Conjugated Linoleic Acid: Biosynthesis, Benefits for Human Health and Its Contents in Milk and Milk Products

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Abstract: Conjugated linoleic acid (CLA) , this acid belongs to a family of geometric and positional isomers of linoleic acid. Unlike natural fatty acids, its double bonds are conjugated which is defined for the presence of alternating double-single-double bonds in the carbon structure. Dairy products are the major source of CLA. This article dealt with the presence and healthy benefit of CLA in milk and its dairy products.
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Introduction

Conjugated linoleic acid is a collective term for a mixture of geometric and positional isomers of octadecanoic acid (18: 2) in which double bonds are conjugated rather than methylene separated as they are in linoleic acid (18: 2, cis-9, cis-12); **(Saliminen *et al.*, 1998).While Chin *et al.* (1992)** defined CLA as a group of fatty acids found predominantly in the milk of ruminant animal such as cow, goats and sheep s. While **Lawson *et al.* (2001)** considered CLA as generic term used to describe positional and geometric isomers of octadecadienoic fatty acids containing conjugated double bonds. They mentioned that, milk and dairy products are the predominant sources of CLA in the rumen diet. However **Lin *et al.* (1995**), considered Conjugated Linoleic acid 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 the Linoleic acid derivatives with cis-9, trans-11-; trans-9, cis-11; trans-9, trans-11-; trans-10, trans-12; trans-10, cis-12 and cis-11, cis-13-octadecadienoic acids as the minor isomers. However, only the isomers that contain trans double bonds are biologically active. Later identifying cis-9, trans-11 CLA, as a milk fatty acid (FA) that contained a conjugated double bond pair. Subsequent research established that, cis-9, trans-11 CLA was the major CLA isomer in ruminant fat which representing about 75-90% of the total CLA, **(Parodi, 2003)** and **(Bauman *et al.*, 2003).** The common name of rumenic acid has been proposed for this isomer because of its unique relationship to ruminants, **(Kramer *et al.*, 1998)**.The term conjugated linoleic acid refers to this whole group of 18 carbon conjugated fatty acids. Alphalinolenic acid goes through a similar biohydrogenation process producing 18:1 trans-11 and 18:0 but does not appear to produce CLA as an intermediate. CLA is a generic term used to describe the isomers of linoleic acid (LA), which is an essential fatty acids found in appreciable amounts in food products derived from ruminant animals, especially in the liquid fraction of milk, **(Collomb *et al.* (2006).**

The Conjugated Linoleic acid is a polyunsaturated fatty acid (PUFA) which comprises a group of positional and geometric isomers derived from Linoleic acid ($C_{18:2}$ n-6). This acid is produced by bacterial fermentation in the digestive tract of ruminant animals, therefore, its main sources are milk and its derivatives, **(Evans *et al.*, 2002).**

Many studies had been performed on the effect of different dietary regimens whish have done on the fatty acids (FAs) profile and CLA of milk fat in cows **(Stanton *et al.*, 2003)** and goats milk **(Chilliard *et al.*, 2006).** Despite of the facts that, the effect of lipid supplement on milk fat synthesis bear some similarities across ruminant species, often goat's and ewe's respond differently to cow's, **(Chilliard *et al.*, 2003).** Furthermore, there was a little information about the consumer acceptability and sensory attributes of CLA enriched milk and cheese made from cow's milk **(Luna *et al.*, 2005).**

Dairy products are the major source of CLA in US diets which represented 60-70%, **(Ritzenthaler *et al.*, 2001)** and the major CLA isomer in milk fat is cis-9, trans-11 CLA which represented 75-90% **(Bauman *et al.*, 2003).**

CLA was found naturally in food products from ruminants refers to a mixture of positional and geometric isomers of linoleic acid (C-9, C-12, 18:2) with two conjugated double bonds at various carbon positions in the FA chain. Each double bond can be cis or trans, but those with one trans double bond are bioactive, **(Jensen, (2002).** It is formed as on intermediate during the biohydrogenation of linoleic acid to stearic acid in the rumen by butyrivibrio fibrisolven, **(Kepler and Tove, (1967),** and other rumen bacteria, **(Kritchevsky, 2000).**

On the other hand, **Lin *et al.* (2005)** comprised a mixture of positional and geometric isomers of octadecad-ienoic fatty acid with conjugated double bonds. These conjugated dienes were found to be responsible, while **Lin *et al.* (1995)** reported that there are mixtures of octadecadienoic acids that have been recently recognized as anti-carcinogens. These compounds are linoleic acid (LA) 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 accounting for the major isomers and cis-9, cis-11; cis-10, cis-12; cis-10; trans-12 and cis-11, cis-13-octadecadienoic acids accounting for minor isomers. **Kepler and Tove, 1967** showed that, cis-9, trans-11 18:2, (the major isomers of CLA) is the first intermediate formed in the biohydrogenation of linoleic acid by the rumen bacteria, butyrivibrio fibrisolvins. This initial reaction involves the isomerization of the cis-12 double bond to trans-11 by cis-9, trans-11 isomerase. The next step in the conversion of this diene to the trans-11 monoene (trans-11 18:1). These initial steps occur rapidly. The conversion of trans-11 (18:1) to (18:0) appears to involved a different group of organisms and occurs at a slower rate, **(Griinari *et al.*, 1997).**

Kennely and Bell, (2003) added that although the cis-9, trans-11 is the predominant CLA isomer in bovine milk, other isomers can be formed with double bonds in position 8/10, 9/11, 10/12 or 11/13. Each of these double bonds can be in a cis or trans configuration giving a range of possible CLA isomers.

