

Conjugated linoleic acid of dairy foods is affected by cows' feeding system and processing of milk

Juan Pablo Avilez Ruiz¹, Marcelo Wladimir Alonzo², Manuel Delgado Pertinhez^{3*}

¹Catholic University of Temuco/School of Veterinary Medicine, Montt 56 – Temuco – Chile.

²Milk Company, Francisco del Campo s/n – Osorno – Chile.

³University of Sevilla/School of Agricultural Engineering – Agroforestry Science Dept., Ctra. Utrera km 1 – 41013 – Sevilla – Spain.

*Corresponding author <pertinhez@us.es>

Edited by: Paulo Cesar Sentelhas

Received February 10, 2015

Accepted June 28, 2015

ABSTRACT: The distribution of conjugated linoleic acid (CLA) in dairy products commercially available in Chile is poorly understood. This study aimed to assess the content of CLA in dairy cow products from Chile and the effect of processing fresh milk into dairy products. Samples of raw milk were categorized into two groups based on the animal feeding system utilized by the dairy farm: 1) grazing based systems (Los Lagos region); and 2) housing systems using total mixed ration (TMR) diets (Los Angeles region). Simultaneously, commercial samples of condensed milk, powdered milk, butter and Gouda cheese were analyzed. Furthermore, samples of raw milk and processed products (powdered and sweetened condensed milk) were also analyzed. Dairy farms based on grazing systems had higher levels of CLA in raw milk than TMR farms. In addition, average values of CLA were 1.72 g 100 g⁻¹ of total fatty acids, in spring milk in the Los Lagos region, and 0.42 g 100 g⁻¹ in summer milk, in the Los Angeles region. Similarly, the CLA content of dairy products was higher than that of raw milk. Milk processing affected the transferring of CLA from fresh milk into the final products. Sweetened condensed milk presented lower CLA values than raw and powdered milk. In conclusion, this study indicates the importance of the production systems to the CLA content as well as the effects of milk processing into dairy products. To sum up, more research is needed to elucidate the exact effect of the processing conditions of dairy products on the CLA content.

Keywords: CLA, functional foods, grazing, milk quality

Introduction

Conjugated linoleic acid (CLA) has been indicated as one of the most potentially beneficial fatty acids (FA) for human health (Collomb et al., 2006). The most effective strategy for multiplying the content of CLA in raw milk involves supplementing ruminant feed with different oils or oilseeds with high levels of linoleic and linolenic acids (Hervás et al., 2008). Feeding ruminants under grazing conditions (natural and/or improved pastures) can also provide high CLA content in raw milk (Gómez-Cortés et al., 2009; Avilez et al., 2012).

In Chile, cattle production systems are based, for the most part, on grazing (generally, southern Chile), whereas a minority of systems correspond to housing dairy farms that use total mixed ration (TMR) diets (generally, central Chile). At present, there are no studies regarding the content and proportion of CLA isomers in the Los Lagos region, the largest milk production area in the country. Furthermore, the distribution of CLA in dairy products commercially available in Chile is unknown. However, it is well accepted that milk undergoes changes during its preparation or processing, which may include moderate or severe heat treatments, that can lead to undesirable changes in lipids and proteins (Herzallah et al., 2005). Semma (2002) reported that milk lipids could undergo chemical and physical changes during processing and storage, such as auto-oxidation and formation of *trans* FA. Nevertheless, the results of the effects of processing conditions, storage, and packaging on the CLA content of various types of dairy products are unclear (Collomb et

al., 2006; Bisig et al., 2007). As for cheeses, reports and reviews present results for individual varieties, often in the belief that CLA levels may vary due to different processing conditions. Herzallah et al., (2005) reported CLA decreases of 21 and 53 % in cheeses heated in a microwave oven for 5 and 10 min, respectively. These effects are likely to be small and variations in CLA levels are similar to the levels in raw milk (Gómez-Cortés et al., 2009). However, other studies detected new CLA isomers in ripened cheeses (Werner et al., 1992; Lavillonière et al., 1998; Sehat et al., 1998) and it was hypothesized that biohydrogenation of linolenic acid in cheese could lead to the formation of CLA isomers as intermediates. Thus, the present study aimed to assess the contents of total and individual CLA isomers of cow dairy products in Chile, both in raw milk received at commercial dairy plants and its derived products destined for human consumption. Additionally, the effect of processing fresh milk into dairy products on the CLA content was also assessed.

