

Source of Variation of Conjugated-Linoleic-Acid Contents in Dairy Products

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Abstract

Samples of bovine milk (raw, thermized, or pasteurized) and of cheeses (raw and pasteurized) at 60 d of ripening were analyzed for contents in CLA. Detected values varied between 1.45 ± 0.20 mg/g in raw milk, 1.44 ± 0.05 mg/g lipid in thermized milk and 1.40 ± 0.11 mg/g in pasteurized milk, and 9.6 ± 0.5 mg/g in pasteurized cheeses and 10.8 ± 4.2 mg/g in raw-milk cheeses. Our results indicate that although processing factors such as heating and ripening affect the CLA contents of a dairy product, the major source of its variation among products may be the intrinsic amount of CLA present in the raw-milk.

Keywords: Pasteurization; Traditional cheese; Thermization; Ripening; Pasture; CLA; Dairy products

Introduction

The fat component of bovine milk is on average 3 to 5% of its composition, and most of it is in the form of triacylglyceroids, reported as having bioactivity that results in specific physiological effects [1-5]. One such component is the conjugated linoleic acid (CLA) which chemically refers to various positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12- octadecadienoic acid), for which the two double bonds have a conjugated arrangement instead of methylene interruption. CLA may have a powerful ant carcinogenic, immunomodulation, growth promoting, lean body mass-enhancing and antidiabetic properties [6-8], consequently, there is a high interest on understanding what factors affect the amount of CLA in a food product.

In bovine raw milk, the CLA values may vary from 0.2% to 3.7% of total milk fat, depending on animals' diet, [9,10], its physiological state and season, while cheeses are reported to have much higher values [11,12]. Milk from ruminants fed predominantly on pasture is known as being in general richer in CLA. In fact Ponnampalam et al., [13] reported higher values of CLA for milk and meat products from Australia and New Zealand than the equivalent products from elsewhere. This was, attributed to the greater access to lush pasture, throughout the year by Australasian cattle. Similarly, in Azores islands, dairy cows are essentially pastured-fed all year round, and [14,15], reported the presence of higher contents of CLA in milk from Azores, as compared to milk counterparts from mainland Portugal. Approximately one million tons of milk are produced in Azores annually, most of which is transformed into a number of varieties of raw-milk and pasteurized-milk cheeses.

Cheese processing at industrial level, requires usually heat treating the milk, either by thermization - a generic description of a range of sub-pasteurization heat treatments (57 to $68^\circ\text{C} \times 10$ to 20 s) that reduces the number of spoilage bacteria in milk with minimal heat damage [16] or, most commonly, by pasteurization (65 to $75^\circ\text{C} \times 15$ s to 30 min) a more severe treatment that leads to a safer milk and products, but also changes the milk microflora that may be involved in CLA synthesis. While Shantha et al. [17] and Boylston et al. [18] reported that heating, agitation of curds and typical microbial enzymatic reactions during cheese ripening may alter the contents of CLA in milk or cheese, Khanal and Olson [19] and Panghyová et al. [20] refer that the post-harvest related factors have a minor role in influencing the CLA content of a dairy product. Understanding the main source of variation of CLA among products may help shedding light on how to obtain final products with maximum contents of CLA.

The aim of this study was thus to investigate how much the contents of CLA vary among different dairy products.

Materials and Methods

Milk sampling and cheeses samples

Milk sample - raw, thermized or pasteurized - were collected from twelve local small-scale dairies immediately before their use in cheese processing, and transferred into individual flasks. Simultaneously samples of cheeses made from raw milk ($n=7$) or pasteurized milk ($n=5$), both at different ripening stage were also collected at the same dairies. All samples were transported in cooled containers to our laboratory, and at arrival they were stored refrigerated (5°C) until analyzed for their CLA content.