In other words, the double bonds are located every two carbons **(O'shea *et al.*, (1998); Bauman *et al.*, 1999).** Near 20 different positional and geometric isomers of CLA have been reported. Those isomers have several positions for the double bonds in the 18-carbon chain. Some of these geometric configurations are: cis-trans, trans-cis, cis-cis and trans-trans, **(Sehat *et al.*, 1998).**

The presence of CLA in milk fat was early first noted in the 1930s by scientists at the University of Reading, United kingdom, **(Booth *et al.*, 1933, Moore 1939** and **Parodi 1977**). Several studies had suggested that, there may be little accumulation of CLA in the rumen. Although, it is accepted that, CLA is formed in the rumen, there is good evidence that much of the cis-9, trans-11 CLA found in bovine milk is actually synthesized with the mammary gland from 18: 1 trans-11 **(Griinari and Bauman, 1999)**.

Sheep milk is rich in CLA isomers which are melodically active components influencing human health. There are four geometric CLA isomers pairso: cis, trans; tans-cis; trans, trans and cis, cis. **(AgnieszkaRozbicka- Wieczorker, *et al.*, 2013).**

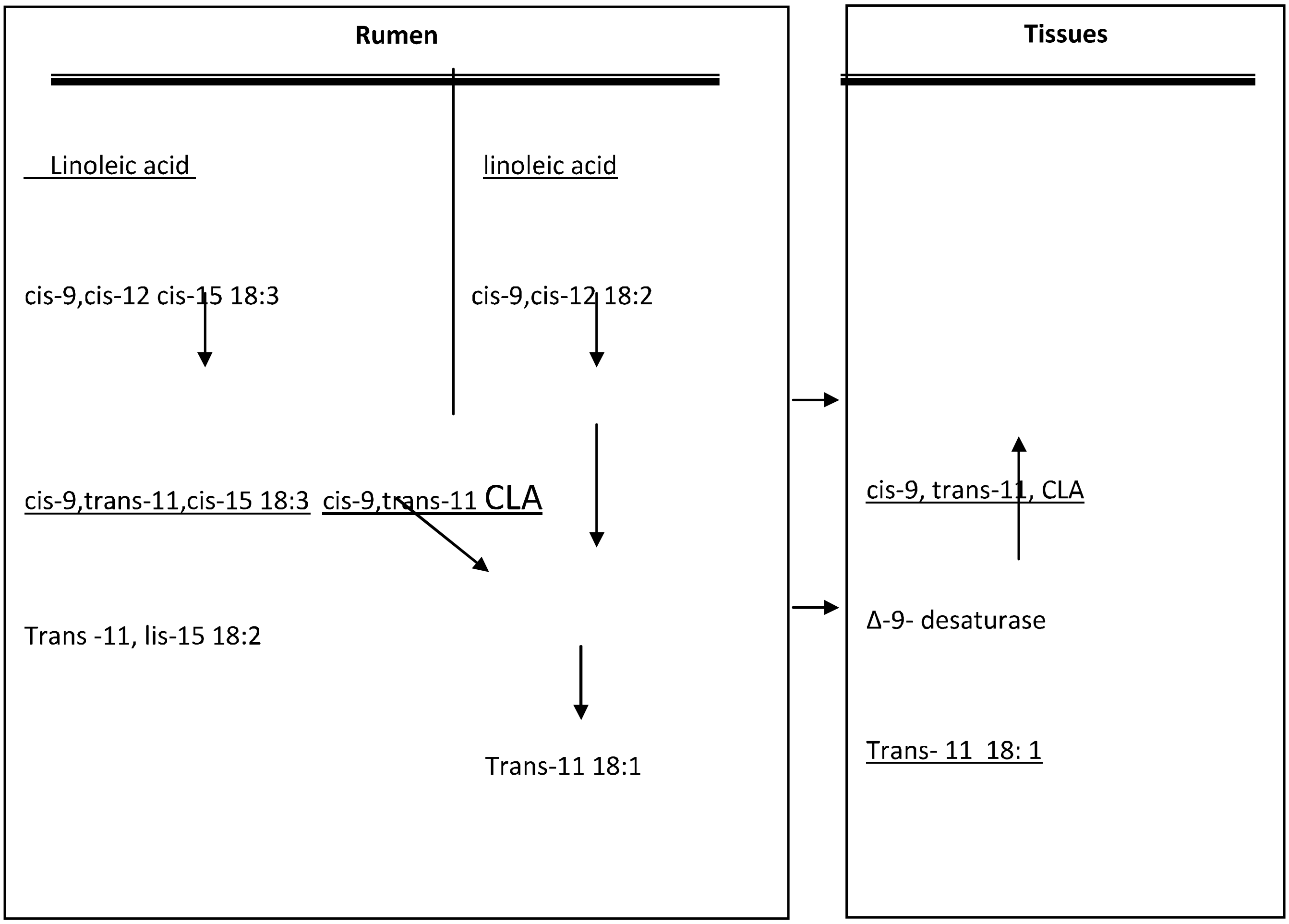
Milk from ruminants therefore contains more CLA than that of non-ruminants. The amount of CLA found in whole milk is generally about 4.5 to 5.5 mg/g. fat, although variation of a much as 2.5 to 18 mg/g. fat has been reported, **(Chilliard *et al.*, 2001).**

