Advances in Dairy Research

Fernández D, et al., J Adv Dairy Res. 2015, 3:4
http://dx.doi.org/10.4172/2329-888X.1000147

Research Article Open Access

Variation of Fatty Acid Content in Zamorano-Type Ovine Cheese According to the Milk Conjugated Linoleic Acid Content

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Rec date: Sep 03, 2015; **Acc date:** Sep 30, 2015; **Pub date:** Oct 02, 2015

Abstract

A total of 30 Zamorano-type cheeses were manufactured in order to study the effects of milk conjugated linoleic acid (CLA) content, ripening time, and interactions between both effects on fatty acid (FA) profile. Cheeses elaborated from milk with a high CLA content showed higher contents of vaccenic, oleic, alpha-linolenic (ALA), CLA, monounsaturated (MUFA) and polyunsaturated (PUFA) FA, and lower contents of capric, lauric, myristic, palmitic, estearic, linoleic, and saturated (SFA) FA than cheese from milk with a low CLA content. Content of unsaturated C18 FA along with MUFA and PUFA groups increased throughout the ripening, while SFA content decreased. The interactions between milk CLA content and ripening time were significant for palmitic, linoleic, CLA, SFA, and omega-6/omega-3 ratio in Zamorano-type cheese. Decreases in cheese omega-6/omega-3 ratio were obtained from milk with a high CLA content; this ratio worsened over ripening in cheeses from low-CLA milk. In conclusion, these results emphasize the importance of the initial CLA content in milk with regard to improve the lipid profile in cheese for consumption.

Keywords: Sheep; Cheese; Fatty acids; Conjugated linoleic acid

Introduction

The world cheese production in 2010 was 20.698 thousand tonnes, 50% of which were produced in European countries [1], the mean consumption of cheese in several Mediterranean countries being greater than 20 kg per person and year. Ewe milk is a product that is high in fat and protein and is mainly used for commercial or artisanal cheeses. Fatty acids (FA) in cheese are becoming more and more important because of the link between the FA content in the diet and certain illnesses. Nevertheless, fat in dairy foods also contain compounds which can improve health like butyrate, sphingolipids, conjugated linoleic acid (CLA) and omega-3 [2,3]. The CLA is a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bond system. Omega-3 FA are a form of essential polyunsaturated fat, the most nutritionally notable being alpha-linolenic acid (C18:3 *cis*-9, *cis*-12, *cis*-15; ALA) in milk and cheese products. Biomedical studies with animal models have evidenced several profitable properties (anticarcinogenic, antiatherogenic, antioxidant, antiobesity, antidiabetic, etc) attributed to CLA [4,5], omega-3 [6,7], and other FA, which have been related to human health because of their unsaturated nature [8,9]. In addition, the relationship among FA in the final product is also important. Western diets are deficient in omega-3 FA, and have excessive amounts of omega-6 FA compared with the diet on which human beings evolved and their genetic patterns were established [10]. In addition, excessive amounts of omega-6 polyunsaturated FA and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 (a lower omega-6/omega-3 ratio), exert suppressive effects [11]. These aspects are of great interest in the cheese, which is a fat-product highly consumed in industrialized countries. In this context, the cheeses made from ewe's milk are characterized by a greater CLA content than those from other species [12] and there is evidence that the consumption of naturally enriched cheese in ALA, CLA and vaccenic FA possesses beneficial properties, since it ameliorates the plasma lipid profile and reduces endocannabinoid biosynthesis in hypercholesterolaemic subjects [13].

Factors influencing variation of FA content in ovine milk, such as flock, lactation stage, parity number, season or day of testing, have been studied in a previous paper [14], but very little information is disposable on the transference of these FA from milk to cheese and on the evolution of lipid profile during ripening [15]. In this sense, the variation of cheese lipid profile in function of milk CLA content could be of interest with view to final FA content because the cheese represents the main source of CLA for human consumption and an attempt should be made to know the relationship between the lipid profiles of milk and cheese and monitor their stability during cheese elaboration and ripening. Thus, we hypothesized that CLA level of milk is a main factor improving the final lipid profile of cheese for consumption.

Zamorano cheese is one of the best known among the ripened Spanish cheeses protected with designation of origin; it is a semi-hard non-cooked cheese, salted in brine, and produced in the Castilla and León region (Spain) from ewe´s milk. The shape is cylindrical (height and diameter less than 14 and 24 cm, respectively) and the weight range from 2.8 to 3.0 kg.

