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Original article

Fatty acid composition and nutritional value of fat in three PDO ewe's milk Portuguese cheeses

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Abstract – The aim of this study was to evaluate the composition and nutritional quality of fat in three Protected Designation of Origin (PDO) ewe's milk Portuguese cheeses: Azeitão, Nisa and Évora. Fatty acid (FA) composition of cheeses and raw milks was determined by gas chromatography (GC) and individual conjugated linoleic acid (CLA) isomers were analyzed by Ag^+-HPLC. Fat from Azeitão, Nisa and Évora cheeses showed some differences in FA composition, in particular for oleic acid contents: 15.4, 17.1 and 19.7 g·100 g^{-1} of FA, respectively, for Azeitão, Évora and Nisa cheeses with extended ripening. Also, some differences were observed in CLA isomer contents, with values for total CLA reaching 1.15 g·100 g^{-1} of FA for Nisa cheeses, while Azeitão cheeses revealed lower levels for total CLA (0.90 g·100 g^{-1} of FA). Values for oleic and stearic acids, monounsaturated fatty acids (MUFA) and CLA were significantly higher for Nisa cheeses. Also, significant differences were found between milk and cheese samples, especially for MUFA (21.0 g·100 g^{-1} of FA for milk and 17.5 g·100 g^{-1} of FA for 3-month-old cheese) and *trans* fatty acids (TFA), with 3.98 g·100 g^{-1} of FA for milk and 4.57 g·100 g^{-1} of FA for 3-month-old cheeses from Azeitão. The two major CLA isomers (*cis*-9,*trans*-11 and *trans*-7,*cis*-9) also seem to be affected by the processing of milk into cheese, particularly for Nisa cheeses, as suggested by the significant differences obtained for *cis*-9,*trans*-11 levels (68.7 mg·g^{-1} of fat for milk and 73.8 mg·g^{-1} of fat for 3-month-old cheeses) and for *trans*-7,*cis*-9 levels (15.8 mg·g^{-1} of fat for milk and 10.1 mg·g^{-1} of fat for 3-month-old cheeses). In addition, the relatively high contents of CLA and low values of the n-6/n-3 ratio suggest good health-related parameters. However, the values of 0.06 obtained for the relation polyunsaturated/saturated fatty acids (PUFA/SFA index) were consistently below the recommended guidelines for the human diet (0.45).

ewe's milk cheese / conjugated linoleic acid (CLA) / fatty acid / composition

摘要 – 三种 PDO 葡萄牙羊奶干酪乳脂肪的脂肪酸组成和营养。本文评价了三种原产地名号保护 (PDO) 葡萄牙羊奶干酪 (Azeitão 干酪、Nisa 干酪和 Évora 干酪) 脂肪的组成和营养品质。采用气相色谱法 (GC) 测定干酪和原料乳的脂肪酸组成和银离子高效液相色谱法 (Ag^+-HPLC) 测定共轭亚油酸 (CLA) 异构体。Azeitão, Nisa 和 Évora 干酪脂肪的脂肪酸组成有一定的差异,特别是 Azeitão, Évora 和 Nisa 干酪中油酸占脂肪酸的含量分别 15.4, 17.1 和 19.7 g·100 g^{-1}。同样三种干酪共轭亚油酸异构体含量有一定的差异, Nisa 干酪中总共轭亚油酸占脂肪酸的量达到 1.15 g·100 g^{-1}, 而 Azeitão 干酪中总共轭亚油酸的含量较低 (0.90 g·100 g^{-1} FA) 。Nisa 干酪的油酸、硬脂肪酸、单不饱和脂肪酸

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(MUFA) 和共轭亚油酸含量显著地高于其他两种干酪。原料奶和 Azeitão 干酪脂肪酸含量之间存在显著性的差异, 特别是单不饱和脂肪酸 (原料奶: 21.0 g·100 g^{-1} FA, 成熟 3 个月干酪: 17.5 g·100 g^{-1} FA) 和反式脂肪酸 (原料奶: 3.98 g·100 g^{-1} FA, 成熟 3 个月干酪: 4.57 g·100 g^{-1} FA) 。在一定程度上, 干酪加工条件可以显著地影响两个主要共轭亚油酸异构体 (c9，t11 和 t7，c9) 的含量, 特别是 Nisa干酪, 其原料奶和干酪在 *cis*-9 及 *trans*-11 (原料奶: 68.7 mg·g^{-1} 脂肪, 成熟 3 个月干酪: 73.8 mg·g^{-1} 脂肪) 和 *trans*-7 及 *cis*-9 (原料奶: 15.8 mg·g^{-1} 脂肪, 成熟 3 个月干酪: 10.1 mg·g^{-1} 脂肪) 含量上有显著性的差异。尽管三种干酪的多不饱和酸与饱和脂肪酸比值 (0.06, PUFA/SFA 指数) 低于人类饮食推荐值 (0.45), 但较高的共轭亚油酸含量和低比例的 n-6/n-3 对人体健康还是有益的。