Milk from ruminant animals has been an important component of the human diet for thousands of years. Ruminants have the unique ability to take organic material that is indigestible to humans and convert it into milk, a food with high nutritional value. Ancient civilizations learnt to explain this potential through the domestication of various species of ruminant including the cow, sheep, goat, buffalo and camel animals. Strategies that alter the basic composition of milk are a much more recent endeavor, encouraged by the rapid advancement of scientific knowledge over the past century, **(Kennelly and Bell, 2003).**Thus, CLA concentrations is particularly high in milk from ruminants where it can reach a value of 0.65% of total lipids, **(Fritsch and Steinhart, 1998).**

Mechanism of Conjugated Linoleic Acid Formation in Rumen and Mammary Glands

Conjugated linoleic acid, a natural component of milk fat is produced in the rumen during the formation of stearic acid because of the microbial biohydrogenation of polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acids, **(Kepler and Tove, 1997).**

Various researchers were reported the mechanisms of CLA formation in rumen and mammary glands but, the major pathways for rumen biohydragenation of linoleic and linolenic acid are shown in (Fig 1) as reported by **(Bauman *et al.*, 2003).** The metabolic pathway proposed for the formation of CLA isomer includes the isomerization and biolydrogenation of unsaturated fatty acids by rumen bacteria and the desaturation of vaccenic acid (C 18:1, trans-11) by Δ- desaturase in the mammary gland, the incomplete biuohydrogenation of linoleic acid in the rumen provides the surplus of CLA found in milk fat, (**Collomb, *et al.*, 2006**). It can be noted that, cis-9 trans-11 CLA is the first intermediate in the pathway and it is formed only during the biohygdrogenation of linoleic acid. In contrast, vaccenic acid is an intermediate formed from both linoleic and linolenic acids. Vaccenic acid and CLA are both present in ruminant fat and it was generally assumed, they were of ramen origin and represented intermediates that had escaped complete biohydrogenation.



(Fig.1): Pathways for raminal and endogenous synthesis of cis-9, trans-11 ClA in dairy cow. (Bauman et. al. 2003)

However, cis-9 trans-11 CLA is only a transitory intermediate in rumen biohydrogenation, whereas vaccenic acid tends to accumulate based on this and other considerations. **(Griinari and Bauman, 1999)** proposed that, endogenous synthesis could be an important source of cis-9, trans-11 CLA in milk fat with synthesis involving the enzyme Δ-9- desaturase and vaccenic acid as the substrate. The importance of endogenous synthesis of cis-9, trans-11 CLA had been examined in a serious of in vivo investigations encompassing a range of diets characteristic of lactating dairy cows, **(Bauman *et al.* 2001).** Although 18:2, C-9, trans-11 is the major CLA formed in the rumen from linoleic acid in the diet, other CLA can be produced when X- linoleic acid or trans fatty acids are present in the animal diet, **(Salminen *et al.*, 1998).** Studies have shown that, the intestinal bacteria of rodents are capable of converting unsaturated fatty acids to conjugated linoleic acid, **(Chin *et al.*, 1994).** Processing has little effect on CLA, so the content in food products in related to CLA concentration in the starting fat, **(Parodi, 2003).**

Potential Benefits of Conjugated Linoleic Acid on Human Health and Its Intake

Conjugated linoleic acid (CLA) is a component of milk fat that has been shown in recent years to have numerous potential benefits. Interest in the levels of CLA in human diet have increased in the recent years because of accumulating evidence largely based on animal studies which suggest potential health benefits of CLA, **(Banni and Martin, 1998).** Anti-carcinogenic properties of CLA have been reported against rodent mammary and colon cancer models as well as in vitro models of human melanoma colorectal and breast cancer. Potentially beneficial actions on body composition and on immune function have also been reported. Less consistent have been reported of putative hypocholesterdaemic, antiatherogenic actions of CLA, **(IpC. *et al.*, 1994 and** Kennelly **& Bell, 2003).** It is interesting to highlight that, there is natural presence of CLA in the lipid fraction of milk, which has been regarded as beneficial for the human health. Such benefits include its cancer prevention properties **(Belury, 1995),** positive effects on the cardiovascular system (**Nicolosi *et al.*, 1997).** Furthermore, in a study conducted in Colombia, it was reported, that CLA also has positive effects on prim gravid women with risk of preeclampsia.

Biomedical studies with animal models had shown that, this isomer cis-9,trans-11 CLA is produced as an intermediate in the rumen biohydrogenation of linoleic acid but not of linolenic acid. However, it is only a transient intermediate, and it is the major source of milk fat from endogenous synthesis, **(Lock and Bauman, 2004).** The intake of CLA in humans is of interest because of the potential health benefits , these fatty acids may confer.