Materials and Methods

Experimental samples

Raw milk - Milk samples were collected from two regions in Chile considered representative of two different dairy production systems, based on feeding strategy: grazing based systems (Los Lagos region, Osorno) and housing systems using TMR diets (Bio-Bio region, Los Angeles). The predominant genotype of dairy cow in southern Chile corresponds to a Chilean Black Friesian, multiparous cow (6.6 ± 1.8 calvings), with an

average milk production of 17.8 ± 3.20 kg d⁻¹, milk fat percentage of 3.67 ± 3.6 and protein percentage of 3.42 ± 1.4 . Raw milk samples were collected from the tanks of a Chilean milk company, located in the Osorno sector (Los Lagos region, 6 reception tanks) and the Los Angeles sector (Bio-Bio region, 5 reception tanks). Each tank had a capacity of 120,000 L and milk was kept at 8 °C. Three samples of 100 mL were collected in each sector at 15-day intervals during the summer (n = 18), autumn (n = 18), and spring (n = 18), according to the protocol laid down by the International Dairy Federation (IDF, 1995). These samples were transported to the laboratory in isolated-thermo boxes at 4 °C and subsequently stored at -80 °C until analysis.

Dairy products - Collection of dairy products was made over three years. Samples of sweetened condensed milk (sample weight: 0.40 kg) were obtained from a Chilean milk company plant located in Los Angeles (two samples per month, n = 72). Powdered milk samples (sample weight: 4 kg) were obtained from the same milk company but now in another plant located in Osorno (two samples per month, n = 72). In addition, samples of commercial butter (sample weight: 0.12 kg, n = 14) were obtained and Gouda cheese (sample weight: 1 kg, n = 14), the most consumed cheese in Chile produced in the two major dairies in southern Chile. All samples were transported in isolated-thermo boxes at 4 °C to the laboratory and subsequently stored at 4 °C until analysis.

CLA is found in dairy products made from fresh milk. Three samples of raw milk from the tanks of milk company plants located in both the Osorno and Los Angeles regions were collected at 10-day intervals for 10 months (n = 90), in accordance with the protocol laid down in the International Dairy Federation. In addition, samples of processed products previously made from raw milk were also collected, which included powdered milk (three samples per month, n = 30) obtained by evaporation (55-70 °C) and spray-drying (the air is filtered and heated to 150-250 °C) and sweetened condensed milk obtained by evaporation at 100 °C (three samples per month, n = 30). All samples were shipped in isolated-thermo boxes at 4 °C to the laboratory and subsequently stored at 4 °C until analysis.

Determination of CLA content and composition

The CLA content of isomers (*cis*-9, *trans*-11; *trans*-10, *cis*-12; *cis*-10, *cis*-12) in raw milk and dairy product samples were determined by the Folch method, using a mixture of chloroform and methanol (2:1, v:v). Individual CLA isomers were identified by comparing their retention times with those of an authenticated standard FA mix. The fatty acids were analyzed by gas chromatography after transesterification of FA to FA methyl esters as previously described by Avilez et al., (2012). Briefly, FA methyl esters were analyzed by gas chromatography, Flame Ionization Detector (FID), a capillary column SP-2560 (100 m, 0.25 mm i.d. with 0.20 µm thickness in the stationary phase) using He as the tracer gas. Gas chroma-

tography conditions were as follows: the injection volume was 0.5 µL, a split injection was used (70:1, v:v); ultrapure hydrogen was the carrier gas; and the injector and detector temperatures were 250 and 300 °C, respectively. The initial temperature was 70 °C (maintained for 1 min), increased by 5 °C min⁻¹ to 100 °C (held for 3 min), then by 10 °C min⁻¹ to 175 °C (held for 40 min), and then again by 5 °C min⁻¹ to 220 °C (maintained for 19 min) for a total run time of 86.5 min. Data were then quantified using the HPCHEM Stations software, and expressed as a percentage of area according to the total FA identified.

Statistical analysis

CLA content data were analysed using the general linear model (GLM) in the SPSS for Windows 18.0 package. For the distribution of CLA content in dairy products, a global or partial comparison of means was analyzed, including the fixed effect of type of product, as well as the dairy production systems and season of sample harvesting (for raw milk), or the year of harvest (for processed products). For the effect of processing raw milk into dairy products on the CLA content, the fixed effects of the type of product and month were considered. Pairwise comparisons of means were carried out, where appropriate, using Tukey's honest significant difference tests, considering a level of significance of 5 %.