The objectives of this study were: first, to determine the transfer of main FA and FA groups from ewe's milk to Zamorano-type cheese in function of milk CLA level, second, to evaluate the importance of ripening time on cheese FA content, and third, to study the interactions between both effects. The results of the study will provide valuable information for future efforts to maximize the content of CLA and other beneficial FA in cheese products.

Materials and Methods

Sampling and cheese making

From the recording system of bulk tank milk lipid profile implemented in the flocks of the Association National of Churra sheep (ANCHE) in Castilla and León region (Spain), 3 dairy sheep flocks with high, medium and low CLA content were selected for this experiment. From bulk tank milk of these 3 flocks, 3 batches (150 L of milk each) of 5 Zamorano-type cheeses per batch were manufactured in duplicate (75 L of milk per manufacture) in order to study the effect of several factors influencing the FA content in the cheese. The effects tested were the FA content provided by the milk of high, medium, and low CLA content, and the ripening time. The 3 batches corresponded with the design shown in Table 1. All cheeses were made according to the next cheese making procedure. Pasteurized ewe´s milk with calcium chloride (0.2 g/L) is warmed to 30-32ºC and a commercial lyophilized starter culture (MA11, 0.8%; Table 1) was added after milk pasteurization. After 30 min of milk acidification, 25 mL of commercial lamb rennet (80% chymosin; 75 Rennet Units) were added per 100 L of milk. Coagulation took place over 35–40 min and the curd was cut until a size of a grain of rice. The curd was then heated to 37–38ºC, being continuously stirred until it reached the desired consistency.

Batch no.	CLA content (g/100 g of total fatty acids)	Heat treatment	Coagulation type	Starter culture (0.8%)	Ripening time (days)
1	High C18:2 *cis*-9, *trans*-11 (CLA): 1.83 C18:2 *trans*-10, *cis*-12 (CLA): 0.04	Pasteurized milk	Enzymatic	MA11[1]	1, 60, 120, 180, 240
2	Low C18:2 *cis*-9, *trans*-11 (CLA): 0.48 C18:2 *trans*-10, *cis*-12 (CLA): 0.01	Pasteurized milk	Enzymatic	MA11[1]	1, 60, 120, 180, 240
3	Medium C18:2 *cis*-9, *trans*-11 (CLA): 0.82 C18:2 *trans*-10, *cis*-12 (CLA): 0.02	Pasteurized milk	Enzymatic	MA11[1]	1, 60, 120, 180, 240

Table 1: Design of three batches of Zamorano cheese manufactured from ovine milk with high, low and medium CLA content.

[1]CHOOZIT™MA11LYO25DCU (Danisco, Sassenage, France): *Lactococcus lactis* subsp. *lactis* + Lc. *lactis* subsp. *cremoris*.

The whey was drained off and the curd was transferred to cylindrical moulds 14 cm deep by 24 cm diameter, and pressed over 4–5 hr until they reach a pH of 5.4–5.5. The curds with their characteristic cylindrical shape were salted in brine (17–18 ºBaumé; pH 5.4 and temperature 8-10 ºC) for between 16 and 18 hr. Finally, the cheeses were stored in ripening rooms at a temperature of approximately 10–12 ºC and a relative humidity of 85% for 240 days.

For each batch, duplicate one whole cheese of 3 kg was sampled at the ripening days indicated in Table 1. The cheese rind was discarded and the cheeses were minced and stored in hermetic containers at -30ºC until they were analyzed. The total number of cheeses analyzed was 30.

Milk samples were transported to the laboratory under refrigeration, were taken from each batch prior to addition of rennet and analyzed within 24 hr of sampling.

Analytical methods

The methods used in the analysis of basic chemical composition of milk were total solids by IDF Standard 021 [16]; protein by IDF Standard 020-5 [17]; fat by IDF Standard 105 [18]; lactose by IDF Standard 214 [19]. The pH was directly measured with a pHmeter GLP 22 (Crison, Barcelona, Spain). Titratable acidity was measured according to AOAC Standard 947.05 [20].

Dry matter, fat and salt in cheese were determined according to IDF Standards 004 [21] and 222 [22], and AOAC Standard 935.43 [23], respectively. The pH and titrable acidity of cheeses were measured using AOAC Standards 14022 [24] and 920.124 [25], respectively. Cheese water activity was determined by Aqualab Dew Point Analyzer (Decagon Devices Inc., Pullman, WA, USA).