As biomedical studies with animal models expanded in scope, an impressive range of additional health effects were discovered for CLA, including anti-diabetogenic, antiatherogenic, immunomodulation, anti-obesity and modulation of bone growth**, (Belury, 2002).** The anti-obesity effects of CLA are due to the trans-10 cis-12 isomer; while this isomer can vary in milk fat, it never represents more than, 1 or 2% of total CLA, and food products derived from ruminants are this isomer to have biological effects on body fat **(Belury, 2002).**

Biomedical rehearses using animal models had also identified many beneficial health effects of CLA. In particular, the cis-9, trans-11 CLA isomer has been identified as a potent anti-carcinogen for many types of cancer, **(Belury, 2002).** Whil **Banni *et al.*, 2003** has been shown to prevent development and cause regression of atherosclerotic lisions in animal models for Coronary Heart Disease (CHD) **(Toomy *et al.*, 2003).** Consequently, surveys indicated a consumer interest in food products that are enriched in CLA **(Lynch *et al.*, 2005).**

Human diet comprises of milk and milk products in both developed and developing parts of globe. Milk fat is the major energy sources in Indian diet but due to the fear of hypercholesterolemia, saturated fats have led to avoidance of dietary fats especially of animal origin. However, milk contains a number of components with a beneficial properties, one such compound associated with the fat phase is conjugated linoleic acid (CLA) which has potential health benefits toward human beings, **(Chinnadurai and Tyagi, 2012).**

The levels of CLA intake that would result in beneficial properties is 3g/d. as extrapolated from animal studies, **(IPC *et al.*, 1994).** However, **Fritsche and Steinhart, 1998** reported that, CLA intake for German men and women to be only 350 and 430 mg/d respectively.

Cis-9, trans-11 and trans-10, cis-12 are the two isomers with known physiological importance including anticarcinogrenic, antidiabetic, antilipogenic and antiatherosclerotic effects. Positive effects of CLA on immune function and bone modeling have also been reported. by **Khanal, 2004.**

Biologically active of CLA isomers (cis -9, trans-11), octadecadienoic acid, (C 18: 2 cis-9, trans-12) have shown beneficial effects on human health and can be considered as therapeutic nutrients with protective effects against various common diseases such as obesity, atherosclerosis, chronic inflammatory and cancer diseases, **(Khanal, 2004; Parodi, 2004).** According to the published reports, the recommended CLA intake in order to achieve beneficial effects on health ranges between 0.7 and 6.8g. /day,**(Belury, 2002).** Interesting health benefits have been attributed to the intake of conjugated linoleic acid (C 18:2, cis-9, trans-11) which is the main isomer of linoleic acid is present in bovine milk. Among those benefits are: cancer, prevention and cardiovascular diseases. Although an adequate nutrition of cows has permitted to increase the amount of CLA in their milk, there is variation in CLA concentrations among cows consuming the same diet**, (Julian *et al.*, 2010).**

Beneficial fatty acid content in milk fat from grazing sheep usually shows two nadirs, one in winter, at the beginning of lactation, when the herbage on offer is usually low and one at pasture maturing phase. In early lactation period, diet supplementation with grain based concentrates is widespread in sheep farms. However, the use of this kind of supplements after brings about the depression of some beneficial FA of the resulting dairy products. Maximizing the nutritional value of milk and cheese fat can be achieved by the addition of whole oilseeds or plant oils rich in linoleic and/or linolenic acids, the main precursors of milk PUFA, CLA and C18:1, trans-11 **(Dhiman *et al.*, 2000).**

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, yoghurt, 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)**.

Some Factors Affecting Conjugated Linoleic Acid Contents in Milk and Dairy Products

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).**

The contents of CLA in milk and dairy products are affected by different factors such as breed, age of dairy cow, stage of lactation, seasonal variation, feeding (green and posture). Some researchers had reported the variation associated with breed. W**hite *et al.*, 2001** found that, Holstein cows tended to have a higher concentration of CLA in their milk than Jersey cows.

In another study, milk from Brown Swiss cows was reported to contain more CLA than Holstein milk, although Brown Swiss appeared to be less responsive to dietary manipulation, **(White lock *et al.*, 2002).** Variation in Δ-9 desaturase may explain much of this difference between breeds. Age of the dairy cows and stage of lactation may also influence the milk CLA content to some degree but the effect of these parameters has not been well characterized. Seasonal variations on milk CLA have been known for some time and these appear related to this. Fresh pasture resulted in a two to three fold increase in CLA of milk fat, but the effect diminishes as the pasture matures **(Stanton *et al.*, 1997).** The effect of breed (Holstein vs. Brown Swiss) was examined in a large study and no differences were observed. Although some have proposed breed differences in CLA content of milk fat, **(Lawless *et al.*, 1999),** These, if breed differences; do exist in CLA content of milk fat and desaturase index, they must be minor compared with the effect of diet and individual animal variations **(Palmquist *et al.*, 2004).**

On the other side, in dairy products, the amount of CLA present varied according to the breed, feeding conditions and subsequent processing. In milk fat, CLA content had been shown to vary from 0.24 to 2.8% from winter to summer, reflecting the effect of pasture feeding on linoleic acid intake and subsequent CLA accumulation in the rumens, **(Chin *et al.*, 1992).**

