavour of a washed-curd, dry-salted cheese. CLA levels remained unchanged over 4 months of ripening, but the addition of linoleic acid-rich safflower oil to the cheese curd increased the concentration of free linoleic acid generated in the cheese but the total CLA content did not change. The CLA contents were significantly higher in fermented milks showing shorter fermentation times demonstrated by *Streptococcus thermophilus* TA040-*Bifidobacterium animalis* ssp. *lactis* HN019 and BB12 in comparison with co-cultures of *Streptococcus thermophilus* TA040-*Bifidobacterium animalis* ssp. *lactis* BL04 or B94 and *Streptococcus thermophilus* TA040-*Lactobacillus delbrueckii* ssp. *bulgaricus* LB340 (Florence *et al.*, 2009). Pickle white cheeses were produced from whole milk with five different probiotic cultures (*Enterococcus faecium*, *Lactobacillus paracasei*, *Bifidobacterium longum*, *Bifidobacterium bifidum* and *Lactobacillus acidophilus*) (Gursoy *et al.*, 2012). CLA-content of cheeses ranged from 3.52 to 3.92 mg/g and probiotic differences and storage process had not affected the CLA contents of the samples statistically (Gursoy *et al.*, 2012). A study demonstrated that the type of applied starter culture (Ceska-star Y508 culture) and storage time affected the content of CLA in fermented milk drinks (Paszczyk *et al.*, 2016). The mean content of CLA in fresh drinks reached 3.60 mg/g fat, after 6 days 3.85 g/g fat and after 13 day storage 3.89 mg/g, respectively. Renes *et al.* (2019) achieved 1.19 times higher CLA content in sheep cheese because the addition of autochthonous CLA-producing *Lactobacillus plantarum* TAUL 1588 and *Lactobacillus casei* ssp. *casei* SS 1644 strains.

DAIRY PRODUCTS ENRICHED IN CLA

Bell and Kennelly (2011) summarized the potential of “designer milk” for the production of dairy products. According to Meraz-Torres and Hernandez-Sanchez (2012) all dairy products were a source of CLA and can be considered as functional foods. CLA-enriched fresh, ripened and other dairy products were developed in order to enhance their nutritional and biological values. The need, the interest and the possibilities to produce dairy products from CLA-enriched milk are reviewed below.

CLA-enriched fresh dairy products

The effects of added conjugated linoleic acid (CLA) (1 and 2% in the form of triglyceride oil) on the sensory, chemical, and physical characteristics of 2% total fat fluid milk were studied by Campbell *et al.* (2003). They established lower consumer acceptability for CLA-fortified milks compared to control milks, but the addition of chocolate flavour increased acceptability. Similar results were obtained by Jones *et al.* (2005) for CLA-enriched ultra-high temperature (UHT) treated milk, which sensory profile differed slightly from those of the control sample. Although the sensory profiles of the CLA-enriched milk and fresh cheese differed from those of the control product with respect to some attributes, the overall impression and flavour did not differ. Rodríguez-Alcalá and Fontecha (2007) evaluated the changes of CLA isomers composition in fresh dairy products (milk, fermented milk, yoghurt, fresh cheese, milk-juice blend) after processing and storage. They detected a significant decrease of total CLA in fresh cheese samples after 10 weeks of refrigerated storage, but not in CLA isomers C18:2 *cis*-9, *trans*-11 and C18:2 *trans*-10, *cis*-12. In the same research, the disappearance of some of the minor CLA isomers and a significant increase of C18:2 *trans*, *trans* isomers from *cis*, *trans*; *trans*, *cis*; and *cis*, *cis* in fermented milk, yogurt, and milk-juice blend were established. Gutiérrez (2016) revised the effects of technological treatments on CLA content variations in milk and fermented milks. Some substrates (maltodextrine and fructooligosaccharides) in combination with probiotic starter cultures showed to be an effective method for enhancing the concentration of CLA in fermented milks (Akahn *et al.*, 2007). In contrary, it has been reported that the addition of sucrose, fructose and lactose (60 g/L) led to an inhibition of the CLA production in yogurts elaborated using *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Lin, 2000).

According to Van Nieuwenhove *et al.* (2007a) sunflower oil supplementation increased CLA concentrations in fresh cheeses in combination with appropriate adjunct cultures (*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*). CLA was found to be significantly positively correlated with *trans*-vaccenic acid and negatively correlated with linoleic acid in fermented milks (Florence *et al.*, 2009). Other authors observed that the higher the linoleic acid in the sample the higher concentration of conjugated linoleic acid (Okur and Guzel-Seydim, 2012). A positive correlation between the CLA and linoleic acid contents of *Lactobacillus paracasei* and *Lactobacillus acidophilus* cheeses was confirmed by Gursoy *et al.* (2012). Addition of oily CLA preparation slightly increased the quantity of the separated whey in fermented milks enriched in CLA and decreased the viscosity of the product (Liutkevičius *et al.*, 2009). According to the same authors, the addition of CLA in microcapsules powder form caused better sineretical properties and the increase in viscosity of the product. They established that the addition of CLA (0.25-0.75 %) independent of its form had no significant influence on the odour, taste, textural properties and acceptability of the fermented milk.