Results and discussion

CLA content of commercial dairy products

Table 1 presents the CLA content in raw milk of bulk tank commercial dairy plants and in dairy products. Raw milk presented total CLA mean values between 1.72 g 100 g⁻¹ total FA in spring milk from the Osorno region and 0.42 g 100 g⁻¹ in summer milk from the Los Angeles region. The total CLA content of dairy products showed average values with greater variability, ranging from 0.88 g 100 g⁻¹ of total FA in cheese up to 1.50 g 100 g⁻¹ in butter; 1.49 g 100 g⁻¹ in sweetened condensed milk and 1.97 g 100 g⁻¹ in powdered milk. Comparing total CLA content and its isomers in all products tested, the dairy products presented higher contents ($p \leq 0.01$) when compared with raw milk, except for spring milk from the Osorno region. Similarly, the amount of CLA in raw milk differed ($p \leq 0.05$) by season and geographic region, with higher levels during the spring and in the Osorno region, but no differences were observed between regions during autumn. Furthermore, differences between years were observed for sweetened condensed milk and powdered milk, with higher CLA levels in the second year when compared to the first and third ones.

A study conducted in Germany reported maximum CLA values in raw milk of 1.16 g 100 g⁻¹ of total FA (Fritsche and Steinhart, 1998). In France, Laloux et al., (2007) reported 0.04 g 100 g⁻¹, and 0.72 g 100 g⁻¹ of total FA was reported in Portugal (Martins et al., 2007). These values were lower than those found in the present study (0.89 g 100 g⁻¹ of total FA in Los Angeles, and 1.48

Table 1 – Conjugated linoleic acid contents (CLA) in raw milk of bulk tank commercial dairy plants and in dairy products.

Product	CLA Total				CLA Isomer		
	n	Mean ± SEM ²	Minimum	Maximum	Cis-9, trans-11 Mean ± SEM	Trans-10, cis-12 Mean ± SEM	Cis-10, cis-12 Mean ± SEM
g 100 g ⁻¹ total fatty acids							
Raw milk ¹							
Housing systems (summer)	18	0.41 ± 0.1 ^{2e}	0.28	0.49	0.26 ± 0.0 ^{6f}	0.15 ± 0.06 ^c	0.00
Housing systems (autumn)	18	0.82 ± 0.08 ^d	0.74	0.91	0.32 ± 0.15 ^e	0.33 ± 0.09 ^b	0.18 ± 0.23 ^e
Housing systems (spring)	18	0.93 ± 0.28 ^d	0.41	1.33	0.49 ± 0.02 ^d	0.44 ± 0.02 ^a	0.00
Grazing based systems (summer)	18	0.61 ± 0.01 ^e	0.59	0.62	0.40 ± 0.01 ^e	0.21 ± 0.00 ^c	0.00
Grazing based systems (autumn)	18	0.89 ± 0.14 ^d	0.74	1.01	0.48 ± 0.04 ^d	0.00	0.41 ± 0.12 ^d
Grazing based systems (spring)	18	1.72 ± 0.07 ^b	0.55	2.96	0.81 ± 0.44 ^a	0.00	1.19 ± 0.41 ^a
Dairy products							
Butter	14	1.50 ± 0.41 ^b	1.26	2.22	0.92 ± 0.38 ^a	0.56 ± 0.24 ^a	0.53 ± 0.21 ^c
Cheese	14	0.88 ± 0.55 ^d	0.37	1.47	0.47 ± 0.34 ^d	0.00	0.41 ± 0.21 ^d
Condensed milk (year 1)	24	1.03 ± 0.24 ^d	0.73	1.46	0.44 ± 0.15 ^d	0.05 ± 0.06 ^d	0.55 ± 0.09 ^c
Condensed milk (year 2)	24	1.49 ± 0.49 ^b	1.14	2.20	0.74 ± 0.23 ^b	0.00	0.75 ± 0.29 ^b
Condensed milk (year 3)	24	1.31 ± 0.26 ^c	0.96	1.61	0.79 ± 0.55 ^b	0.57 ± 0.60 ^a	0.10 ± 0.20 ^f
Powdered milk (year 1)	24	1.43 ± 0.26 ^b	1.06	2.14	0.68 ± 0.11 ^c	0.18 ± 0.10 ^c	0.57 ± 0.15 ^c
Powdered milk (year 2)	24	1.97 ± 0.37 ^a	1.61	2.59	0.97 ± 0.23 ^a	0.00	0.10 ± 0.16 ^f
Powdered milk (year 3)	24	1.46 ± 0.20 ^b	1.24	1.79	0.73 ± 0.13 ^b	0.24 ± 0.33 ^b	0.54 ± 0.13 ^c
Effects, p ³							
Type of product ⁴		**			**		**
Dairy production systems ⁵		**			*		**
Season of sample harvesting ⁵		*			*		*
Year of harvest ⁶		*			*		*

Mean values within a column with different superscripts are different ($p \leq 0.05$); ¹Milk samples were taken in two regions of Chile, with dairy cattle production systems mainly differentiated by feeding strategy: grazing based systems (Los Lagos region, Osorno) and housing based in total mixed ration systems (Bio-Bio region, Los Angeles); ²Standard error of mean; ³* $p \leq 0.05$; ** $p \leq 0.01$; ⁴Raw milk, butter, cheese, condensed milk, powdered milk; ⁵For raw milk; ⁶For processed products.

g 100 g⁻¹ of total FA in Osorno). However, CLA values from previous studies refer only to *cis-9, trans-11* isomer in the German study, total CLA in the French study, and 17 isomers with a greater proportion of *cis-9, trans-11*, in the study conducted in Portugal.