Furthermore, variation in CLA content in milk of cattle and sheep has been associated with several factors such as stage of lactation, parity, **(Kelley *et al.*, 1998)** and breed, **(Secchiari *et al.*, 2001), (White *et al.*, 2001).** However, diet is the most important factor infloucing milk CLA concentration, **(Bauman and Griinair, 2000)**, **(Chilliard *et al.*, 2000).** In particular, the CLA concentration is higher in milk from animals fed pasture than those fed dry diets, **(Dhiman *et al.*, 1999),** and decreases with increasing growth stage of forage or maturity, **(Chouinarel *et al.*, 1998).** Such a relevant effect of the diet could be partly responsible for the higher milk CLA content in sheep in comparison with cow, **(Banni *et al.*, 1996).**

To date, studies have indicated that, the diet of the ruminant animal has a significant effect on CLA levels in milk fat. Lipid substrates that have been added to the bovine diet and proven to be successful for enhancing CLA concentration in milk fat include plant oils rich in PUFA and fish oils rich in long-chain PUFA the former through enhancement of biohydrogenation substrates, and later by affecting pathways of rumen biohydrogenation, **(Chilliard *et al.*, 1999), (Dhiman *et al.*, 2000).**

The concentration of CLA in bovine milk fat could vary quite substantially depending on the feeding strategy adopted. For instance pasture feeding has been found to result in a much higher milk fat CLA concentration (TMR) based on conserved forage and grain, **(White *et al.*, 2001).**

Milk fat is the richest natural dietary source of CLA. Milk contains an range 4.5mg. CLA/g. fat **(Kelley *et al.*, 1998) .** Recent studies had shown that, CLA content of milk fat can be markedly enhanced by dietary manipulation especially those involving dietary addition of plant oils which are high in unsaturated fatty acids, (**Griinari and Bauman, 1999**).

So, diet is the major determinant of milk CLA content and over the last decade, numerous experiments had been carried out with the objective of enhancing milk fat content of CLA, (**Bauman *et al.*, 2001).**

Physical factors that affect milk fat content of CLA had also been examined striking. Even when diet and other physiological variables are similar, there is still a two to three fold range among individuals in the milk fat concentration of CLA **(Peterson. *et al.*, 2002).**

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Dietary factors that affect rumen bacteria involved in biohydrogenation either directly or indirectly via changes in the rumen environment can also affect CLA content of milk fat. A combination of dietary supply of PUFA and modification of rumen environment can be especially effective in increasing CLA content of milk fat, (Bauman ***et al.***, 2001). Also diet can markedly affect milk fat CLA content and there were also substantial differences, among individual cows, **(Lock and Bauman, 2004).**

Finally, diet has a major effect on milk fat content of CLA and is also a wide variation among individuals through modification of dairy cow diets.

(Dhiman *et al.*, 1999), reported that CLA concentration of milk to be 22.1mg/g. fat with pasture feeding compared to 3.8 mg/g. fat with TMR feeding. **Kelley and Bauman, 1996** supplemented the basal diet with 53g/kg dry matter (DM) of peanut oil (high oleic acid), sunflower oil (high linoleic acid) or linseed oil (high linolenic acid).

Dietary intake levels of CLA: The ability to quantity CLA content of human diet had previously been limited by lack of accurate data on CLA content of animal food products produced under varying conditions had become available allowing estimates of human intakes to be made, **(Chin *et al.*, 1992).** This study also measured adipose tissue CLA content which was shown to significantly correlate with both dietary CLA content and milk intake, **(Williams, 2000)**.

CLA content of whole milk varies from 0.30-0.55g/l00g fatty acids. The CLA content of milk products from ruminants can be increased through manipulation of the diet such as grazing on posture or feeding feed source rich in linoleic and linolenic acids, **(Jahries *et al.*, 1997) and (Lock and Garns worthy, 2002).**

The predominant source of CLA in human diets is ruminant – derived food product, with dairy products contributing about 75% of the total, **(Ritzenthaler *et al.*, 2001; Parodi, 2003).**

CLA concentrations were 13.3, 24.4 and 16.7 mg/g. milk fat respectively. The increase in CLA levels observed with the sunflower oil treatment represented levels approximately 500% greater than those typically seen in traditional diets, **(Chouinard *et al.*, 1998),** fed diets supplemented with 4% DM of calcium salts of fatty acids form canola oil, soybean oil, or linseed oil. The resulting milk CLA concentrations were 13.0, 22.0, 19.0 mg/g. fat for canola oil, soybean oil and linseed oil respectively, and 3.5 mg/g. fat for control. Soybean oil which is high in linoleic acid was most effective at increasing CLA. It appears that, the availability of the oils to the rumen microbes is an important determinant of subsequent CLA production. **(Chouinard *et al.*, 2001)** showed that, processing soybeans especially by extrusions increased milk CLA above that obtained by feeding ground soybeans.