羊奶干酪 / 共轭亚油酸 (CLA) / 脂肪酸 / 组成

Résumé – Composition en acides gras et valeur nutritionnelle de la matière grasse de trois fromages portugais avec Dénomination d'Origine Protégée. L'évaluation de la composition et de la qualité nutritionnelle de la matière grasse de trois fromages portugais au lait de brebis d'Appellation d'Origine Protégée, Azeitão, Nisa et Évora, a fait l'objet de cette étude. La composition en acides gras (AG) des fromages et des laits crus utilisés dans la fabrication a été déterminée par chromatographie en phase gazeuse et les différents isomères de l'acide linoléique conjugué (CLA) séparés par chromatographie liquide haute performance à l'argent. La composition en acides gras des fromages Azeitão, Nisa et Évora présentait quelques différences, en particulier pour la teneur en acide oléique qui était respectivement de 15,4, 17,1 and 19,7 g·100 g^{-1} d'acides gras dans les fromages à affinage prolongé. Les acides oléique et stéarique, les acides gras mono-insaturés et les CLA étaient significativement plus abondants dans le Nisa. De plus, des différences significatives ont été trouvées entre le lait et le fromage d'Azeitão, en particulier pour la teneur en acides gras mono-insaturés (21,0 g·100 g^{-1} d'AG pour le lait et 17,5 g·100 g^{-1} d'AG pour le fromage à 3 mois d'affinage) et en acides gras *trans* (3,98 g·100 g^{-1} d'AG pour le lait et 4,57 g·100 g^{-1} d'AG pour le fromage à 3 mois d'affinage). Le procédé de transformation a affecté les deux isomères majeurs du CLA (*cis*-9,*trans*-11 et *trans*-7,*cis*-9), en particulier pour le Nisa, comme le montrent les différences significatives obtenues pour le *cis*-9,*trans*-11 (68,7 mg·g^{-1} de matière grasse pour le lait et 73,8 mg·g^{-1}de matière grasse pour le fromage à 3 mois d'affinage) et pour le *trans*-7,*cis*-9 (15,8 mg·g^{-1} de matière grasse pour le lait et 10,1 mg·g^{-1}de matière grasse pour le fromage à 3 mois d'affinage). De plus, les valeurs relativement élevées en CLA et les faibles valeurs du ratio n-6/n-3 suggèrent des caractéristiques nutritionnelles positives pour les fromages étudiés. Au contraire, les valeurs de l'index PUFA/SFA étaient inférieures à celles recommandées sur le plan nutritionnel.

fromage au lait de brebis / acide linoléique conjugué / acides gras / composition

1. INTRODUCTION

The analysis of fatty acid (FA) composition is essential for the nutritional quality evaluation of the lipid fraction in foods. Milk and dairy products, mainly cheese, are usually associated with high levels of long-chain saturated FA (SFA), mainly palmitic (16:0) and stearic (18:0) acids [13]. In the case of ewe's milk and cheese, higher values of medium-chain triacylglycerols, made up of FA with 6–10 atoms of carbon, especially capric acid (10:0), are characteristic of their lipids [3, 22]. These volatile FA are usually released during cheese ripening, although only at low levels, and are responsible for the characteristic flavor of ewe's and goat's cheeses [10, 24]. In spite of the high levels of SFA in milk fat, milk and cheese are known to play an important role in human nutrition and, more recently, were also recognized as a source of biologically-active substances [7, 25].

Conjugated isomers of *cis*-9,*cis*-12 octadecadienoic acid (linoleic acid), commonly known as conjugated linoleic acid (CLA), are a family of positional and

geometric isomers of linoleic acid, with conjugated double bonds, i.e., double bonds separated by a single carbon-carbon linkage rather than by the usual methylene group [7]. A large number of reports refer to the potential beneficial effects on health of CLA, mainly in animal models of human diseases and in cultures of various types of cells [19, 28, 32]. Some of the CLA isomers (*cis*-9,*trans*-11 and *trans*-10,*cis*-12) exhibit interesting biological activities that include anticarcinogenic, anti-obesity, antidiabetogenic, anti-atherogenic, immunomodulation and modulation of bone growth [4].

It is well established that milk and milk products are one of the major dietary sources of CLA. The main CLA isomer in ruminant fats is the *cis*-9,*trans*-11 CLA (rumenic acid), which comprises about 75–90% of total CLA in milk and dairy products [7, 16]. It is formed as an intermediate during biohydrogenation of the ingested polyunsaturated FA in the rumen and from the endogenous desaturation of vaccenic acid (*trans*-11 18:1) in the mammary gland [16, 17]. A total of 70% of rumenic acid in milk fat is derived from this last process [32].

Azeitão with Protected Designation of Origin (PDO), Évora PDO and Nisa PDO cheeses are traditional Portuguese ewe's milk cheeses made from fresh raw milk, using *Cynara cardunculus* L. extract as coagulant. They have a considerable commercial relevance and are distinguished not only by their physical characteristics (they are usually associated with a smooth and creamy paste), but also by their unique flavor [27].

When compared with the number of reported studies on CLA from bovine milk fats, studies on CLA from sheep's milk origin are scarce [28–30]. In addition, CLA isomers are usually not specified in food composition tables. Thus, the evaluation of the FA composition and the CLA isomer contents of three important Portuguese PDO cheeses from the South of Portugal, produced with milk from ewes reared in traditional farming systems based on grazing, was investigated. Moreover, the nutritional value of their lipids was also assessed.

2. MATERIALS AND METHODS

2.1. Samples and sampling procedure

Cheeses were made by local producers, by traditional techniques, all based on the utilization of fresh raw milk, from animals reared in traditional farming systems based on grazing. *Cynara cardunculus* L. extract was used as coagulant for the three types of cheese. The main differences among the three manufacture and ripening processes are summarized in Table I.

Cheese samples were collected at their minimum ripening times of 20, 30 and 45 days for Azeitão, Évora and Nisa cheeses, respectively. Samples of the raw ewe's milk used in the cheese-making process were collected just before the beginning of manufacture. In addition, cheese samples were taken 3 months after the minimum ripening times.

Milk samples were collected aseptically and transported frozen to the laboratory. Cheese samples were transported under refrigerated conditions. At the laboratory, the rind was removed and discarded and samples were grated finely. All samples were kept frozen at –20 °C until they were analyzed.

Analyses were carried out on ten replicates of each type of cheese (Azeitão, Évora and Nisa) and milk (from Azeitão, Évora and Nisa manufactures), performing a total of 90 samples: 30 milk samples, 30 cheeses with minimum ripening time and 30 cheeses with 3 months of extended ripening.

Table I. Differences in technological parameters for the three PDO cheeses' manufacture and ripening.

