Food Processing & Technology

Kongo, et al,. J Food Process Technol 2014, 5:12
http://dx.doi.org/10.4172/2157-7110.1000404

Research Article Open Access

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

Marcelino Kongo J*, João Leite, Ana Borges and Duarte Ponte

INOVA, Institute for Technological Innovation of the Azores, Road Sao Goncalo, 9540-504 Ponta Delgada, Azores, Poland

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 triacilgliceroids, 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°C×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°C×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 (5ºC) 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 benzine 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

***Corresonding author:** Marcelino Kongo J, INOVA, Institute for Technological Innovation of the Azores, Road Sao Goncalo, 9540-504 Ponta Delgada, Azores, Poland, Tel: +351 296 20 17; E-mail: mkongo@inovacores.pt

Received November 06, 2014; **Accepted** December 03, 2014; **Published** December 10, 2014

Citation: Kongo JM, Leite J, Borges A, Ponte D (2014) Source of Variation of Conjugated-Linoleic-Acid Contents in Dairy Products. J Food Process Technol 5: 404. doi:10.4172/2157-7110.1000404

Citation: Kongo JM, Leite J, Borges A, Ponte D (2014) Source of Variation of Conjugated-Linoleic-Acid Contents in Dairy Products. J Food Process Technol 5: 404. doi:10.4172/2157-7110.1000404

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) whit a Flame Ionizantion 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 whit helium as carrier gas, with the oven initial temperature at 60ºC. After 4 min the temperate was raised to 175°C at a rate of 20ºC/min and kept at the same temperature for 70 min. The temperature was then increased by 4ºC/min to until 220ºC and was kept at that temperature for 36 min. The injector temperature was set at 250ºC with a splitless 20:1, and two injections were performed for each case. The detector FID was at 300ºC 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 cisi-9, trans-11, a biological active isomer [7],

Milk sample type	Content in CLA (mg/g fat)
Raw	$1.45 \pm 0.20^{a*}$ (n=12)
Thermized	$1.43 \pm 0.05^{a*}$(n= 12)
Pasteurized	$1.40 \pm 0.11^{a*}$ (n= 12)

*Rows with different letters are significantly different

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

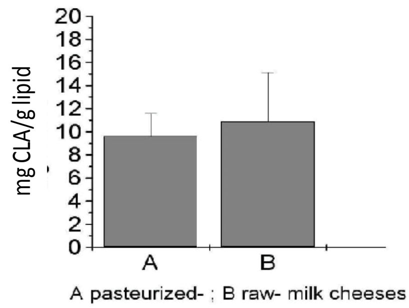

Figure 1: Total CLA measured in Azorian raw cheeses (n=7) and pasteurized cheeses (n=5) at 60 d ripening

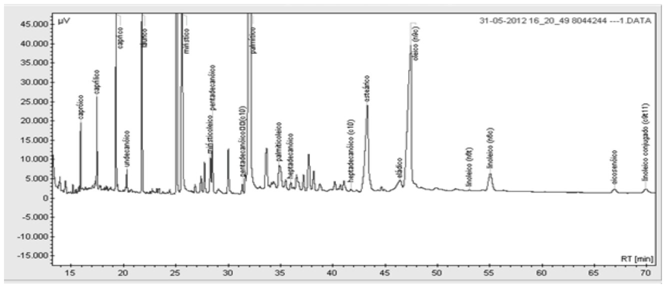

Figure 2: Chromatogram showing a lipidic profile of an azorian cheese with miristic, palmític, stearic and oleic fatty acids as the main components

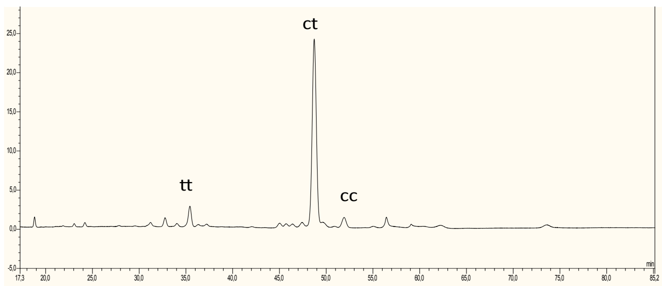

Figure 3: CLA isomers in an Azorian cheese (tt: trans-trans, ct:cis-trans, cc: cis-cis)

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 ma y be associated to the intrinsic content of CLA present in raw milk.

Acknowledgments

We thank Fundo Regional para a Ciência and the Programa Proconvergência via Governo Regional dos Açores who funded this research under the Project Seproqual Inovação.