The amount of CLA and type of CLA isomers produced as a result of feeding supplemental fat; varied to large extent depending on the ruminal conditions. A study at Cornell University using supplemental fat, found that CLA levels in milk were halved when the forage: concentrate ratio of the diet was changed from 50: 50 to 20: 80, **(Kelley and Bauman, 1996).** Dietary fish oil supplementation had also been found to increase the concentration of CLA in bovine milk from 0.2-0.6 % in control diets to 1.5-2.7% in supplemented diets, **(Chilliard *et al.*, 2001).**

Moreover, studies using fish supplementation that reported milk CLA values showed that, the increase in CLA was almost exclusively in cis-9, trans–11 isomere, **(Chilliard *et al.*, 1999; Offer *et al.*, 1999).** Feeding fish oil in combination with a source of 18:2 or 18:3 would therefore be expected to increase level of milk CLA much more than would be achieved with 18:2 or 18:3 alone. Butter from milk containing these higher than average levels of CLA and other polyunsaturated fatty acids (PUFA) is softer and the flavor characteristics of both milk and Butter appear to be unchanged by altering milk fatty acid profile, **(Baer *et al.*, 2001) and (Ramaswamy *et al.*, 2001).**

Decandia *et al.* (2007), studied the effect of different systems on fatty acid composition and volatile compound content in goat milk, thin survey was carried out on three Sarda goat herds during the milking period, with the aim to investigate the effect of different feeding systems on milk composition, with particular emphasis to fatty acid and volatile compound content. Individual milk yield and composition were different in all herds ($P > 0.05$), as well as the bulk milk FAs composition, particularly long chain fatty acids (C 17: 0 – C 18:3). The levels of CLA (C 18:2, cis-9, trans-11) and vaccenic acid (C 18:1, trans-11) were higher when pooling all data, a relationship was found between CLA and vaccenic acid. The feeding system based on pasture confirmed effect on CLA content in milk, in particular when the pasture in characterized by a high proportion of herbages with high botanical variability, **(Cabiddu *et al.*, 2005).**

The CLA content of raw milk itself could affect by feeding and these changes could be significant. The CLA levels could be influenced by several measures: feeding of fresh pasture (Kelley et al., 1998); addition of oils such as rapeseed, sunflower or soybean, **(Dhiman *et al.*, 1999).** Milk produced by grass-fed cows had a higher content of CLA than the milk of cows fed low forage diets, **(Singh and Sachan, 2011).**

Conjugated Linoleic Acid Contents in Milk and Its Products

Conjugated linoleic acid is widely pound in many foods, including dairy products. Contents of CLA in animal products are much higher than in plant oils.

Among the animal products, CLA contents are generally higher in ruminant tissues than no ruminant tissues; and dairy products are recognized as major dietary sources of CLA. Its contents of most dairy products range from 2.5 to 7.0 mg/g of lipid, **(Lin *et al.*, 1995).** Most of dairy products contain different CLA isomers of which 85% to 95% consist of rumenic acid (cis-9, trans-11), octadecaolienoic acid, (C18:2, cis -9 trons-11) in amounts ranging from 6 to 16 mg/g. of total fat. CLA content of dairy products is decreased by processing, storage or cooking and hence, its concentration in food depends primarily on the concentration in the raw materials. That CLA is produced in the rumen during the biohydrogenation process has been known for a long time **(Kennelly and Bell, 2003).**

CLA enriched dairy products have been evaluated for taste, organoleptic properties and storage characteristics in comparison with standard dairy products. Off – flavors due to fatty acid oxidation are of prime concern because of the shift toward greater instauration of milk fat. Post pasteurization indicated no differences in sensory and triangle fast testes. Similar results had been observed by others with milk that had a less marked enrichment of CLA **(Ramaswamy *et al.*, 2001).**

CLA in dairy products which represents the major source in human diet, is present primarily as 18:2, C-9, trans-11 conjugated fatty acids are formed as partial hydrogenation products during raminal hydrogenation by the action of specific bacterial isomerases and industrial hydrogenation by the action of hydrogen in the presence of catalyst: the latter reactor is essentially similar to auto oxidation-mediated conjugated reactions where free radical reactions lead to shifting and recombination of double bonds adjacent to the site of free radical attack, **(Ramaswamy *et al.*,2001).**

The key to increasing milk-CLA is to increase rumen vaccenic acid output allowing for increased endogenous synthesis in the mammary gland. Maximizing rumen output of vaccenic acid can be achieved in two ways; by increasing the supply of, 18-carbon PUFA precursors and by inhibiting vaccenic acid reduction to stearic acid. Increasing the dietary supply of 18-carbon PUFA substrates is most easily achieved by the addition of plant oils high is linoleic and or linolenic acids.

The effects of different types and amounts of plant oils had been investigated and a range of plant oils had been shown to be effective in increasing milk CLA content. Plant oils are often added to dairy cattle diets as calcium salts of free fatty acids (FFA) and fed calcium salts of canola, soybean and linseed oil. All three oils increased CLA content of milk fat: however, those containing the greatest amounts of linoleic and linlenic acids; (Soybean and Linseed respectively) caused the greatest increases, (**Chouinard *et al.*,2001**).

Cheeses: in the conversion of the milk into cheese, lactic acid bacteria and in some cases propionibacteria are used as starter cultures. The CLA content of cheeses ranged from 3.6 to 8 mg/g of total fat. Blue. Bine, Edam and Swiss cheeses had significantly higher CLA content than other cheeses and yoghurt has a content similar to that of whole milk (**Lin *et al.*, 1995**). Cheese from both oilseeds supplemented groups (C 18:2 and C 18:3) showed similar and significantly higher levels ($P < 0.01$) of both C 18:1 trans-11 and CLA cis-9, trans-11, than other cheeses, as a result of the high content of CLA precursors in oilseeds based concentrates (**Jahries *et al.*, 1997**)..