CLA-enriched ripened dairy products

Various specific health effects due to a consumption of cheese enriched in CLA have been proven. Murru *et al.* (2018) demonstrated that CLA-enriched cheeses, consumed for different periods of time, were an alternative nutritional source of omega-3 highly unsaturated fatty acids in humans. However, CLA-enhanced cheese demonstrated less firmness as compared to the control sample (Jones *et al.*, 2005).

The effects of pasture-based versus indoor TMR feeding systems on the chemical composition, quality characteristics, and sensory properties of full-fat Cheddar cheeses were investigated (O'Callaghan *et al.*, 2017). Pasture-based feeding systems demonstrated a greater 2-fold increase in the concentration of the bioactive conjugated linoleic acid C18:2 *cis*-9, *trans*-11, in comparison with TMR-derived cheeses. This alteration in cheese fatty acid profile resulted in reduced hardness scores at room temperature but enhanced nutritional quality. Mele *et al.* (2011) characterized the composition and the oxidative stability of lipids from Pecorino cheese enriched with CLA (*cis*-9, *trans*-11 CLA, *trans*-11 C18:1 and alpha-linolenic acid was increased by 290%, 197% and 250%, respectively), obtained by supplementing the diet of dairy ewes with extruded linseed. Van Nieuwenhove *et al.* (2007a) stated that ripening and sunflower oil supplementation showed a positive influence on the CLA content in ripened buffalo cheese samples produced by adjunct starter cultures (*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*). Texture and colour were similar in both types of cheese. Luna *et*

al. (2005) found that sensory characteristics of cheeses made with CLA-enriched milk (ewes fed with linseed supplements) did not substantially differ from those made with non-supplemented ewes' milk. CLA total content and isomer profile did not change during ripening. CLA-enriched sheep cheese had no appreciable changes on sensory characteristics in comparison with the control sample (Renes *et al.*, 2019).

Other CLA-enriched dairy products

A recent study evaluated the effects of three widely practiced cows feeding systems in the United States, Europe, and Southern Hemisphere regions on the characteristics, quality, and consumer perception of sweet cream butter (O'Callaghan *et al.*, 2016a). It was found a significantly higher concentration of conjugated linoleic acid (*cis*-9, *trans*-11) in butter produced from pasture-derived milks than TMR butter. They observed alterations in the fatty acid composition of butter contributed to significant differences in textural and thermal properties of the butter. Jones *et al.* (2005) also found less firm CLA-enhanced butter as compared to control product. Gonzalez *et al.* (2003) established that the solid fat

index of high-oleic and high-linoleic milkfat in CLA-enriched butter was lower than the control.

CLA content of fortified milk powder was affected by refrigerated storage and thermal treatment (Rodríguez-Alcalá and Fontecha, 2007).

Few researches about ice cream CLA-fortification were published (Gonzalez *et al.*, 2003). They found that control ice cream mixture had higher viscosity compared with high-oleic and high-linoleic, but the firmness of all ice creams was similar when measured between -17 and -13°C. Enriched anhydrous milk fat (AMF) and fractions (at 25, 20, 15, 10, 5, 0 and -5°C) showed CLA increase by 31% in enriched AMF and 59% after fractionation (liquid fraction at 0°C) when compared to control samples (Herrera-Meza *et al.*, 2012). According to the authors this new technology, called "dry fractionation", was an inexpensive chemical-free process that may be used to produce CLA-rich products such as butter, cream, cookies and bread, in a way that would not be possible with conventional dairy processing equipment.