References

1. Ip C, Scimeca JA, Thompson HJ (1994) Conjugated linoleic acid. A powerful anticarcinogen from animal fat sources. Cancer 74: 1050-1054.
2. Silveira MB, Carraro R, Monereo S, Tébar J (2007) Conjugated linoleic acid (CLA) and obesity. below Public Health Nutr 10: 1181-1186.
3. Parra P, Serra F, Palou A (2010) Moderate doses of conjugated linoleic acid isomers mix contribute to lowering body fat content maintaining insulin sensitivity and a noninflammatory pattern in adipose tissue in mice. J Nutr Biochem 21: 107-115.
4. Gaullier JM, Halse J, Høye K, Kristiansen K, Fagertun H, et al. (2004) Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. Am J Clin Nutr 79: 1118-1125.
5. Gaullier JM, Halse J, Høivik HO, Høye K, Syvertsen C, et al. (2007) Six months supplementation with conjugated linoleic acid induces regional-specific fat mass decreases in overweight and obese. Br J Nutr 97: 550-560.
6. Parodi PW (1999) Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. J Dairy Sci 82: 1339-1349.
7. Ip C, Chin SF, Scimeca JA, Pariza MW (1991) Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res 51: 6118-6124.
8. Chin SF, Liu W, Storkson JM, Ha YL, Pariza MW (1992) Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. Journal of Food Composition and Analysis 5: 185-197.
9. Rego OA, Rosa HJD, Regalo SM, Alves SP, Alfaia CMM, et al. (2008) Seasonal changes of CLA isomers and other fatty acids of milk fat from grazing dairy herds in the Azores. Journal of Science of Food and Agriculture 10: 1855-1859.
10. Kelly ML, Berry JR, Dwyer DA, Griinari JM, Chouinard PY, et al. (1998) Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J Nutr 128: 881-885.
11. Ha Y, Grimm NK, Pariza MW (1989) Newly recognized anticarcinogenic fatty acids: identification and quantification in natural and processed cheeses. Journal of Agricultural and Food Chemistry 37: 75-78.
12. Lin H, Boylston TD, Chang MJ, Luedecke LO, Shultz TD (1995) Survey of the conjugated linoleic acid contents of dairy products. J Dairy Sci 78: 2358-2365.
13. Ponnampalam EN, Mann NJ, Sinclair AJ (2006) Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and trans fatty acids in Australian beef cuts: potential impact on human health. Asia Pacific Journal of Clinical Nutrition 15: 21-29.
14. Leite J, Lima E, Baptista J (2007) Azorean bovine milk conjugated linoleic acid. Effect of green pasture diet, storage and processing temperature. Le lait 87: 167-179.
15. Pestana JM, Martins SIV, Alfaia CMM, Lopes PA, Costa ASH, et al. (2009) Content and distribution of conjugated linoleic acid isomers in bovine milk, cheese and butter from Azores. Dairy Science Technology 89: 193-200.
16. Stepaniak L, Rukke EO (2003) Thermization of milk. Encyclopedia of Dairy Sciences. London, UK: Academic Press.
17. Shantha NC, Decker EA, Ustunol Z (1992) Conjugated linoleic acid concentration in processed cheese. Journal of the American Oil Chemists' Society 69: 425-428.
18. Boylston HL, Luedecke LO, Shultz TD (1999) Conjugated Linoleic Acid content of cheddar-type cheeses as affected by processing. Journal of Food Science 64: 874-878.
19. Khanal RC, Olson KC (2004) Factors affecting Conjugated Linoleic Acid (CLA) content in milk, meat, and egg: a Review. Pakistan Journal of Nutrition 3: 82-98.
20. Panghyová E, Kacenová D, Hajdusková S, Matulová M, Kiss E (2006) Influence of free linoleic acid on the fatty acids profile of fermentation by selected probiotic bacteria. Journal of Food and Nutrition Research 45: 159-165.
21. Regula A, Boncza G, Pustowiak H (2005) The effect of heat trreastment on the free fatty acids in ewe's milk. Biotechnology in Animal Husbandry 21: 237-240.
22. Lobos-Ortega I, Revilla I, Gonzalez-Martin MI, Hernandez-Herrero JM, Vivar-Quintana A, et al. (2012) Conjugated linoleic acid contents in cheeses of different composition during six months ripening. Czech Journal of Food Science 30: 220-226.
23. Garcia-Lopez S, Echeverria E, Tsui I, Balch B (1994) Changes in the content of conjugated linoleic acid (CLA) in processed cheese during processing. Food Research International 27: 61-64.
24. Kepler CR, Tucker WP, Tove SB (1970) Biohydrogenation of unsaturated fatty acids. IV. Substrate specificity and inhibition of linoleate delta-12-cis, delta-11-trans-isomerase from Butyrivibrio fibrisolvens. J Biol Chem 245: 3612-3620.
25. Jiang J, Björck L, Fondén R (1998) Production of conjugated linoleic acid by dairy starter cultures. J Appl Microbiol 85: 95-102.
26. Werner SA, Luedecke LO, Shultz TD (1992) Determination of conjugated linoleic acid content and isomer distribution in three cheddar-type cheeses: effects of cheese cultures, processing, and aging. Journal of Agricultural and Food Chemistry 40: 1017-1021.

Citation: Kongo JM, Leite J, Borges A, Ponte D (2014) Source of Variation of Conjugated-Linoleic-Acid Contents in Dairy Products. J Food Process Technol 5: 404. doi:10.4172/2157-7110.1000404