CLA values found in the present study could be related to the dairy cattle production system of each region, and particularly with the type of diet. In the case of the Los Lagos region (Osorno), the diet is based mainly on grazing, whereas the productive system of the Bio-Bio region (Los Angeles), has housing and TMR diets. There is strong evidence that milk produced from grazing animals had higher CLA concentrations in their milk compared to non or low grazing animals (Butler et al., 2008; Rego et al., 2008; Gómez-Cortés et al., 2009). Also, seasonal differences in CLA content may be related to differences in diet quality, mainly due to ingestion and nutritional composition of the herbage (Dewhurst et al., 2006). Lower CLA content in milk have been observed where cows were grazing mature pastures, and this effect has been attributed to the declining quantity and quality of the herbage (Ward et al., 2003; Avilez et al., 2012). In this respect, CLA content in the milk increased during the spring season when cows were fed on high quality pasture (1.20 g 100 g⁻¹ of total FA) compared to the summer season (0.90 g 100 g⁻¹) and the autumn (1.00 g 100 g⁻¹) (Thomson et al., 2003). In previous studies,

neither sites of sampling nor the type of animal production system deployed had been mentioned as determining factors. In Chile, the levels of CLA from raw milk received in the Osorno region are very important because the volume of milk collected in this region represents around 71 % of the total national milk production. In addition, these values of CLA in raw milk and processed products are higher than those reported in the international literature.

In dairy products, international researchers have reported CLA values of 0.40 g 100 g⁻¹ of total FA in mature cheese and from 0.29 to 0.71 g 100 g⁻¹ in fresh cheese in Germany (Fritsche and Steinhart, 1998); 0.39, 0.80 and 0.16 g 100 g⁻¹ in Beaufort, Blue and Camembert cheeses, respectively, in France (Laloux et al., 2007); and 0.48 g 100 g⁻¹ in cheese without (type not specified) in Portugal (Martins et al., 2007). On the other hand, cheeses, such as Parmesan and Romano, with ripeness over 10 months were among the cheeses with lower amounts of CLA (less than 0.50 g 100 g⁻¹ of total FA; Henning et al., 2006). In the case of butter, values from 0.48 to 0.51 g 100 g⁻¹ have been reported (Mir et al., 2003; Laloux et al., 2007; Martins et al., 2007). In sweetened condensed milk, Chin et al., (1992) indicated values below 0.70 g 100 g⁻¹. Thus, the results presented in this study, for similar products, have higher values than those mentioned above.

Table 1 summarizes the content of CLA isomers. The *cis-9*, *trans-11* presented a range between a minimum of 0.26 g 100 g⁻¹ of total FA in summer for raw milk from the Los Angeles region and a maximum of 1.26 g 100 g⁻¹ in butter from the Osorno region. This isomer represents 53 % of total CLA, with higher values in the Osorno region. In the case of sweetened condensed milk, this isomer ranged from 0.44 to 0.74 g 100 g⁻¹, depending on the year; while cheese and butter had a range of 0.37 - 1.47 and 1.26 - 2.22 g 100 g⁻¹, respectively. The isomer *trans-10*, *cis-12* had a range of 0.15 to 0.44 g 100 g⁻¹ for total FA in raw milk received at the plants. This isomer was not detected in milk samples collected during the autumn and spring in the Osorno region. In dairy products, the levels of this isomer ranged from 0.00 to 0.56 g 100 g⁻¹ of total FA. The isomer *cis-10*, *cis-12* presented higher proportions during springtime in raw milk from Osorno region (1.19 g 100 g⁻¹ of total FA), as well as in condensed and powdered milk (0.75 and 0.57 g 100 g⁻¹ of total FA, respectively). As for the total amount of CLA, this isomer reached about 50 % in sweetened condensed milk and powdered milk, this being the major isomer found in raw milk in springtime from the Osorno region.

In the literature values have been reported of 0.70 g 100 g⁻¹ of total FA for the *cis-9*, *trans-11* isomer for sweetened condensed milk (Shantha et al., 1995); whereas in cheese, values of 0.32 and 0.89 g 100 g⁻¹ have been reported with an average of 0.35 g 100 g⁻¹ (Martins et al., 2007). The same authors reported values of 0.38 for butter, as well as values of 1.32 g 100 g⁻¹ for this product (Shantha et al., 1995).