	Cheese-making (°C / time)	Ripening (time / °C / humidity)
Azeitão cheese (A)	30 °C / 45 min	20 d / 10–15 °C / 85–90%
Évora cheese (E)	30 °C / 20–40 min	30 d / 8–15 °C / 80–95%
Nisa cheese (N)	25 °C / 45–60 min	15–18 d / 8–10 °C / 80–90%
		30–40 d / 10–14 °C / 85–90%

2.2. Fatty acid and CLA analysis

Analytical grade and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany).

Milk fat was extracted according to ISO 14156 [15], except for the extraction mixture used, which was ethyl ether/petroleum ether (1:1) instead of ethyl ether/*n*-pentane (1:1). This small alteration does not cause any changes in triacylglycerol extraction, as was indicated by consecutive successful participations in interlaboratory studies by the FAPAS (Food Analysis Performance Assessment Scheme). Cheese fat was extracted after acidification of samples with 10 mL of HCl (25%), followed by an ethyl ether/petroleum ether mixture extraction [23]. After separation of ether and aqueous phases, with a separating funnel, the ether extracts were evaporated under reduced pressure in a water bath set at 40 °C, for both milk and cheese fat extracts. An aliquot of 100 mg of fat was dissolved in 3 mL of *iso*-octane and a transmethylation was performed with 2 mL of a methanolic sodium hydroxide solution (2 mol·L^{-1}). The tube was capped and shaken for 30 s. The aqueous and organic layers were allowed to separate, and from the top (*iso*-octane) layer, 2 and 10 μL of the resulting fatty acid methyl esters (FAME) were injected directly into the GC (for FA composition) and HPLC (for CLA isomeric profile), respectively.

2.2.1. FAME analysis

Analysis of FAME was performed on a Trace 2000 Thermo Quest CE Instruments gas chromatograph (Thermo Quest Italia, Milan, Italy), with a split/splitless injector and a flame ionization detector (FID). The analytical column was a DB 23 (J & W, Folson, CA, USA) fused silica capillary column, with 60 m × 0.25 mm i.d. and 0.25 μm film thickness. The temperature program was raised from 70 °C to 195 °C at 5 °C·min^{-1}, standing for 10 min, then raised to 220 °C at 5 °C·min$^{-1,}$, and finally standing at that temperature for 30 min. The injector and detector temperatures were set at 220 °C and 280 °C, respectively. The carrier gas was helium at a flow rate of 0.4 mL·min^{-1} (pressure of 70 kPa).

FA were identified by their retention times using a mixture of 36 FAME from Supelco (Mix.C4-C24, Supelco, Bellefonte, PA, USA) and a certified reference anhydrous milk fat (CRM 164) supplied by the Community Bureau of Reference (Commission of the European Communities, Brussels).

The quantification of FAME was performed by the internal normalization method (relative percentages), and calculation of correction factors was done for conversion of the peak area percentages into weight percentages. The correction factors were calculated with the reference material CRM 164. This correction is necessary because of differences in FID

response and discriminations in the chromatographic system between FAME of different molecular weights.

For each FA, the relative composition was expressed in g·100 g^{-1} of total FA (weight %). The CLA content was expressed in mg·g^{-1} of fat by the utilization of a lipid conversion factor: CLA (mg·g^{-1} fat) = weight % × 0.945. The value of 0.945 is the lipid conversion factor for milk and milk products recommended by McCance and Widdowson [11]. This factor takes into account the weight percentage of FA in the triacylglycerol molecules and the weight percentage of triacylglycerols in milk fat, thus enabling the conversion of the values expressed in g·100 g^{-1} FA into mg·g^{-1} fat or, when multiplied by the fat content of the sample, into mg·mL^{-1} of milk or mg·g^{-1} of cheese.

Fat content was determined by the butyrometric method of Gerber-van Gulik [2].

2.2.2. CLA isomer analysis

The methyl esters of CLA isomers were prepared according to a procedure described previously [8] and were individually separated by three connected silver-ion columns in series (Chrom-Spher 5 Lipids, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using a high-performance liquid chromatography system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) as reported previously [1]. Briefly, the mobile phase was 0.1% acetonitrile in *n*-hexane, at a flow rate of 1 mL·min^{-1}, the diode array detector (DAD) was adjusted to 233 nm and volumes of 10 μL were injected by the autosampler. The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial standards (Matreya Inc., Pleasant Gap, PA, USA) as well as with values published in the literature [11]. In addition, the identity of each isomer was controlled by the typical ultraviolet spectra of CLA isomers from the DAD in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09.01 (Agilent Technologies, 2001). The CLA isomers were expressed as a percentage of the sum of identified CLA isomers (% total CLA). Total CLA contents in milk and cheese were determined by the GC analysis of FA, as described previously.

2.2.3. Statistical analysis

Individual fatty acids, partial sums of fatty acids and CLA isomers were compared by parametric methods of analysis of variance, one-way ANOVA, for $P < 0.05$. The Bonferroni test, through the program SPSS – Statistical Package for Social Sciences, version 12.0, was also applied, for comparison between the three types of milk and cheeses or, alternatively, to evaluate the effect of ripening time of each type of cheese.

This study was not based on a two-way ANOVA design (cheese type × ripening time) since the ripening time and the technological processes used varied a lot among the three cheeses analyzed.

3. RESULTS AND DISCUSSION

3.1. Fatty acid composition

Table II (available at http://dx.doi.org/10.1051/dst:2008032) shows FA composition obtained for the milks and the three types of cheeses under study. Ewe's milk fat is characterized by high contents of caproic acid (6:0), caprylic acid (8:0) and, mainly, capric acid (10:0), which are the most characteristic ewe's milk FA [14, 22]. In all analyzed samples, the values obtained for capric acid were equal to or

higher than 6.5% (w/w). Another characteristic of ewe's milk fat, already observed in previous studies (unpublished data) with other Portuguese PDO cheeses (Serra da Estrela cheese), was lower contents of palmitic and oleic (18:1) acids in ewe's milk fat when compared with values for cow's milk fat. This tendency was confirmed in the present study.