Milk fat extraction was carried out by the method of Bligh and Dyer [26] using chloroform and methanol. Fat of cheese samples were extracted by the method of De Jong and Badings [27]. Lipid extracts were stored at −30°C until further analysis. Fatty acid analysis and identification was carried out according to methodology previously describes [14]. Briefly, fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of glycerides (NaOCH3) according to Aldai et al. [28]. FAME were separated by gas chromatography using a Tekno TR-CN 100 capillary column (60 m × 0.25 mm i.d. × 0.2-µm film thickness; Teknokroma, Barcelona, Spain) on a Hewlett-Packard chromatograph (model 6890, Wilmington, DE) equipped with an automatic injector (model 7683, Hewlett-Packard) and a mass selective detector (model 5973, Hewlett-Packard). The injection and detector temperatures were 230ºC. Helium was used as a carrier gas at a flow rate of 1 mL/min. Samples (1 µL) were injected into a split injector (split ratio 10:1).

	Milk					Cheese				
Trait[1,2]	**Median**	**SD**	**Min.**	**Max.**	**CV(%)**	**Median**	**SD**	**Min.**	**Max.**	**CV(%)**
C4:0	2.30	0.21	2.13	2.54	9.38	2.30	0.25	2.05	3.51	11.09
C6:0	2.20	0.10	2.09	2.29	4.61	2.29	0.17	2.03	2.78	7.51
C8:0	3.19	0.20	2.97	3.36	6.26	3.29	0.25	2.40	3.52	7.60
C10:0	8.53	0.13	8.39	8.63	1.51	8.26	0.57	7.33	9.34	6.95
C12:0	4.64	0.04	4.59	4.67	0.94	4.87	0.33	4.38	5.66	6.84
C13:0	0.16	0.01	0.15	0.18	9.35	0.16	0.03	0.10	0.22	21.95
C14:0iso	0.13	0.02	0.11	0.15	16.43	0.15	0.01	0.11	0.17	9.91
C14:0	9.70	0.34	9.31	9.92	3.52	9.68	0.42	9.05	10.51	4.38
C15:0iso	0.40	0.01	0.39	0.41	2.50	0.38	0.03	0.32	0.45	9.10
C14:1 *cis*-9	0.59	0.03	0.56	0.62	5.21	0.65	0.07	0.49	0.77	11.79
C15:0	1.13	0.01	1.12	1.14	0.88	1.24	0.08	1.09	1.38	6.81
C16:0iso	0.43	0.06	0.36	0.47	14.14	0.43	0.06	0.16	0.52	15.20
C16:0	22.21	2.21	19.68	23.73	9.94	21.63	1.98	19.62	25.02	9.17
C17:0	1.12	0.23	0.86	1.32	21.05	0.88	0.20	0.59	1.22	22.59
C16:1 *cis*-9	1.63	0.10	1.52	1.70	6.04	1.49	0.13	1.22	1.77	8.43
C17:0antiso	1.11	0.12	1.00	1.24	11.04	0.98	0.10	0.76	1.21	10.58
C18:0iso	0.14	0.02	0.13	0.16	12.37	0.15	0.03	0.08	0.20	20.88
C17:1 *cis*-10	0.42	0.09	0.32	0.48	21.17	0.36	0.09	0.14	0.47	23.88
C18:0	10.05	0.60	9.46	10.66	5.97	10.28	0.55	9.37	11.29	5.37
C18:1 *trans*-11	2.76	0.24	2.60	3.05	8.91	2.82	0.27	2.39	3.21	9.54
C18:1 *cis*-9	17.11	1.55	16.07	18.90	9.08	17.54	1.45	14.78	19.89	8.28
C18:1 *cis*-11	0.79	0.07	0.74	0.87	9.19	0.80	0.08	0.67	0.92	9.77
C18:1 *cis*-12	0.69	0.07	0.65	0.77	9.60	0.70	0.07	0.59	0.81	9.61
C18:2 *trans*-9, *trans*-12	0.51	0.03	0.47	0.53	6.79	0.70	0.29	0.38	1.18	41.72
C18:2 *cis*-9, *cis*-12	4.14	0.14	3.99	4.26	3.32	3.82	0.51	3.13	4.85	13.49
C20:0	0.37	0.03	0.33	0.39	9.36	0.36	0.04	0.29	0.47	11.10
C18:3 *cis*-6, *cis*-9, *cis*-12	0.04	0.01	0.03	0.06	35.25	0.06	0.01	0.03	0.10	26.68
C20:1 *cis*-9	0.02	0.01	0.01	0.03	50.00	0.03	0.01	0.01	0.05	30.98
C18:3 *cis*-9, *cis*-12, *cis*-15 (ALA)	1.28	0.20	1.05	1.41	15.76	1.50	0.36	0.95	2.04	24.38
C18:2 *cis*-9, *trans*-11 (CLA)	1.04	0.70	0.48	1.83	67.30	1.11	0.48	0.67	1.81	43.00
C18:2 *trans*-10, *cis*-12 (CLA)	0.02	0.01	0.01	0.04	65.46	0.03	0.01	0.01	0.04	38.41
C21:0	0.18	0.06	0.14	0.25	33.79	0.18	0.07	0.11	0.30	40.47
C22:0	0.24	0.05	0.20	0.30	23.27	0.20	0.03	0.13	0.25	16.06
C20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14	0.41	0.05	0.35	0.44	12.13	0.43	0.11	0.22	0.68	24.57