The isomer *trans-10*, *cis-12* would be present in amounts of only 3 to 5 % of the total CLA in milk (Parodi, 1999) and less than 1 % in dairy products (Martins et al., 2007). However, in the present study, the values obtained were higher than those reported in the literature, which is remarkable given its important physiological role. Also, the contents of *cis-10*, *cis-12* isomer were higher than those reported in the literature (less than 0.01 %). Furthermore, it has not been detected in either butter or cheese (Martins et al., 2007). It is thus important, given the high proportion observed in this study, to determine their biological function.

In Germany, human consumption of CLA from dairy products is estimated in 0.24 g d⁻¹ (Fritsche and Steinhart, 1998). In France, it ranges from 0.17 to 0.21 g d⁻¹ (Laloux et al., 2007), and in Portugal from 0.40 to

0.72 g d⁻¹ (Martins et al., 2007). Ritzenthaler et al., (2001) suggested that optimal consumption of CLA should be between 15 and 20 g d⁻¹, whereas rich consumption should be around 65 g d⁻¹ (Park et al., 2001). On the contrary, Ip et al., (1999) suggested an optimal CLA consumption of 3.0 g d⁻¹ for a 70 kg person to reap health benefits. Daily CLA consumption in Chile, considering the contents of CLA found in the present study, ranged between 0.03 and 0.24 g d⁻¹.

Evaluation of the CLA content in dairy products after processing

The amounts of total CLA and each of their isomers in raw milk observed at commercial dairy plants and those found in processed products obtained from that milk are shown in Table 2. A significant interaction between product type and sampling month was observed (Table 3 and Figure 1). In general, raw milk presented higher CLA contents during the autumn (March to May) and springtime (especially October and November) months when compared to the wintertime (especially June and August) months. In contrast, powdered and sweetened condensed milk products presented higher values during the winter months, albeit with greater variability, especially in sweetened condensed milk. Furthermore, sweetened condensed milk collected in most of the months studied (specifically 6 out of the 10 sampling months) had lower CLA content than raw milk and powdered milk although the latter showed similar values to those found in raw milk, and even in 4-5 months of the study, showed higher values, most of them coinciding with the months (winter) of lowest values obtained for raw milk.

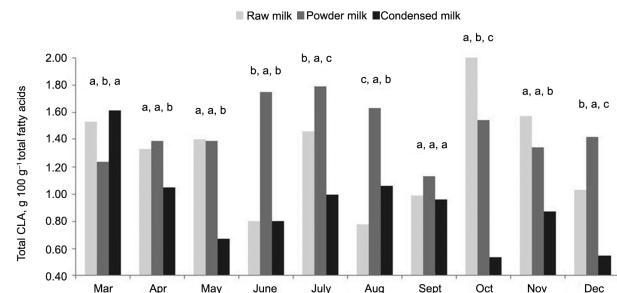


Figure 1 – Contents of total conjugated linoleic acid (CLA) in dairy products (raw milk, powder milk and condensed milk) made from cows' fresh milk. Means within a month (a, b, c) with different letters are different ($p \leq 0.05$).

Table 2 – Contents of conjugated linoleic acid (CLA) in dairy products made from cows' fresh milk.

CLA (g 100 g ⁻¹ total fatty acids)	Product			Effects, p ¹		
	Raw milk	Powdered milk	Condensed milk	T	M	T × M
n	90	30	30			
Total CLA	1.35 ± 0.51	1.45 ± 0.28	0.93 ± 0.61	ns	ns	***
CLA <i>cis-9</i> , <i>trans-11</i>	0.58 ± 0.41	0.73 ± 0.16	0.45 ± 0.35	ns	***	***
CLA <i>trans-10</i> , <i>cis-12</i>	0.67 ± 0.24	0.72 ± 0.20	0.45 ± 0.19	ns	ns	*
CLA <i>cis-10</i> , <i>cis-12</i>	0.77 ± 0.45	0.56 ± 0.27	0.87 ± 0.13	ns	ns	*

¹T, Product type; M, Sample months; T × M interaction; * $p \leq 0.05$; *** $p \leq 0.001$; ns: not significant, $p > 0.05$.

Table 3 – Contents of conjugated linoleic acid (CLA) in dairy products made from cows' fresh milk according to month.