It is well established that the diet plays a major role in FA composition of ruminant milks [6, 29]. Milk from animals grazing pasture is described as having higher values of fat and of some specific FA, such as CLA and *trans* FA. In the present study, all milks and cheeses revealed considerably high values for *trans* FA, mainly elaidic and vaccenic acid (*trans*-9 18:1 and *trans*-11 18:1) contents (> 2.7% w/w). This is a consequence of the grazing system of the animals [21, 26].

The patterns obtained for the different partial sums of FA (Tab. III) reflect the values described above for the major individual FA of each group. For Azeitão cheeses, significant differences were obtained, with ANOVA ($P < 0.05$), between milk and cheese samples for MUFA and TFA. For Évora cheeses, significant differences ($P < 0.05$) were observed between milk and cheese samples for SFA and MUFA. However, for Nisa cheeses no significant differences ($P > 0.05$) were observed between milk and cheese samples for FA composition.

The results from ANOVA ($P < 0.05$) carried out on the three types of cheese also showed significant differences for SFA and MUFA of Nisa cheeses, when compared with Azeitão and Évora. Three-month-old Nisa cheese revealed the lowest values of SFA, which was a consequence of the lower levels of medium-chain, C10 and C12 FA (Tab. II). In contrast, the highest values of MUFA presented by Nisa cheeses, with minimum and extended ripening, were directly related to higher values of oleic acid. Values for PUFA were very similar for the three types of cheese. In addition to the very important dietary factors that affect milk fat composition, differences among cheese-making techniques and ripening times may also explain some of the observed differences, such as the higher values of oleic and stearic acids and MUFA for the Nisa PDO cheeses, which were also the cheeses with the highest ripening time (Tab. I).

3.2. Nutritional value of fat

The results referring to total lipids in milks and cheeses are shown in Table III. Total lipids varied from 5.6 to 6.8 g·100 mL^{-1} product in milks, and from 36.5 to 43.5 g·100 g^{-1} product in cheeses.

The ratios of n-6/n-3 and PUFA/SFA (as defined in Tab. III), which are nutritional indexes widely used to evaluate the nutritional value of fat for human consumption, were calculated and are also presented in Table III.

Current nutritional recommendations are that the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFA, the n-6/n-3 ratio should not exceed 4.0 [5]. In view of the above guidelines, n-6/n-3 ratios in Azeitão (3.38–3.61) and Nisa (3.70–3.74) cheeses are within the recommended values for the human diet, in contrast to Évora cheeses (4.16–4.28), which revealed values above that guideline. These differences are mainly due to distinct sums for the n-3 PUFA, in which grass lipids are rich [20], with Évora cheeses showing lower values for these FA.

Regarding the PUFA/SFA ratio, the values are similar for all analyzed cheeses (0.06–0.07) and, as expected, below the recommended guideline for the human diet. This fact is due to the well-known biohydrogenation of feed unsaturated FA in the rumen [7, 17, 19].

Table III. Total lipids (g·100 mL^{-1} milk and g·100 g^{-1} cheese), respectively, for milk and cheese samples, partial sums of fatty acids (g·100 g^{-1} FA) and nutritional ratios in Azeitão (A), Évora (E) and Nisa (N) milks and cheeses (n = 10). Values obtained at minimum ripening time (1) and after 3 months of extended ripening (2).

	Milk A	Cheese A (1)	Cheese A (2)	Milk E	Cheese E (1)	Cheese E (2)	Milk N	Cheese N (1)	Cheese N (2)
Total lipids	6.8	37.5	36.5	5.6	42.0	41.9	6.6	43.5	39.4
Partial sums									
Σ SFA	$70.2^{a} \pm 2.148$	$70.9^{a}_{x} \pm 1.725$	$73.8^{a}_{\mathbf{x}} \pm 4.443$	$69.2^{\mathbf{a}} \pm 1.164$	$73.2^{\mathbf{b}}_{x} \pm 1.490$	$71.2^{\mathbf{b}}_{\mathbf{x}} \pm 2.389$	$69.7^{a} \pm 2.137$	$69.2^{a}_{x,y} \pm 2.748$	$67.9^{a}_{\mathbf{x,y}} \pm 1.020$
Σ MUFA	$21.0^{a} \pm 2.346$	$18.9^{a,b}_{x} \pm 0.817$	$17.5^{b}_{\mathbf{x}} \pm 1.736$	$20.5^{\mathbf{a}} \pm 0.763$	$18.7^{\mathbf{b}}_{x} \pm 1.272$	$19.4^{\mathbf{b,a}}_{\mathbf{y}} \pm 0.955$	$21.2^{a} \pm 1.466$	$21.0^{a}_{y} \pm 1.969$	$22.1^{a}_{\mathbf{z}} \pm 0.616$
Σ TFA	$3.98^{a} \pm 0.533$	$4.77^{b}_{x} \pm 0.517$	$4.57^{b}_{\mathbf{x}} \pm 0.318$	$5.13^{\mathbf{a}} \pm 0.667$	$4.68^{\mathbf{a}}_{x} \pm 0.575$	$4.82^{\mathbf{a}}_{\mathbf{x}} \pm 0.705$	$4.55^{a} \pm 0.804$	$5.18^{a}_{x} \pm 0.590$	$5.11^{a}_{\mathbf{x}} \pm 0.631$
Σ PUFA	$4.28^{a} \pm 0.297$	$4.43^{a}_{x} \pm 0.231$	$4.11^{a}_{\mathbf{x}} \pm 0.285$	$4.56^{\mathbf{a}} \pm 2.246$	$4.16^{\mathbf{a}}_{x} \pm 0.344$	$4.35^{\mathbf{a}}_{\mathbf{x}} \pm 0.365$	$4.25^{a} \pm 0.266$	$4.49^{a}_{x} \pm 0.178$	$4.60^{a}_{\mathbf{x}} \pm 0.169$
Σ n-6	$2.89^{a} \pm 0.527$	$2.67^{b}_{x} \pm 0.135$	$2.47^{b}_{\mathbf{x}} \pm 0.290$	$2.89^{\mathbf{a}} \pm 0.180$	$2.64^{\mathbf{a}}_{x} \pm 0.265$	$2.73^{\mathbf{a}}_{\mathbf{y}} \pm 0.244$	$2.60^{a} \pm 0.179$	$2.69^{a}_{x} \pm 0.140$	$2.71^{a}_{\mathbf{x,y}} \pm 0.131$
Σ n-3	$0.73^{a} \pm 0.060$	$0.77^{a}_{x} \pm 0.144$	$0.74^{a}_{\mathbf{x}} \pm 0.075$	$0.70^{\mathbf{a}} \pm 0.120$	$0.64^{\mathbf{a}}_{y} \pm 0.125$	$0.69^{\mathbf{a}}_{\mathbf{x}} \pm 0.159$	$0.63^{a} \pm 0.010$	$0.72^{b}_{x,y} \pm 0.044$	$0.73^{b}_{\mathbf{x}} \pm 0.049$
Ratios									
PUFA/SFA	$0.06^{a} \pm 0.005$	$0.06^{a} \pm 0.004$	$0.06^{a} \pm 0.006$	$0.06^{\mathbf{a}} \pm 0.004$	$0.06^{\mathbf{a}} \pm 0.005$	$0.06^{\mathbf{a}} \pm 0.007$	$0.06^{a} \pm 0.005$	$0.06^{a} \pm 0.004$	$0.07^{a} \pm 0.003$
n-6/n-3	$3.39^{a} \pm 0.481$	$3.61^{a} \pm 0.967$	$3.38^{a} \pm 0.687$	$4.25^{\mathbf{a}} \pm 0.712$	$4.28^{\mathbf{a}} \pm 0.774$	$4.16^{\mathbf{a}} \pm 0.894$	$4.15^{a} \pm 0.398$	$3.74^{b} \pm 0.132$	$3.70^{b} \pm 0.172$