The major sources of CLA in the human diet are in milk and its products particularly cheese. Unlike the situations with total fatty acids, margarines and oils make only a very slight contribution to total CLA intakes, **(Fritsche and Steinhart, 1998).** Cheese production offers a range of processing conditions, (temperature, whey proteins, ripening time) which may influence the final CLA content since some result in enhanced production and others increased breakdown of CLA. However, studies suggested the major determinate of CLA content of various natural cheeses is CLA content of the raw materials. Overall, it seems that feeding, rather than processing conditions, controlled CLA content of foods, **(Fritsche and Steinhart, 1998).**

The investigators, (**Jiang *et al.*,1998, Lin *et al.*, 1999; kim and Liu, 2002**) 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 after be different and some conditions of processing and ripening could influence the CLA content of cheeses. It is known that, there is high variability in the content of CLA in bovine milk. This occurs for animals of the same breed and even under the same diet. It has been suggested that, the causes for these variations are charge in the activity of straroyl-CoA desaturase (SCD), the gene coding for the engine and the biohydrogenation process, **(Peterson *et al.*, 2002**). The level in dairy products of substances beneficial to consumer health such as PVFA, CLA cis-9, trans-11 and vaccenic acid (C 18:1, trans-11) increases along with the grass supply, (**Jensen, 2002**).

More extensive investigations had to show if, during yoghurt manufacture and cheese ripening, lactic acid bacteria and porpionibacteria are able to produce CLA in sufficient amounts to be of physiological importance for human nutrition. This should especially in cheeses which show high lipolytic activity (e.g. Blue cheese varieties) and as a result, a high content of free linoleic acid. 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 lacotocoecus lactis, (**Kim and Liu, 2002).**

In the conversion of the milk into cheese, lactic acid bacteria and in some cases propionibacteria are used as starter cultures. The Judicious of suitable stains of lactobacilli, lactococci, streptococci can increase the CLA content of cheese. Indeed, estimated as free linoleic acid can serve as a substrate for CLA production by lactobacillus lactis, (**Kim and Liu, 2002**). In the case of goats the range of CLA content in milk ranged from 6.4 to 7.9 mg/g (**Nudda *et al.*, 2003). Siber *et al.*, (2004**) indicated that; during ripening, mainly in Emmental and Blue cheese, CLA can be formed from linoleic acid through the action of the primary or secondary enlarges including the propionibacteria in Emmental. It is also possible that, the released linoleic acid could be converted to CLA very quickly without inhibiting the growth of propionibercteria. The conjugated linoleic acid (CLA) content of cheeses ranged from 3.59 to 7.76 mg/g of lipid, Blue, Brie, Edam and Swiss cheeses had significantly higher CLA content than the other cheeses. Sharp cheddar cheeses tended to have higher CLA content than the medium cheeses, but the increase was not significant.

Mean fat content in cheeses (Referring to Total solids) made from the milk of ewes fed the enriched and the control rations did not change during the ripening period. Total CLA and other potentially healthy fatty acid levels in ripened cheese were similar to those found in raw milk. Overall, modifications observed as a consequence of cheese manufacturing were less relevant than changes coursed by ration fat supplementation, **(Addis *et al.*, 2005**). Since the processing of milk into cheese did not substantially after the CLA content, the most practical approach for achieving cheeses with high concentrations of CLA would consist of producing ewes milk with a high CLA content by modifying the ration of these animals, (**Nudda *et al.*,2005).**

Cabildu *et al.* (2005), investigated the relationship between feeding regimen and content of CLA in sheep milk and cheese during the years 2000-2002 in South Sardinia on 15 dairy sheep farms characterized by different pedological conditions, pasture composition and supplementation levels. Data showed that, the level of CLA in milk was high (18.46 ± 0.93 mg/g-fat), when the flocks grazed pastures at vegetable phase while it dropped down to (13.34 ± 1.67 mg/g fat) during pasture reproductive affected the milk CLA content. Similar effects of pasture maturity and supplementation levels were shown on CLA content of manufactured cheeses. In conclusion, the level of CLA in milk and cheese was positively related with the amount of sheep racquet maneuvered by the grazed herbage.

According to (**Herzallah *et al.*, 2005),** this reduction of CLA could be due to on oxidation process which results in the formation of hydroperoxides that could curse the conversion or degradation of CLA. The CLA content of cheese heated in a microwave oven for 5 min. decreased by 21% and further heating for 10 min. caused 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 (processed cheese) of total fat (**Dhiman *et al.*, 2005**).On the contrary, **Zhang *et al.*, 2006**, found that, sunflower supplementation was more effective than flaxseeds in increasing milk and cheese CLA concentration in dairy sheep. These differences can likely be attributed to the different FAs precursors ratio in offered feeds used in the various works and to the differences in the basal diet which interact with supplement effect.