C23:0	0.16	0.01	0.15	0.16	3.68	0.13	0.02	0.10	0.18	19.66
C24:0	0.13	0.01	0.11	0.14	12.06	0.10	0.02	0.07	0.13	14.57
	Milk					**Cheese**				
Trait[1,2]	**Median**	**SD**	**Min.**	**Max.**	**CV(%)**	**Median**	**SD**	**Min.**	**Max.**	**CV(%)**
SFA	68.52	2.94	65.12	70.26	4.30	67.95	2.79	64.00	72.79	4.11
SCFA	16.22	0.21	15.99	16.38	1.27	16.13	0.72	14.82	17.84	4.49
MCFA	16.16	0.36	15.75	16.38	2.21	16.49	0.59	15.56	17.44	3.57
LCFA	36.13	2.65	33.07	37.81	7.35	35.32	1.92	32.79	38.49	5.44
MUFA	24.02	2.05	22.48	26.35	8.53	24.40	2.04	20.54	27.54	8.37
PUFA	7.45	0.99	6.57	8.53	13.35	7.65	0.84	6.28	8.96	10.96
Ratio omega-6/omega-3	3.64	0.61	3.23	4.34	16.79	3.10	1.05	1.75	4.96	33.88

Table 2: Basic statistics of milk (n=3) and Zamorano-type cheese (n=30) fatty acid content.

[1]ALA=alfa linolenic acid; CLA=Conjugated linoleic acid; SFA=sum of saturated fatty acids; SCFA=sum of short-chain saturated fatty acids; MCFA=sum of medium-chain saturated fatty acids; LCFA=sum of long-chain saturated fatty acids; MUFA=sum of monounsaturated fatty acids; PUFA=sum of polyunsaturated fatty acids.

[2]Grams per 100 g of total fatty acids.

Reference standards were used to determine recoveries and correction factors for individual FA (Supelco 37 Component FAME MIX and linoleic conjugated acid methyl ester, Sigma-Aldrich, Bellafonte, PA, USA).Identification of fatty acids and trans isomers was carried out by retention time and quantified by comparing the retention times and areas of their peaks to those observed for their respective standards, using nonanoic acid (C9) as an internal standard. The individual FA contents were expressed as weight percentages (g/100 g of total FA). All analyses were made in duplicate.

Dependent variables for the study of variation factors

Although 36 FA were initially determined analytically (Table 2), the statistical study of the variation factors was restricted to the most important 13 FA, 6 groups of FA, and 1 FA index, all of which were treated as dependent variables. The groups, which were based on the saturation level and chain length, were as follows: sum of saturated FA (SFA), sum of short-chain saturated FA (C4 to C10, SCFA), sum of medium-chain saturated FA (C12 to C15, MCFA), and sum of long-chain saturated FA (C16 to C24, LCFA). The 2 remaining groups were the sum of monounsaturated FA (MUFA), and the sum of polyunsaturated FA (PUFA). The index considered was the omega-6/omega-3 ratio, being omega-6 the sum of linoleic (C18:2 *cis*-9, *cis*-12) + gamma linolenic (C18:3 *cis*-6, *cis*-9, *cis*-12) + araquidonic (C20:4 *cis*-5, *cis*-8, *cis*-11, *cis*-14) FA, and omega-3 the ALA (C18:3 *cis*-9, *cis*-12, *cis*-15). The criteria for selecting the 13 FA as dependent variables were as follows: 1) considering the 11 quantitative most important FA, with percentages above 2.0% [14], and 2) including 2 additional FA of biological interest, which are CLA and ALA.