Comparison between milk and cheeses: means within a row with different superscript letters are significantly different ($P < 0.05$).
Comparison between the three types of cheeses with minimum ripening time (regular font style) and with extended ripening time (bold font style): means within a row with different subscript letters are significantly different ($P < 0.05$).

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids.
Σ n-6 = 18:2n-6.
Σ n-3 = 18:3n-3.
n-6/n-3 = n-6/n-3 ratio (18:2n-6/18:3n-3).
PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2n-6 and 18:3n-3)/(sum of 4:0, 6:0, 7:0, 8:0, 9:0, 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].

3.3. CLA contents and isomeric profile

Data on the CLA contents and their isomeric distribution in the fat, of the three types of PDO cheeses under study, are presented in Table IV. The smallest amounts of CLA obtained were for Évora at minimum ripening time (8.32 mg·g^{-1} fat) and Azeitão with extended ripening (8.51 mg·g^{-1} fat). The highest contents of CLA (10.1 and 10.9 mg·g^{-1} fat) were obtained for Nisa PDO cheeses with minimum and extended ripening (Tab. III). In addition, the milk used in Nisa PDO cheese manufacture had the highest value of CLA (9.63 mg·g^{-1} fat) among the three analyzed milks. It is well known that CLA levels in milk and cheese depend strongly on the initial milk fat composition, which is, in turn, very well related to the feeding system [7, 18, 28, 29].

The CLA contents described are relatively high when compared with the values reported in the literature for milk and dairy products, which range from 3.4 to 10.7 mg·g^{-1} of total fat [9]. In a study on dairy products from Italy, values of 8.11 mg·g^{-1} of total fat, for Fontina Valdostana cheese, a cow's milk cheese, were reported [24]. The same author described for a ewe's milk cheese (Pecorino cheese) values of 7.77 mg·g^{-1} fat. Other values described in the literature, for different types of cheese, are: 3.59–7.96 mg·g^{-1} [19], 5.05–5.39 mg·g^{-1} [31], and the highest values, of 16 and 19 mg·g^{-1} fat, were reported for Feta Greek cheeses [33].

Biohydrogenation of PUFA in the rumen leads, in a final step, to the formation of stearic acid [17, 19] and, since CLA is an intermediate of this process, it is interesting to observe that Nisa cheeses (the samples with the highest values of CLA) also have the highest values of stearic acid, with 10.5 and 10.7 g·100 g^{-1} FA, for minimum and 3 months of extended ripening, respectively. In addition, the highest values for total MUFA were obtained for the same type of milk and cheese (Tab. III). Also, the cheeses with lower values of CLA, Azeitão with 3 months of extended ripening and Évora with minimum ripening time (8.51 and 8.32 mg·g^{-1} fat, respectively), are also those with lower values of stearic acid (8.04 and 8.94 g·100 g^{-1} FA, respectively).

Regarding the relative proportions of individual CLA isomers (Tab. IV), the percentages of the main isomer, rumenic acid (*cis*-9,*trans*-11), varied from 67–74% (w/w). These values are lower than some values referred to in the literature for the same CLA isomer: 75–90% in cow's milk fat [7, 16] and 76–82% in ewe's milk fat [21]. The *cis*-9,*trans*-11 CLA isomer presented significantly higher values ($P < 0.05$) for Nisa cheeses with extended ripening time, relative to other cheese types. The second quantitatively most important CLA isomer in the analyzed samples was the *trans*-7,*cis*-9 isomer (8.5–16%), which co-eluted with minor amounts of the *trans*-8,*cis*-10 isomer. The third quantitatively most predominant CLA isomer obtained in this study was the *trans*-11,*trans*-13 isomer, with percentages from 3–5%. In contrast to our values, some authors [17] described the *trans*-10,*cis*-12 as the third most predominant isomer in ruminant milks. This last CLA isomer has been associated with some beneficial biological properties by some authors [7, 17], while others suggest possible deleterious effects in man [32]. In ewe's milk and cheese samples from the present study, this particular isomer was present in percentages lower than 1% relative to the total CLA isomers.