Manchego cheese is the most important and well known ewe's milk cheese in Spain. It is made from whole milk of Manchega breed of sheep and ripened for a period of at least 3 months. This study was to produce CLA-enriched ewe's milk together with increased levels of α-linolenic acid without impairing milk production. This work also studied whether CLA- enriched Manchego cheese from linoleic and α-linolenic acids- fed ewes milk would be similar in consumer acceptability attributes and specific characteristics to that of milk and cheese with low levels of CLA., (**Luna *et al.*, 2008).** They found that, the processing of milk into cheese did not substantially alter CLA content, the most practical approach for achieving cheeses with high concentration of CLA would consist of producing ewes milk with a high CLA content by modifying the ration of these animals.

Margherita *et al.* (2009), studied the effect of different fat- enriched concentrates on fatty acid profile of cheese from grazing dairy sheep, the results showed that, the concentrates of oilseeds from both sunflower and linseed sources determined high C18:1 trans-11 CLA cis-9 trans-11 cheese fat content. Feeding management is the mainly is flouncing factor of milk and cheese fatty acid profile. There are also reports of dairy products to which CLA has been added to increase their original content, **(Lopes *et al.*, 2009).**CLA in milk has been shown to be stable under normal cooking and storage conditions however, a significant reduction between raw and UHT milk samples from 10.18 to 7.96mg/g has been detected in some studies, (**Costa *et al.*, 2011).** In the case of sheep milk, the CLA content in individual samples varied from 17.8 to 56.5 mg/g fat, the highest CLA concentrations in sheep milk products were found in white brined cheese (35.6 mg/g -1) followed by yoghurt (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 the diet, (**Mihailova and Odjakova, 2011**).

Meraz-Torres and Hemandez-Sanchez (2012) reported that, 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 could be modified by direct addition of this nutraceatical compounds or by the additions of a precursor as linoleic or linolenic acids and a CLA-producing bacterial train. An added value could be obtained if the producer strain is a probiotic microorganism.

Marialice *et al.* (2013) studied the availability of food sources of CLA in the homes of Brazilian families, the fat content of the selected foods was taken from tables of food composition and that of CLA from papers published in international journals. By crossing these data, it was possible to found that, the availability of CLA in Brazilian households is of 1.8 mg/g -1 of fat using as food sources cow's milk and its derivatives. This value is below the estimated one for CLA perform its health beneficial function which would be around 3.5mg/day. However, it is similar to that found for the conscriptions of CLA in other countries.

Fermented dairy Products:

Several studies reported elevated CLA levels in fermented milk products: in Dahi, 226.5 mg/g -1 fat compared to the 5.5mg/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 *et al.*, 1995),** and they found no statistically significant difference between milk and yoghurt on fat basis and between milk and yoghurt with 1.0% and 3.25 fat. The conjugated linoleic acid (CLA) content of the fermented dairy products ranged from 2.82 to 1.66 mg/g of lipid and cultured buttermilk had the highest content. The conjugated linoleic acid content of sour fluid milks ranged from 3.38 to 6.39 mg/g of lipid and were not significantly different from one the another, (**Lin *et al.* 1995**).

It was hypothesized that, CLA concentration could increase during milk fermentation. CLA content in yoghurt could be increased by using certain lactic acid bacteria, (Sieber, et al., 2004). Different strains of lactobacillus acidophilus, lactobacillus plantarum, lactococcus lactis have been reported to enhance CLA concentration during milk fermentation, (**Shantha *et al.*, 1995),** typical values of CLA in fermented milk range from 3.41 to 9.12 mg/g. of fat.

In addition, several strains of lactobacillus, propionibacterium, Bifidobacterium and Entercoccus are able to from CLA from linoleic acid and thus could be used to increase CLA level in fermented dairy products such as yoghurt and cheese. It appears likely that lactic acid bacteria and especially propionibacteria can from CLA during cheese ripening because free linoleic acid is formed in the ripening process.

The CLA content of cow's milk shows a large variation depending on the type of management, breeds and season of the year. Thus, the CLA content in dairy products such as yoghurt is a reflection of the raw materials that gave rise to them. The process of pasteurization of milk seems to incurrence the CLA content, and these observed that yoghurt made with pasteurized milk showed higher CLA content (5.25 mg CLA.-1 fat_ compared to that where unpasteurized milk was used (4.40 mg CLA. g-1 fat.) **Kim and Liu, 2002. Boylston and Beitz, 2002**, they found the 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 days or 7 days. **Julian *et al.* (2011)** reported that, concentration of conjugated linoleic acid of the main commercial kumis consumed and distributed in Colombia as well as the concentration of CLA of an artisanal Kumis elaborated with two different types of milk (Skim liquid and powder reconstituted). Conjugated linoleic acid C18.2 cis-9 trans-11) contents expressed as mg. rumminic acid/g. fat ranged from 7.63 ± 0.96 to 22.62 ± 3.85. Also, the main fatty acids of kumis samples were identified and quantified.

Results indicated that, kumis is a food product that could be used for supplying important amounts of conjugated linoleic acid human diet. Concentration of CLA in commercial samples of kumis has been evaluated for the first time in Colombia. Results in this study demonstrated that, CLA content of the analyzed kumis samples was slightly higher than the ones previously reported for various dairy products. The calculated values of CLA intake for each kumis portions indicated that, this dairy product could provide up to 47% of the estimated dairy CLA intake of 254.5 g/day. This fact suggested that, in order to incense the daily CLA intake in the Colombian population at least one kumis portions should be added to the daily diet, (**Julian *et al.*, 2011).**

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