Statistical analysis

The statistical analysis was carried out by the GLM procedure of SAS [29]. The model used was:

$$Y_{ijk}=\mu+C_i+R_j+CR_{ij}+e_{ijk}$$

where Y_{ijk} refers to the 20 dependent variables of cheeses; that is, 13 FA, 6 groups of FA, and 1 FA index previously described. The factor C_i was the CLA level of milk (3 levels: high, low, and medium; Table 1). The factor R_j was the ripening time of cheese (5 levels: 1, 60, 120, 180, 240 days; Table 1). The factor CR_{ij} was the interaction between CLA level of milk and cheese ripening time. The factor e_{ijk} was the residual effect. Water activity, pH, titratable acidity, total solids, fat and salt content of cheeses were non-significant co-variables for the most of the dependent variables, and were excluded from the statistical models.

This research was carried out in accordance with EU Directive 2010/63/EU for animal experiments.

Results and Discussion

The statistics of the FA studied in milk and cheese are shown in Table 2. Similarly to other studies in ovine milk [14,30], some of the SFA, such as palmitic (C16:0, 22.2% and 21.6%), stearic (C18:0, 10.1% and 10.3%), capric (C10:0, 8.5% and 8.3%), myristic (C14:0, 9.7% and 9.7%), and lauric (C12:0, 4.6% and 4.9%) acids had relatively high contents in milk and cheese, respectively (Table 2). Similarly the contents of oleic acid (C18:1 *cis*-9, 17.1% and 17.5%) and linoleic acid (C18:2 *cis*-9 *cis*-12, 4.1% and 3.8%) were also high in milk and cheese, respectively. The C18:2 *cis*-9 *trans*-11 CLA isomer had a similar content in milk (1.04%) and cheese (1.11%), lower than the content of vaccenic (C18:1 *trans*-11, 2.8% and 2.8%) and ALA (C18:3 *cis*-9, *cis*-12, *cis*-15, 1.3% and 1.5%) acids. High SFA (68.5% and 68.0%), LCFA (36.1% and 35.3%) and MUFA (24.0% and 24.4%) contents, and low PUFA (7.5% and 7.7%) content and omega-6/omega-3 ratio (3.6 and 3.1) were also observed in ovine milk and cheese, respectively. Quantitative contents in FA, FA groups and FA index were very similar in both products; thus, a close relationship could be predicted between the milk and cheese lipid profiles.

Table 3 showed the test of significance for all studied effects on cheese FA profile. The CLA content and ripening time significantly contributed to variation of final FA profile. The importance of CLA content x ripening time interaction was lesser.

The CLA content of milk significantly contributed ($p<0.001$) to variation of the CLA and other FA related (ALA, linoleic, oleic, and vaccenic) in the cheese because the relationship among the unsaturated C18 FA family [14]. Also, CLA content of milk significantly influenced ($p<0.001$) the variation of MCFA and LCFA (C12:0 to C18:0), such as lauric, myristic, palmitic, and stearic FA (Tables 3 and 4).

Variable[1]	CLA content (df=2)	Ripening time (df=8)	Interaction (df=8)	R^2
C4:0	0.47NS	0.78NS	0.69NS	0.39
C6:0	5.36*	1.23NS	3.93*	0.76
C8:0	1.64NS	2.09NS	2.10NS	0.65
C10:0	4.65*	12.52***	1.71NS	0.83
C12:0	32.17***	5.57**	2.65*	0.88
C14:0	22.93***	14.16***	0.99NS	0.88
C16:0	89.36***	24.39***	7.28***	0.96
C18:0	37.77***	2.42NS	1.15NS	0.86
C18:1 *trans*-11	259.53***	61.70***	8.08NS	0.98
C18:1 *cis*-9	107.35***	27.76***	2.30NS	0.96
C18:2 *cis*-9, *cis*-12	127.84***	11.71***	9.29***	0.96
C18:3 c-9, c-12, c-15 (ALA)	225.40***	7.79**	3.48*	0.97
C18:2 *cis*-9, *trans*-11 (CLA)	2838.84***	13.21***	4.84**	0.99
SFA	156.98***	38.46***	5.18**	0.97
SCFA	4.25*	5.06**	0.43NS	0.93
MCFA	45.00***	24.25***	3.93*	0.94
LCFA	75.20***	12.67***	3.89*	0.94
MUFA	143.35***	34.61***	3.70*	0.97
PUFA	45.28***	10.98***	3.56*	0.92
Ratio omega-6/omega-3	480.84***	0.87NS	4.94**	0.98