The sums of the *cis,trans* and *trans,cis* isomers contributed 88–92% of total CLA in all analyzed milks and cheeses, while total *trans,trans* contributed only 8–12%. No *cis,cis* CLA isomers were detected in any analyzed sample.

Finally, based on the few differences observed in CLA contents and the ANOVA

Table IV. CLA contents (mg·g^{-1} fat) and their individual isomers (% total CLA) in Azeitão (A), Évora (E) and Nisa (N) milks and cheeses. Values were obtained at minimum ripening time (1) and after 3 months of extended ripening (2).

	Milk A	Cheese A (1)	Cheese A (2)	Milk E	Cheese E (1)	Cheese E (2)	Milk N	Cheese N (1)	Cheese N (2)
Total CLA content[1]	8.59 ^{a} ± 0.130	9.35 ^{b} ± 0.127	8.51 ^{a} ± 0.083	9.16 $^{\mathbf{a}}$ ± 0.088	8.32 $^{\mathbf{a}}$ ± 0.098	8.79 $^{\mathbf{a}}$ ± 0.137	9.63^{a} ± 0.108	10.1 ^{a} ± 0.140	10.9 ^{a} ± 0.085
CLA isomers[2]									
*t*12,*t*14	1.80^{a} ± 0.100	2.34^{a} ± 0.359	2.34^{a} ± 0.547	2.41$^{\mathbf{a}}$ ± 0.785	2.21$^{\mathbf{a}}$ ± 0.592	2.53$^{\mathbf{a}}$ ± 0.580	1.77^{a} ± 0.532	1.92^{a} ± 0.214	2.11^{a} ± 0.530
*t*11,*t*13	3.22^{a} ± 2.252	5.07^{b} ± 0.811	5.17^{b} ± 0.281	3.20$^{\mathbf{a}}$ ± 0.532	3.13$^{\mathbf{a}}$ ± 0.515	3.49$^{\mathbf{a}}$ ± 0.629	2.88^{a} ± 1.036	3.30^{a} ± 0.426	3.34^{a} ± 0.336
*t*10,*t*12	1.61^{a} ± 0.486	1.14^{a} ± 0.168	1.07^{a} ± 0.391	0.85$^{\mathbf{a}}$ ± 0.126	0.80$^{\mathbf{a,b}}$ ± 0.122	1.02$^{\mathbf{b}}$ ± 0.235	0.68^{a} ± 0.360	1.06^{a} ± 0.303	0.91^{a} ± 0.366
*t*9,*t*11	1.54^{a} ± 0.592	1.05^{b} ± 0.185	1.00^{b} ± 0.068	0.65$^{\mathbf{a}}$ ± 0.114	0.99$^{\mathbf{a}}$ ± 0.513	0.76$^{\mathbf{a}}$ ± 0.163	0.52^{a} ± 0.198	0.69^{a} ± 0.147	0.62^{a} ± 0.193
*t*8,*t*10	1.62^{a} ± 0.473	1.33^{a,b} ± 0.162	1.28^{b} ± 0.079	0.98$^{\mathbf{a}}$ ± 0.075	1.06$^{\mathbf{b}}$ ± 0.318	1.29$^{\mathbf{b}}$ ± 0.111	0.60^{a} ± 0.297	1.29^{b} ± 0.300	1.21^{b} ± 0.198
*t*7,*t*9	1.12^{a} ± 0.264	0.90^{a,b} ± 0.184	0.76^{b} ± 0.160	0.64$^{\mathbf{a}}$ ± 0.104	0.76$^{\mathbf{a}}$ ± 0.328	0.82$^{\mathbf{a}}$ ± 0.223	1.13^{a} ± 0.427	0.69^{b} ± 0.242	0.62^{b} ± 0.233
*t*6,*t*8	1.21^{a} ± 0.735	0.58^{b} ± 0.200	0.46^{b} ± 0.079	0.45$^{\mathbf{a}}$ ± 0.078	0.44$^{\mathbf{a}}$ ± 0.190	0.57$^{\mathbf{a}}$ ± 0.181	0.37^{a} ± 0.124	0.48^{a} ± 0.139	0.49^{a} ± 0.187
Total *trans,trans*	12.1^{a} ± 2.185	12.4$^{a}_{x}$ ± 1.142	12.0^{a} ± 1.313	9.17$^{\mathbf{a}}$ ± 1.391	9.39$^{\mathbf{a}}_{y}$ ± 1.128	10.5$^{\mathbf{a}}$ ± 1.106	7.94^{a} ± 2.008	9.45$^{a}_{y}$ ± 1.308	9.31^{a} ± 1.454
c/*t*12,14	1.37^{a} ± 0.508	1.74^{a} ± 0.906	1.61^{a} ± 0.819	1.04$^{\mathbf{a}}$ ± 0.677	1.40$^{\mathbf{a}}$ ± 0.713	1.63$^{\mathbf{a}}$ ± 0.786	0.66^{a} ± 0.218	1.03^{b} ± 0.180	1.29^{b} ± 0.483
*t*11,*c*13	2.31^{a} ± 1.759	3.63^{a,b} ± 0.787	4.12^{b} ± 1.187	2.78$^{\mathbf{a}}$ ± 1.283	3.00$^{\mathbf{a}}$ ± 1.311	2.65$^{\mathbf{a}}$ ± 0.752	2.55^{a} ± 0.455	2.85^{a,b} ± 0.194	2.95^{b} ± 0.299
*c*11,*t*13	0.49^{a} ± 0.166	0.29^{a} ± 0.076	0.43^{a} ± 0.411	0.33$^{\mathbf{a}}$ ± 0.446	0.52$^{\mathbf{a}}$ ± 0.478	0.45$^{\mathbf{a}}$ ± 0.116	0.12^{a} ± 0.164	0.36^{b} ± 0.171	0.21^{b,a} ± 0.153
*t*10,*c*12	0.77^{a} ± 0.249	0.64^{a} ± 0.310	0.79^{a} ± 0.647	0.68$^{\mathbf{a}}$ ± 0.507	0.31$^{\mathbf{a}}$ ± 0.511	0.43$^{\mathbf{a}}$ ± 0.194	0.77^{a} ± 0.167	0.37^{b} ± 0.247	0.32^{b} ± 0.140
*c*9,*t*11	67.2^{a} ± 2.795	67.8$^{a}_{x}$ ± 2.465	70.5$^{b}_{\mathbf{x}}$ ± 3.576	69.7$^{\mathbf{a}}$ ± 2.832	72.1$^{\mathbf{b}}_{y}$ ± 5.181	70.8$^{\mathbf{b}}_{\mathbf{x}}$ ± 1.918	68.7^{a} ± 2.991	70.6$^{a,b}_{x,y}$ ± 1.708	73.8$^{b}_{\mathbf{y}}$ ± 1.570
*t*6,*c*8	2.90^{a} ± 0.718	2.03^{b} ± 0.440	2.09^{b} ± 0.627	3.64$^{\mathbf{a}}$ ± 1.315	2.61$^{\mathbf{b}}$ ± 0.963	2.42$^{\mathbf{b}}$ ± 0.461	3.45^{a} ± 0.944	2.62^{a,b} ± 1.066	2.06^{b} ± 0.409
*t*7,*c*9[3]	12.8^{a} ± 1.474	11.4$^{a,b}_{x,y}$ ± 1.142	8.46$^{b}_{\mathbf{x}}$ ± 2.510	12.6$^{\mathbf{a}}$ ± 3.761	10.7$^{\mathbf{a}}_{x}$ ± 3.884	11.1$^{\mathbf{a}}_{\mathbf{y}}$ ± 2.735	15.8^{a} ± 3.984	12.7$^{b}_{y}$ ± 0.934	10.1$^{b}_{\mathbf{y}}$ ± 0.564
Total *cis*/*trans*	87.9^{a} ± 2.185	87.6$^{a}_{x}$ ± 1.142	87.9 ^{a} ± 1.313	90.8$^{\mathbf{a}}$ ± 1.391	90.6$^{\mathbf{a}}_{y}$ ± 1.127	89.5$^{\mathbf{a}}$ ± 1.104	92.1^{a} ± 2.007	90.5$^{a}_{y}$ ± 1.309	90.7^{a} ± 1.453