Month	CLA total			CLA <i>cis</i> -9, <i>trans</i> -11			CLA <i>trans</i> -10, <i>cis</i> -12; CLA <i>cis</i> -10, <i>cis</i> -12		
	Raw milk	Powdered	Cond. ¹	Raw milk	Powdered	Cond.	Raw milk	Powdered	Cond.
	g 100 g ⁻¹ total fatty acids								
Mar	1.53 ^B	1.24 ^B	1.61 ^A	0.58 ^{b, C}	0.58 ^{b, C}	0.97 ^{a, A}	0.95 ^{a, A}	0.66 ^{b, B}	0.64 ^{b, A}
Apr	1.33 ^B	1.39 ^B	1.05 ^B	0.51 ^{b, C}	0.81 ^{a, A}	0.47 ^{b, C}	0.83 ^{a, B}	0.59 ^{b, C}	0.58 ^{b, A}
May	1.40 ^B	1.39 ^B	0.67 ^C	0.67 ^{b, C}	0.86 ^{a, A}	0.30 ^{c, C}	0.70 ^{b, B}	0.53 ^{b, C}	0.37 ^{c, B}
June	0.80 ^C	1.75 ^A	0.80 ^C	0.24 ^{b, D}	0.84 ^{a, A}	0.43 ^{b, C}	0.56 ^{b, C}	0.90 ^{a, A}	0.37 ^{c, B}
July	1.46 ^B	1.79 ^A	1.00 ^B	1.03 ^{a, B}	0.89 ^{a, A}	0.5 ^{b, B}	0.44 ^{b, C}	0.90 ^{a, A}	0.43 ^{b, B}
Aug	0.78 ^C	1.63 ^A	1.06 ^B	0.18 ^{c, D}	0.78 ^{a, A}	0.53 ^{b, B}	0.64 ^{b, C}	0.85 ^{a, A}	0.53 ^{b, B}
Sept	0.99 ^B	1.13 ^C	0.96 ^B	0.25 ^{b, D}	0.50 ^{a, C}	0.50 ^{a, C}	0.74 ^{a, C}	0.63 ^{a, B}	0.46 ^{b, B}
Oct	2.15 ^A	1.54 ^A	0.54 ^C	1.32 ^{a, A}	0.69 ^{b, B}	0.29 ^{c, D}	0.82 ^{a, C}	0.85 ^{a, A}	0.24 ^{c, C}
Nov	1.57 ^B	1.34 ^B	0.87 ^B	0.41 ^{b, C}	0.63 ^{b, B}	0.52 ^{a, B}	1.16 ^{a, A}	0.71 ^{b, B}	0.35 ^{c, C}
Dec	1.03 ^C	1.42 ^B	0.55 ^C	0.40 ^{b, C}	0.71 ^{a, B}	0.19 ^{c, D}	0.63 ^{b, C}	0.72 ^{a, B}	0.43 ^{c, C}

Means within a row (a, b, c) or column (A, B, C, D) with different superscripts are different ($p \leq 0.05$); ¹Cond.: condensed milk.

Variation in CLA concentration in dairy products is essentially a function of their original concentration (in raw milk). Nevertheless, the results of the effect of processing conditions, storage, and packaging on the CLA content of various types of dairy products are unclear. Coakley et al., (2007) reported that *cis*-9, *trans*-11 and *cis*-10, *trans*-12 isomers were not significantly affected by milk processing to produce cheese made with standard processes (pasteurization of milk and subsequent cooling at 30 °C, 6 months of ripening of cheese at 8 °C). However, Shantha et al. (1995) detected a decrease in total CLA in cheese samples after 10 wks of refrigerated storage (4 °C). Also, Herzallah et al., (2005) reported decreases in CLA content of 21 and 53 % in cheeses heated to 94 ± 1.0 °C in a microwave oven for 5 and 10 min, respectively. These authors also observed a decrease in *trans* isomers of pasteurized cheese at 63 °C for 30 min or heated in a microwave at 96 °C for 5 min. The decline of CLA content in milk after heating may be due to the action of free radicals, which are formed as a result of lipid oxidation (Leung and Liu, 2000). Other studies have detected new CLA isomers in ripened cheeses (Werner et al., 1992; Lavillonière et al., 1998; Sehat et al., 1998), and it has been hypothesized that biohydrogenation of linolenic acid in cheese could lead to the formation of CLA isomers as intermediates (Gnädig et al., 2004). Moreover, refrigerated storage and thermal treatment resulted in significant declines in or even disappearance of a number of minor CLA isomers, plus a significant increasing of *trans-trans* isomers from both *cis-trans*, *trans-cis*, and *cis-cis* isomers especially in CLA-fortified milk powder as well as in fermented milk, yogurt, and milk-juice blends (Rodríguez-Alcalá and Fontecha, 2007). In this study, CLA contents increased in powdered milk, especially in those months in which the original raw milk had lower CLA values, which could be explained by increases in the *trans* isomers, though only three isomers were analysed. However, more research is needed to elucidate the exact effect of processing and storage conditions of milk and dairy products on the CLA content.