Table 3: Analysis of variance (F values, significance and R^2 of the model) for fatty acid content in Zamorano-type cheese.

[1]ALA=alfa linolenic acid; CLA=Conjugated linoleic acid; SFA=sum of saturated fatty acids; SCFA=sum of short-chain saturated fatty acids; MCFA=sum of medium-chain saturated fatty acids; LCFA=sum of long-chain saturated fatty acids; MUFA=sum of monounsaturated fatty acids; PUFA=sum of polyunsaturated fatty acids.

NS: Non-significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$.

With the only exception of linoleic acid, Table 4 showed increased values ($p<0.001$) of unsaturated C18 FA family in cheeses manufactured from milk with high CLA content in comparison with those values found in cheeses obtained from milk with low CLA content. In addition, milk CLA content affected the cheese content of stearic, palmitic, myristic and lauric FA ($p<0.01$ to $p<0.001$), but in a negative sense (Table 4), probably due to the negative correlations among CLA content and C12, C14, C16 and C18 saturated FA contents.

Variable[1,2]	Low	Medium	High	p-value
C4:0	2.30^a	2.36^a	2.24^a	NS
C6:0	2.38^a	2.21^b	2.28^{ab}	*
C8:0	3.31^a	3.35^a	3.19^a	NS
C10:0	8.47^a	8.29^{ab}	8.02^b	*
C12:0	4.98^a	5.10^a	4.55^b	***
C14:0	9.95^a	9.76^a	9.34^b	***
C16:0	22.75^a	22.47^a	19.66^b	***
C18:0	10.49^a	10.69^a	9.65^b	***
C18:1 *trans*-11	2.68^b	2.67^b	3.12^a	***
C18:1 *cis*-9	16.71^b	16.81^b	19.10^a	***
C18:2 *cis*-9, *cis*-12	4.16^a	4.05^a	3.25^b	***
C18:3 *cis*-9, *cis*-12, *cis*-15 (ALA)	1.07^c	1.55^b	1.88^a	***
C18:2 *cis*-9, *trans*-11 (CLA)	0.73^c	0.83^b	1.77^a	***
SFA	69.66^a	69.24^a	64.96^b	***
SCFA	16.46^a	16.21^{ab}	15.73^b	*
MCFA	16.79^a	16.71^a	15.99^b	***
LCFA	36.41^a	36.32^a	33.24^b	***
MUFA	23.32^b	23.25^b	26.62^a	***
PUFA	7.01^c	7.51^b	8.43^a	***
Ratio omega-6/omega-3	4.40^a	2.94^b	1.96^c	***

Table 4: Least squares means for fatty acid content in Zamorano-type cheese by CLA content level of milk (low, medium and high).

[1]ALA=alfa linolenic acid; CLA=Conjugated linoleic acid; SFA=sum of saturated fatty acids; SCFA=sum of short-chain saturated fatty acids; MCFA=sum of medium-chain saturated fatty acids; LCFA=sum of long-chain saturated fatty acids; MUFA=sum of monounsaturated fatty acids; PUFA=sum of polyunsaturated fatty acids.

[2]Grams per 100 g of total fatty acids.

[a,b,c]Means in the same row with different superscripts differ ($p<0.05$).

NS: Non-significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$.

As a whole, cheeses elaborated from milk with a high CLA content showed increased ($p<0.001$) vaccenic, oleic, ALA, CLA, MUFA and

PUFA contents and decreased lauric, myristic, palmitic, estearic, linoleic, SFA, MCFA, LCFA contents and omega-6/omega-3 ratio (Table 4).Indeed, according results evidenced in Table 4, omega-6/omega3 ratio was much improved in the cheeses with a high CLA content (1.96) in comparison with cheeses with a low CLA content (4.40).