Fat extraction

An aliquot of 10 g of each milk sample was extracted with 5 mL of hydrochloric acid 25% (p/v) and 5 mL of ethanol in a mortar. In order to obtain a full extraction of the fat, the sample was transferred to an extraction tube. Then, 20 mL of diethyl ether and 20 mL of petroleum benzene were added and the sample was shaken vigorously after adding each solvent. When the two layers were clear, the supernatant was transferred to a 250 mL flask and the extraction was repeated as before. Solvents were evaporated with a rotary evaporator and the residues were removed under a nitrogen stream. The flask was weighed in order to obtain the fat content of the test samples. Next the residues were dissolved in 4 mL of hexane and transferred to a 10 mL vial. Fat extraction for cheeses samples was performed as indicated above after the entire block of cheese sample was shredded, blended and prepared for fat extraction.

FAME derivatization

Methyl ester solutions of fatty acids (FAME) were obtained by alkaline trans-esterification with methanolic potassium

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hydroxide solution 2 ml/L (0,33M) according to NP EN ISO 5509 (2003). Quantification of FAME was fulfilled according to the method described by Leite et al. [14] by Gas Chromatography (GC) with a Flame Ionization Detector (FID).

CLA analysis

The chromatographic separation was performed in a 100 m long Varian Wcot Fused Silica Coating CP-Sil 88 column, id 0.25 with helium as carrier gas, with the oven initial temperature at 60°C. After 4 min the temperature was raised to 175°C at a rate of 20°C/min and kept at the same temperature for 70 min. The temperature was then increased by 4°C/min to until 220°C and was kept at that temperature for 36 min. The injector temperature was set at 250°C with a splitless 20:1, and two injections were performed for each case. The detector FID was at 300°C and the identification of peaks was based in relative retention times and matching of mass spectral data from sample with data bases from the equipment libraries. In the present work the targets of our analysis were the *cis* 9- and *trans* -11 isomers which are considered the most abundant CLA isomers.

Statistical analyze

The data obtained was subject to statistical analysis via the software-package MicroCal Origin, version 2.8 (MicroCal Software, Northampton, MA, USA).

Results and Discussion

The average content of CLA found in the twelve milk samples are shown in Table 1, and they varied from 1.45 ± 0.21 mg/g lipids in raw milk, 1.44 ± 0.06 mg/g lipids in thermized milk, and 1.40 ± 0.11 mg/g lipids in pasteurized milk samples. These values agree with those reported by Pestana et al. [15] and Regula et al. [21] who reported that pasteurization, depending on its type, causes different changes in the free fatty acid profiles of ewes' milk, which in general has a higher content of CLA as compared to cows' milk [22]. However, Shantha et al. [17] and Garcia-Lopez et al. [23] reported that the application of heat enhanced the formation of linoleic acid radicals and increased CLA content during the production of natural and processed cheeses. The average content of CLA detected during the present work, in raw, thermized and pasteurized were not significantly different.

Figure 1 shows the contents of CLA detected in raw-milk cheeses and pasteurized-milk cheese samples at 60 d of ripening. The contents of CLA detected in raw-milk cheeses (10.8 ± 4.2 mg/g lipid) and in pasteurized-milk cheeses (9.6 ± 0.5 mg/lipid), were not significantly different (p > 0.05). However, Shantha et al. [17] reported an enhancement of CLA formation at elevated temperatures during preparation of processed cheeses. The difference with our data may results from the fact in the present work the heating processing was applied to milk and at a much lower temperature. The highest value of CLA (17.27 mg/g lipid) was detected in a sample of Pico raw-milk cheese and the lowest (8.61 mg/g lipid) in Flores raw-milk cheese sample, while among pasteurized-milk cheeses, the highest value of CLA (13.11 mg/g lipid) was detected in São Miguel cheese.

In the present work we also found (not shown) a trend of increase in CLA contents as the cheeses (both raw and pasteurized) aged. Kepler [24], Jiang et al., [25] attributed this effect to the enzymatic activity from starters and non-starter bacteria on the fat component of the cheeses, as lipolysis produces free fatty acids, which are the precursors of CLA formed through the microbiological hydrogenation pathway.

Ha et al. [11], Chin et al. [8] and Werner et al. [26] report that the

effects of aging on CLA formation is dependent on the cheese type and length of aging, while Boylston et al. [18] reported that the contents of CLA in Cheddar-type cheeses might be controlled by stage and conditions of processing. Finally, Khanal and Olson, [19], suggested that post-harvest factors are of minor importance in influencing the contents of CLA in dairy products, which agrees with our findings.