Conclusion

Higher values were found in raw milk obtained from regions with dairy farms based on grazing systems compared to regions with dairy farms based on total mixed ration and housing. Likewise, dairy products had significantly higher CLA content than raw milk. The most important CLA isomer across regions was the *cis*-9, *trans*-11 isomer, whereas the amounts of the isomers *trans*-10, *cis*-12 and *cis*-10, *cis*-12 were higher than those reported in the literature. Consumption of dairy products in Chile is lower than other countries, but the high amounts of CLA found in Chilean dairy products studied provide a level that is adequate for human health.

Milk processing significantly affected the transferring of CLA from raw milk into dairy products (sweetened condensed milk and powdered milk). For both, total CLA and isomers, sweetened condensed milk presented lower CLA contents than the original raw milk and powdered milk. Instead, powdered milk showed similar or above values to those observed in raw milk. More research is needed to elucidate the exact effect of processing and storage conditions of milk and dairy products on CLA content.

Acknowledgments

The authors thank CONICYT (the National Commission for Scientific and Technological Research) and the milk company for funding this research (FONDEF Project N° D02I1135 "Development of products with high content of functional active principles from cows' milk and its derivatives").

References

- Avilez, J.; Escobar, P.; Diaz, C.; von Fabek, G.; Matamoros, R.; García, F.; Alonzo, M.; Delgado-Pertíñez, M. 2012. Effect of extruded whole soybean dietary concentrate on conjugated linoleic acid concentration in milk in Jersey cows under pasture conditions. Spanish Journal of Agricultural Research 10: 409-418.