Variable[1,2]	Ripening time (days)					p-value
	1	60	120	180	240	
C4:0	2.47^{a}	2.25^{a}	2.24^{a}	2.28^{a}	2.24^{a}	NS
C6:0	2.31^{a}	2.28^{a}	2.22^{a}	2.36^{a}	2.26^{a}	NS
C8:0	3.23^{a}	3.20^{a}	3.46^{a}	3.36^{a}	3.18^{a}	NS
C10:0	8.38^{b}	8.49^{ab}	8.87^{a}	7.78^{c}	7.79^{c}	***
C12:0	4.84^{bc}	5.03^{ab}	5.05^{a}	4.70^{c}	4.76^{c}	**
C14:0	9.75^{b}	9.83^{b}	10.09^{a}	9.34^{c}	9.40^{c}	***
C16:0	22.91^{a}	22.50^{ab}	21.86^{b}	20.52^{c}	20.35^{c}	***
C18:0	10.24^{a}	10.16^{a}	10.08^{a}	10.41^{a}	10.52^{a}	NS
C18:1 *trans*-11	2.66^{c}	2.72^{b}	2.74^{b}	2.99^{a}	3.00^{a}	***
C18:1 *cis*-9	16.69^{b}	17.09^{b}	16.93^{b}	18.51^{a}	18.49^{a}	***
C18:2 *cis*-9, *cis*-12	3.67^{b}	3.66^{b}	3.71^{b}	4.05^{a}	4.02^{a}	***
C18:3 *cis*-9, *cis*-12, *cis*-15 (ALA)	1.47^{b}	1.41^{b}	1.42^{b}	1.61^{a}	1.60^{a}	**
C18:2 *cis*-9, *trans*-11 (CLA)	1.07^{b}	1.08^{b}	1.09^{b}	1.16^{a}	1.18^{a}	***
SFA	69.44^{a}	68.96^{a}	69.07^{a}	66.17^{b}	66.12^{b}	***
SCFA	16.40^{a}	16.21^{a}	16.80^{a}	15.78^{b}	15.47^{b}	**
MCFA	16.49^{b}	16.72^{b}	17.05^{a}	16.00^{c}	16.20^{c}	***
LCFA	36.55^{a}	36.03^{a}	35.22^{b}	34.39^{c}	34.44^{c}	***
MUFA	23.17^{b}	23.73^{b}	23.67^{b}	25.70^{a}	25.73^{a}	***
PUFA	7.39^{b}	7.31^{b}	7.26^{b}	8.14^{a}	8.16^{a}	***
Ratio omega-6/omega-3	3.08^{a}	3.03^{a}	3.04^{a}	3.16^{a}	3.18^{a}	NS

Table 5: Least of squares means for cheese fatty acid content by ripening time (days) in Zamorano-type cheese.

[1]ALA=alfa linolenic acid; CLA=Conjugated linoleic acid; SFA=sum of saturated fatty acids; SCFA=sum of short-chain saturated fatty acids; MCFA=sum of medium-chain saturated fatty acids; LCFA=sum of long-chain saturated fatty acids; MUFA=sum of monounsaturated fatty acids; PUFA=sum of polyunsaturated fatty acids.

[2]Grams per 100 g of total fatty acids.

[a,b,c,d]Means in the same row with different superscripts differ ($p<0.05$). NS: Non-significant; **$p<0.01$; ***$p<0.001$.

These results evidenced that cheeses elaborated from milk with a high CLA content improved their lipid profile from the point of view of human health, by increasing the content of CLA, ALA and unsaturated FA and decreasing omega-6/omega-3 ratio.

The effect of ripening time is showed in Table 5. The content of all unsaturated C18 FA along with MUFA and PUFA groups increased ($p<0.01$ to $p<0.001$) throughout the ripening, while SFA content decreased ($p<0.01$ to $p<0.001$).Therefore, cheese with long ripening periods showed a healthier lipid profile than those with short ripening ones due to beneficial FA enrichment over ripening. These results were also in agreement with those obtained by [31] for the transfer of C18:1 and C18:2 FA isomers from ewe's milk to Peccorino Toscano cheese.