Figure 2 is a typical chromatogram obtained from an azorian cheese, showing Miristic, Palmitic, Estearic and Oleic fatty-acids as main components, and Figure 3 shows that the predominant CLA isomer we found was the *cis*-9, *trans*-11, a biological active isomer [7],

Milk sample type	Content in CLA (mg/g fat)
Raw	1.45 ± 0.20 ^a (n=12)
Thermized	1.43 ± 0.05 ^a (n= 12)
Pasteurized	1.40 ± 0.11 ^a (n= 12)

*Rows with different letters are significantly different

Table 1: Values (average ± SD) of CLA detected in raw, thermized and pasteurized milk

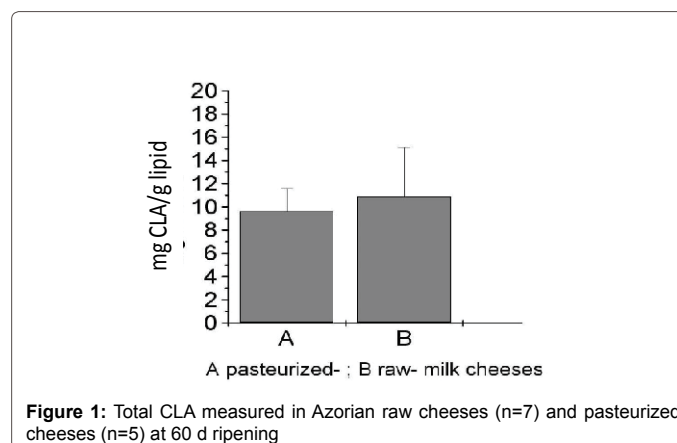


Figure 1: Total CLA measured in Azorian raw cheeses (n=7) and pasteurized cheeses (n=5) at 60 d ripening

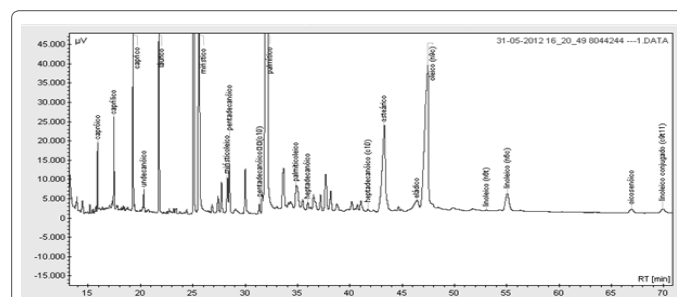


Figure 2: Chromatogram showing a lipidic profile of an azorian cheese with miristic, palmitic, stearic and oleic fatty acids as the main components

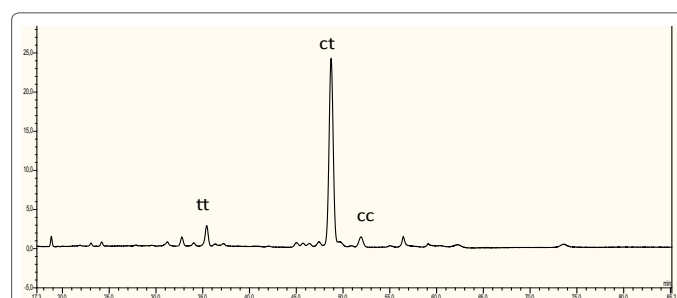


Figure 3: CLA isomers in an Azorian cheese (tt: trans-trans, ct:cis-trans, cc: cis-cis)

which also agrees with results reported by Pestana et al. [15].

Our data confirm previous reports that azorian dairy products have on average a high content in CLA. We also found that heating slightly decrease the contents of CLA present in milk, while ripening increases the contents of CLA in both raw-milk cheeses and pasteurized milk cheeses. We concluded that that post-harvest factors may be of minor importance in influencing the contents of CLA in dairy products, and consequently, its variation among products may be associated to the intrinsic content of CLA present in raw milk.

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