Comparison between milk and cheeses: means within a row with different superscript letters are significantly different ($P < 0.05$).
Comparison between the three types of cheeses with minimum ripening time (regular font style) and with extended ripening time (bold font style): means within a row with different subscript letters are significantly different ($P < 0.05$).

[1] Values obtained from GC analysis using the lipid conversion factor of 0.945.
[2] Values obtained from Ag^{+}-HPLC.
[3] This CLA isomer co-eluted with minor amounts of the *t*8,c10 isomer.

($P < 0.05$) results (Tab. IV) for most of the isomers, we may assume that the processing of milk into cheese does not have much influence on cheeses' final CLA levels [7]. However, for the *cis*-9,*trans*-11 CLA isomer, a general tendency for an increase was found, when comparing values from processed cheeses, with those obtained for the respective raw milk. In contrast, the *trans*-7,*cis*-9 CLA isomer revealed an opposite behavior, with a tendency to decrease with the processing of milk into cheese.

4. CONCLUSION

Analysis of fat from Azeitão, Nisa and Évora PDO ewe's milk cheeses, all from the South of Portugal, revealed some differences for FA profiles and CLA isomer contents. The main differences found were higher values of oleic and stearic acids, and of MUFA and CLA, for Nisa cheeses. All animals were reared in traditional farming systems based on grazing. Also, *Cynara cardunculus* L. extract was used as coagulant for the three types of cheese. The main difference in the cheese manufacture techniques was the longer ripening time for Nisa cheeses, relative to the other two types. So, more work must be undertaken in order to assess the relative contribution of the factors which might affect FA composition and CLA isomer contents of these cheeses, mainly the influence of ripening parameters, such as time and temperature.

From a nutritional point of view, the results obtained from fat of the three types of cheese, when compared with the values described in the literature for ruminant milk cheeses, revealed relatively high CLA contents and the n-6/n-3 ratios (except for Évora cheeses, which are slightly above) are within the recommended values for the human diet. In contrast, values for the PUFA/SFA index were consistently below the recommended guideline for the human diet, which is a characteristic of ruminant foods.

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REFERENCES

[1] Alfaia C., Quaresma M., Castro M., Martins S., Portugal A., Fontes C., Bessa, R., Prates J., Fatty acid composition, including isomeric profile of conjugated linoleic acid, and cholesterol in Mertolenga-PDO beef, J. Sci. Food Agric. 86 (2006) 2196–2205.

[2] Ardö Y., Polychroniadou A., Laboratory manual for chemical analysis of cheese. *COST 95* Improvement of the quality of the production of raw milk cheeses EUR 18890, 1999.

[3] Assenat L., Composition et propriétés, in: Luquet F.M. (Ed.), Laits et Produits Laitiers : Vache. Brebis. Chèvre, Tome 1, Les Laits : De la Mamelle à la Laiterie, Technique et Documentation, Lavoisier, Paris, France, 1985, pp. 281–318.

[4] Belury M.A., Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action, Ann. Rev. Nutr. 22 (2002) 505–531.

[5] British Department of Health, Nutritional aspects of cardiovascular disease, Report on Health and Social Subjects N° 46, HMSO, London, UK, 1994.