Variable[1,2]	Ripening time (days)					p-value
	1	60	120	180	240	
C16:0						7.28***
High CLA	19.67^{a}	19.74^{a}	19.62^{a}	19.64^{a}	19.63^{a}	
Low CLA	24.74^{a}	24.36^{a}	22.60^{b}	21.67^{b}	20.36^{c}	
Medium CLA	24.32^{a}	23.39^{a}	23.36^{a}	20.23^{b}	21.06^{b}	
C18:2 *cis*-9, *cis*-12						9.29***
High CLA	3.46^{a}	3.33^{a}	3.17^{b}	3.13^{b}	3.14^{b}	
Low CLA	3.94^{b}	3.88^{b}	4.00^{b}	4.37^{a}	4.62^{a}	
Medium CLA	3.61^{d}	3.76^{dc}	3.95^{c}	4.64^{b}	4.30^{a}	
C18:2 *cis*-9, *trans*-11 (CLA)						4.84**
High CLA	1.81^{a}	1.72^{b}	1.73^{bc}	1.79^{ac}	1.80^{a}	
Low CLA	0.67^{c}	0.74^{ab}	0.71^{bc}	0.75^{ab}	0.80^{a}	
Medium CLA	0.72^{c}	0.77^{bc}	0.81^{b}	0.93^{a}	0.93^{a}	
SFA						5.18**
High CLA	65.25^{ab}	65.03^{ab}	66.26^{a}	64.04^{b}	64.20^{b}	
Low CLA	71.13^{a}	71.43^{a}	70.39^{a}	68.26^{b}	67.08^{b}	
Medium CLA	71.93^{a}	70.41^{b}	70.57^{b}	66.19^{c}	67.07^{c}	
Ratio omega-6/omega-3						4.94**
High CLA	1.99^{ab}	2.11^{ab}	2.14^{a}	1.79^{ab}	1.76^{b}	
Low CLA	4.30^{b}	4.03^{b}	4.09^{b}	4.72^{a}	4.85^{a}	
Medium CLA	2.95^{a}	2.94^{a}	2.91^{a}	2.96^{a}	2.94^{a}	

Table 6: Main interactions between milk CLA level and ripening time for fatty acid content in Zamorano-type cheese.

[1]CLA=Conjugated linoleic acid; SFA=sum of saturated fatty acids.

[2]Grams per 100 g of total fatty acids.

[a,b,c,d]Means in the same row with different superscripts differ ($p<0.05$).

**$p<0.01$; ***$p<0.001$.

In addition, several authors [12,31,32] found that C18:2 *cis*-9, *trans*-11 isomer increased between 10% and 26% on total content of

CLA during ripening of different cheeses, which is also compatible with our results. This CLA increase has been associated to hydrogen donors in processed cheese [33]. Nevertheless, in other studies [34] the *cis*-9, *trans*-11 CLA decreased as consequence of biohydrogenation or of double bonds isomerisation, while the concentration of *trans*-10, *cis*-12 CLA increased.

The interactions between milk CLA content and ripening time were important effects ($p<0.01$) for palmitic, linoleic, CLA, SFA, and omega-6/omega-3 ratio in Zamorano-type cheese (Table 6).Thus, linoleic and CLA FA increased over ripening for cheeses manufactured from milk with low and medium CLA levels, whereas linoleic decreased and CLA showed a small variation over ripening from milk with a high CLA level. An opposite trend was found for SFA. The ratio omega-6/omega-3 increased ($p<0.05$) throughout the ripening in cheeses manufactured from milk with a low CLA content, and remained unchanged for medium CLA contents. These interactions have not been studied up to now and would explain some discrepancies about the CLA evolution over ripening found by several authors [12,31,32,34].

Conclusions

The lipid profile of ovine cheese was significantly influenced by CLA content of milk and ripening time. The highest vaccenic, oleic, ALA, CLA, MUFA and PUFA contents were for cheeses elaborated from milk with a high CLA content, and at the end of ripening time. On the contrary, SFA were highest in cheeses from a low-CLA milk and at beginning of ripening. Omega-6/omega-3 ratio was more beneficial in cheeses originated from milk with a high CLA content; this ratio worsened over ripening in cheeses from a low-CLA milk. As a whole, these results emphasize the importance of the initial CLA content in milk with regard to improve the lipid profile in cheese for consumption.

Acknowledgments

This work was supported by the Spanish Ministry of Science (Madrid, Spain; project AGL2005-04321) and the Castilla and León regional government (Junta de Castilla y Leon, Valladolid, Spain) by a grant for research groups of excellence (UIC number 056). The authors are grateful to ANCHE farmers for their participation in this study.

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