- Bisig, W.; Eberhard, P.; Collomb, M.; Rehberger, B. 2007. Influence of processing on the fatty acid composition and the content of conjugated linoleic acid in organic and conventional dairy products: a review. *Lait* 87: 1-19.
- Butler, G.; Nielsen, H.; Slots, T.; Seal, C.; Eyre, M.; Sanderson, R.; Leifert, C. 2008. Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. *Journal of the Science of Food and Agriculture* 88: 1431-1441.
- Chin, S.; Liu, W.; Storkson, J.M.; Ha, L.; Pariza, M. 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.
- Coakley, M.; Barrett, J.; Murphy, R.; Ross, R.; Stanton, C. 2007. Cheese manufacture with milk with elevated conjugated linoleic acid levels caused by dietary manipulation. *Journal of Dairy Science* 90: 2919-2927.
- Collomb, M.; Schmid, A.; Sieber, R.; Wechsler, D.; Ryhänen, E. 2006. Conjugated linoleic acids in milk fat: variation and physiological effects. *International Dairy Journal* 16: 1347-1361.
- Dewhurst, R.J.; Shingfield, K.J.; Lee, M.R.; Scollan, N.D. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* 131: 168-206.
- Fritsche, J.; Steinhart, H. 1998. Amounts of Conjugated Linoleic Acid (CLA) in German foods and Evaluation of daily intake. *Z Lebensm Unters Forsh* 206: 77-82.
- Gnädig, S.; Chamba, J.F.; Perread, E.; Chappaz, S.; Chardigny, J.M.; Rickert, R.; Steinhart, H.; Sébédio, J.L. 2004. Influence of manufacturing conditions on the conjugated linoleic acid content and the isomer composition in ripened French Emmental cheese. *Journal of Dairy Research* 71: 367-371.
- Gómez-Cortés, P.; Frutos, P.; Mantecón, A.R.; Juárez, M.; de la Fuente, M.A.; Hervás, G. 2009. Effect of supplementation of grazing dairy ewes with a cereal concentrate on animal performance and milk fatty acid profile. *Journal of Dairy Science* 92: 3964-3972.
- Henning, D.R.; Baer, R.J.; Hassan, A.N.; Dave, R. 2006. Major advances in concentrated and dry milk products, cheese and milk fat-based spreads. *Journal of Dairy Science* 89: 1179-1188.
- Herzallah, S.M.; Humeid, M.A.; Al-Ismail, K.M. 2005. Effect of heating and processing methods of milk and dairy products on conjugated linoleic acid and *trans* fatty acid isomer content. *Journal of Dairy Science* 88: 1301-1310.
- Hervás, G.; Luna, P.; Mantecón, A.R.; Castañares, N.; de la Fuente, M.A.; Juárez, M.; Frutos, P. 2008. Effect of diet supplementation with sunflower oil in milk production, fatty acid profile and ruminal fermentation in lactating dairy ewes. *Journal of Dairy Research* 75: 399-405.
- International Dairy Federation [IDF]. 1995. Milk and Milk Products. Guidance on Sampling. FIL-IDF, Brussels, Belgium. (Standard No. 50C).
- Ip, C.; Banni, S.; Angioni, E.; Carta, G.; McGinley, J.; Thompson, H.J.; Barbano, D.; Bauman, D. 1999. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *Journal of Nutrition* 129: 2135-2142.
- Laloux, L.; du Chaffaut, L.; Razanamahefa, L.; Lafay, L. 2007. *Trans* fatty acid content of foods and intake levels in France. *European Journal of Lipid Science and Technology* 109: 918-929.
- Lavillonnière, F.; Martin, J.C.; Bougnoux, P.; Sébédio, J.L. 1998. Analysis of conjugated linoleic acid isomers and content in French cheeses. *Journal of the American Oil Chemists Society* 75: 343-352.
- Leung, Y.H.; Liu, R.H. 2000. *Trans*-10, *cis*-12-conjugated linoleic acid isomer exhibits stronger oxyradical scavenging capacity than *cis*-9, *trans*-11-conjugated linoleic acid isomer. *Journal of Agriculture and Food Chemistry* 48: 5469-5475.
- Martins, S.V.; Lopes, P.A.; Alfaia, C.M.; Ribeiro, V.S.; Guerreiro, T.V.; Fontes, C.M.; Castro, M.F.; Soveral, G.; Prates, J.A. 2007. Contents of conjugated linoleic acid isomers in ruminant-derived foods and estimation of their contribution to daily intake in Portugal. *British Journal of Nutrition* 98: 1206-1213.
- Mir, P.S.; Okine, E.K.; Goonewardene, L.; He, M.L.; Mir, Z. 2003. Effects of synthetic conjugated linoleic acid (CLA) or bio-formed CLA as high CLA beef on rat growth and adipose tissue development. *Canadian Journal of Animal Science* 83: 583-592.
- Park, Y.; Albright, K.J.; Cai, Z.Y.; Pariza, M.W. 2001. Comparison of methylation procedures for conjugated linoleic acid and artifact formation by commercial (trimethylsilyl) diazomethane. *Journal of Agriculture and Food Chemistry* 49: 1158-1164.
- Parodi, P.W. 1999. Conjugate linoleic acid and other anticarcinogenic agents of bovine milk fat. *Journal of Dairy Science* 82: 1339-1349.
- Rego, O.A.; Rosa, H.J.; Regalo, S.M.; Alves, S.P.; Alfaia, C.M.; Prates, J.A.; Vouzela, C.M.; Bessa, R.J. 2008. Seasonal changes of CLA isomers and other fatty acids of milk fat from grazing dairy herds in the Azores. *Journal of the Science of Food and Agriculture* 88: 1855-1859.
- Ritzenthaler, K.L.; McGuire, M.K.; Falen, R.; Shultz, T.D.; Dasgupta, N.; McGuire, M.A. 2001. Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. *Journal of Nutrition* 131: 1548-1554.
- Rodríguez-Alcalá, L.M.; Fontecha, J. 2007. Fatty acid and conjugated linoleic acid (CLA) isomer composition of commercial CLA-fortified dairy products: evaluation after processing and storage. *Journal of Dairy Science* 90: 2283-2290.
- Sehat, N.; Kramer, J.K.; Mossoba, M.M.; Yurawecz, M.P.; Roach, J.G.; Eulitz, K.; Morehouse, K.M.; Ku, Y. 1998. Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. *Lipids* 33: 963-971.
- Semma, M. 2002. *Trans* fatty acids: properties, benefits and risks. *Journal of Health Science* 48: 7-13.
- Shantha, N.C.; Ram, L.N.; O'Leary, J.; Hicks, C.L.; Decker, E.A. 1995. Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. *Journal of Food Science* 60: 695-697.
- Thomson, N.A.; Chand, A.; Kay, J.K. 2003. Predicting $\Delta 9$ -desaturase activity and the association with conjugated linoleic acid (CLA) concentration in bovine milk. *Proceedings of the New Zealand Society of Animal Production* 63: 25-30.
- Ward, A.T.; Wittenberg, K.M.; Froebe, H.M.; Przybylski, R.; Malcolmson, L. 2003. Fresh forage and solin supplementation on conjugated linoleic acid levels in plasma and milk. *Journal of Dairy Science* 86: 1742-1750.
- Werner, S.A.; Luedecke, L.O.; Shultz, T.D. 1992. Determination of conjugated linoleic acid content and isomer distribution in three Cheddar-type cheeses: effects of cheese cultures, processing and aging. *Journal of Food Chemistry* 40: 1817-1821.