Figure 3 represents some examples of CLA-enriched dairy products

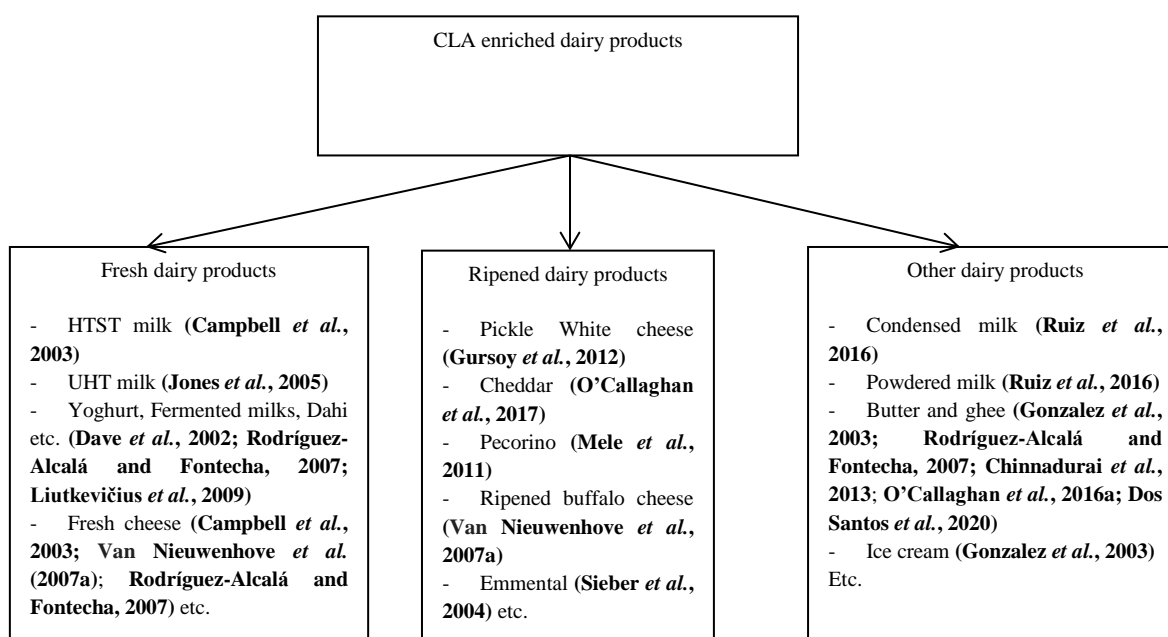


Figure 3 Examples of CLA-enriched dairy products

POTENTIAL HEALTH BENEFITS OF CLA-ENRICHED DAIRY PRODUCTS

Gama *et al.* (2015) findings suggested that dairy products enriched in *cis*-9, *trans*-11 CLA may be useful in the treatment of and respectively correlated significantly with memory enhancement. Biglu *et al.* (2017) demonstrated by meta-analysis that milk enrichment with CLA was an effective action against cancer. Limong *et al.* (2018) studied the impact of cheese rich in CLA on LDL-Cholesterol by dietary intervention in older people. They found that participants who consumed Pecorino cheese, rich in CLA did not register an increase in lipid levels. Pintus *et al.* (2013) proved that sheep cheese naturally enriched in α -linolenic, conjugated linoleic and vaccenic acids improved the lipid profile and reduced anandamide in the plasma of hypercholesterolaemic subjects. Mele *et al.* (2011) established that changes in lipid composition did not increase the amount of cholesterol oxidation products and slightly increased the thiobarbituric acid-reactive substances. This suggestion demonstrated that CLA-enriched cheese did not represent any potential risk related to increased intake of lipid oxidation products. Lordan and Zabetakis (2017) discussed the anti-inflammatory properties of CLA-enriched milk, yogurt, and cheese. Barros *et al.* (2017) evaluated the effect of diet CLA-enriched butter on intestinal damage and inflammatory response and stated that this product exacerbated the 5-FU-induced intestinal damage. Penedo *et al.* (2013) stated that intake of butter naturally enriched with *cis*-9, *trans*-11 conjugated linoleic acid reduced systemic inflammatory mediators in healthy young adults. Chinnadurai *et al.* (2013) proved that high CLA-enriched ghee/clarified butter (19.54 mg/g fat) increased the antioxidant and antiatherogenic potency in female Wistar rats. Ritzenthaler *et al.* (2005) proved that consumption of conjugated linoleic acid (CLA) from CLA-enriched cheese did not alter milk fat or immunity in lactating women. According to Rodríguez-Alcalá *et al.* (2013) CLA-enriched milk powder reversed hypercholesterolemic risk factors in hamsters. Recent research demonstrated that the dietary *cis*-9, *trans*-11-conjugated linoleic acid enriched

from butter reduced breast cancer progression *in vivo* (Zeng *et al.*, 2020). Stefanson *et al.* (2014) have found that feeding butter with elevated content of *trans*-10, *cis*-12 conjugated linoleic acid to lean rats did not impair glucose tolerance or muscle insulin response.

CONCLUSION

The most recent data concerning CLA-enriched dairy products were presented in this review. While there are a lot of data for the possibilities to enrich dairy products with CLA, this area needs further investigations on some issues. Further studies are needed to determine the most appropriate group of dairy products for CLA enrichment. Additional studies should be performed to determine new antioxidants, their respective concentrations and methods for prevention of CLA oxidation. CLA content variations of different dairy products enriched with CLA remain still not completely clear. It is still unknown why some strains of microorganisms produce greater amounts of CLA than others. The influence of the substrate in CLA formation must be further clarified. There is a need of more recent findings in the physiological effects of nutritionally desirable CLA and its isomers, presented in dairy enriched products, on human organism. There is a need of improved knowledge about the improvement of milk fat composition through various factors as feeding regime, production system, breed, or stage of lactation. All of these concerns are in correspondence with the requirements of the growing market of functional foods.

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