[6] Cabiddu A., Addis M., Pinna G., Decandia M., Sitzia M., Piredda G., Pirisi A., Molle G., Effect of corn and beet pulp based concentrates on sheep milk and cheese fatty acid composition when fed Mediterranean fresh forages with particular reference to conjugated linoleic acid *cis*-9, *trans*-11, Anim. Feed Sci. Technol. 131 (2006) 292–311.

[7] Collomb M., Schmid A., Sieber R., Wechsler D., Ryhanen E., Conjugated linoleic acids in milk fat: Variation and physiological effects, Int. Dairy J. 16 (2006) 1347–1361.

[8] Destaillats F., Angers P., Directed sequential synthesis of conjugated linoleic acid isomers from d7,9 to d12,14, Eur. J. Lipid Sci. Tech. 105 (2003) 3–8.

[9] Dhiman T.R., Nam A.L., Factors affecting conjugated linoleic acid content in milk and meat, Crit. Rev. Food Sci. Nutri. 45 (2005) 463–482.

[10] Fernández-Garcia E., Carbonell M., Calzada J., Nunez M., Seasonal variation of the free fatty acids contents of Spanish ovine milk cheeses protected by a designation of origin: A comparative study, Int. Dairy J. 16 (2006) 252–261.

[11] Food Standards Agency, Information Bulletin on Methods of Analysis and Sampling for foodstuffs, Food Standards Agency 1 (2001) 25–26.

[12] Fritsche S., Rumsey T.S., Yurawecz M., Ku Y., Fritsche J., Influence of growth promoting implants on fatty acid composition including conjugated linoleic acid isomers in beef fat, Eur. Food Res. Technol. 212 (2001) 621–629.

[13] German J.B., Dillard C.J., Composition, structure and absorption of milk lipids: A source of energy, fat-soluble nutrients and bioactive molecules, Crit. Rev. Food Sci. Nutr. 46 (2006) 57–92.

[14] Goudjil H., Fontecha J., Luna P., de la Fuente M.A., Alonso L., Juárez M., Quantitative characterization of unsaturated and trans fatty acids in ewe's milk fat, Lait 84 (2004) 473–482.

[15] ISO 14156 / IDF 172, Milk and milk products – Extraction methods for lipids and liposoluble compounds, 2001.

[16] Kay J.K., Mackle T.R., Auldist M.J., Thomson N.A., Bauman D.E., Endogenous synthesis and enhancement of conjugated linoleic acid in pasture-fed dairy cows, Proceedings of the New Zealand Society of Animal Production 62 (2002) 12–15.

[17] Khanal R.C., Dhiman T.R., Biosynthesis of conjugated linoleic acid (CLA): a review, Pakistan J. Nutr. 3 (2004) 72–81.

[18] Ledoux M., Chardigny J.M., Darbois M., Soustre Y., Sébédio J.L., Laloux L., Fatty acid composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers, J. Food Comp. Anal. 18 (2005) 409–425.

[19] Lin H., Boylston T.D., Chang M.J., Luedecke L.O., Shultz T.D., Survey of the conjugated linoleic acid contents of dairy products, J. Dairy Sci. 78 (1995) 2358–2365.

[20] Palmquist D.L., The feeding value of fats, in: Orskov E.R. (Ed.), Feed Science: World Animal Science (Disciplinary Approach B4), Elsevier Publishers, Amsterdam, the Netherlands, 1988, pp. 293–311.

[21] Park Y.W., Juárez M., Ramos M., Haenlein G.F., Physico-chemical characteristics of goat and sheep milk, Small Rum. Res. 68 (2007) 88-113.

[22] Partidário A.M., Queijo Serra da Estrela: avaliação das características químicas e sensoriais. Estudo da fracção lipídica, Ph.D. Thesis, Instituto Superior Técnico, Technical University of Lisbon, Portugal, 1998.

[23] Partidário A.M., Barbosa M., Vilas-Boas L., Free fatty acids, triglycerides and volatile compounds in Serra da Estrela cheese. Changes throughout ripening, Int. Dairy J. 8 (1998) 873–881.

[24] Prandini A., Sigolo S., Tansini G., Brogna N., Piva G., Different level of conjugated linoleic acid (CLA) in dairy products from Italy, J. Food Comp. Anal. 20 (2007) 472–479.

[25] Prates J.A., Mateus C., Functional foods from animal sources and their physiologically active components, Rev. Med. Vet. 53 (2002) 155–160.

[26] Precht D., Molkentin J., Frequency distributions of conjugated linoleic acid and trans fatty acids contents in European bovine milk fats, Milchwissenschaft 55 (2000) 687–691.

[27] Ribeiro J.C.S., Nutritional quality characterization of the lipid fraction from three

ewe's milk cheeses from the South of Portugal, Mcs Thesis, Technical University of Lisbon, Portugal, 2004.

[28] Sampelayo M.R., Chilliard Y., Schmidely P., Boza J., Influence of type of diet on the fat constituents of goat and sheep milk, Small Ruminant Res. 68 (2007) 42–63.

[29] Tsiplakou E., Mountzouris K.C., Zervas G., Concentration of conjugated linoleic acid in grazing sheep and goat milk fat, Livest. Sci. 103 (2006) 74–84.

[30] Tsiplakou E., Mountzouris K.C., Zervas G., The effect of breed, stage of lactation and parity on sheep milk fat CLA content under the same feeding practices, Livest. Sci. 105 (2006) 162–167.

[31] Werner S., Luedecke L., Shultz T., Determination of conjugated linoleic acid content and isomer distribution in three Cheddar-type cheeses: effects of cheese cultures, processing and aging, J. Agric. Food Chem. 40 (1992) 1817–1821.

[32] Whale K., Heys S., Rotondo D., Conjugated linoleic acids: are they beneficial or detrimental to health?, Progr. Lipid Res. 43 (2004) 553–587.

[33] Zlatanos S., Laskaridis K., Feist C., Sagredos A., CLA content and fatty acid composition of Greek Feta and hard cheeses, Food Chem. 78 (2002) 471–477.