

Fatty acids profile of Serra da Estrela PDO cheeses and respective atherogenic and thrombogenic indices

Fatty acids
profile

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Abstract

Purpose – This study aims to determine the physicochemical and fatty acids composition of Serra da Estrela cheese (SEC), as well as health-related lipid indices, like the atherogenic and thrombogenic indices, and to evaluate the influence of producer, geographical origin and production date.

Design/methodology/approach – All 24 SEC produced between November 2017 and March 2018 were collected at selected certified producers and analyzed by NIR spectrophotometer and by GC. Data were statistically evaluated by chemometric tools.

Findings – In all evaluated SEC, 23 fatty acids were quantified. Cheese origin influenced nutritional and health-related lipid indices). The cheeses were characterized by a relative high abundance of saturated fatty acids (67-76%), followed by a medium content of monounsaturated fatty acids (17-25%) and by low level of polyunsaturated fatty acids (5-7%). A putative positive association between cheese consumption and healthy lipid indices could be reached.

Practical implications – The contents of some medium and long chain fatty acids as well as of nutritional and health indices were influenced by cheese producer, geographical origin and production date pointing out the need for standardizing production procedures.

Social implications – The SEC plays a key role in the local economy, being an endogenous product with unique sensory characteristics and nutritional potential, for which the knowledge of the lipids profile and health indices is of utmost relevance.

Originality/value – SEC is an iconic Portuguese cheese with Protected Designation of Origin. Based on the results, like health-related lipid indices, evaluated for the first time, a positive association between cheese consumption and healthy lipid indices could be envisaged.

Keywords Atherogenic index, Fatty acids profile, Omega-6:omega-3 ratio, Serra da Estrela cheese, Thrombogenic index

Paper type Research paper

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Compliance with Ethics Requirements.

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1. Introduction

Serra da Estrela cheese (SEC) is a Protected Designation of Origin (PDO) traditional Portuguese cheese produced from raw milk of autochthonous sheep (“ChurraMondegueira” and “Bordaleira”). SEC is the finest among the Portuguese traditional cheeses and is appreciated worldwide, being preferentially consumed as a soft cheese, with an average maturation of 30-45 days, although some consumers prefer to consume it as a hard cheese, after at least 6 months of storage (Carocho *et al.*, 2015; Macedo and Malcata, 1996; Partidário *et al.*, 1998). Its production is limited to the eponymous mountains in the centre of Portugal, in a specific region. It is manufactured using an aqueous extract of the wild thistle (*Cynara cardunculus*), without deliberate addition of any starter culture. Even though SEC has very precise and legally defined physicochemical and sensorial characteristics, many extrinsic factors may condition them. Among them we might consider the maintenance and the eating qualities of the “ChurraMondegueira” and “Bordaleira” sheep that might directly influence the milk chemical and microbiological characteristics and consequently the final cheese composition (Balthazar *et al.*, 2017; Jaramillo *et al.*, 2008; Sanz Sampelayo *et al.*, 2007). The ecotypes of the wild thistle (*Cynara cardunculus*) (Correia *et al.*, 2014) and the natural and heterogenous microflora associated with the processing and maturation, also contributes to the final characteristics of SEC.

In the EU as well as in Portugal two main diseases groups linked to the circulatory system, namely ischaemic heart diseases (also known as coronary heart diseases, including heart attacks) and cerebrovascular diseases (such as strokes) are the leading cause of death among adults. Therefore, awareness campaigns were developed in order to decrease the ingestion of sugar and fat by the population. Consequently, consumers have become more concerned about the food fat content. Producing cheese with an improved fatty acid (FA) profile and to know exactly the FA composition of endogenous and traditional cheeses could significantly increase economic returns to farmers (Vera *et al.*, 2009). It is well known that in cheese we might find saturated fatty acids (SFA) like myristic and palmitic acids, which are usually related to the increase of blood plasma cholesterol concentration and for a growing incidence in coronary heart diseases (Legrand and Rioux, 2015; Lottenberg *et al.*, 2012). In contrast, differences in metabolic handling of fatty acids may support that medium chain fatty acids (MCFA) hold potential as weight loss agents with unique nutritional and physiologic properties (Terada *et al.*, 2012). Cheese fat also contains health-promoting components associated with unsaturated fatty acids (UFA) including conjugated linoleic acid (CLA), vaccenic, α -linolenic and oleic acids and *n*-3 fatty acids, which contribute to the reduction of the risk of cardiovascular diseases, showing anticarcinogenesis, immunomodulation, and antiatherosclerosis properties as well as lean body mass-enhancing properties, beside inhibiting degenerative cellular proliferation and reduce obesity and cardiovascular diseases (Calder, 2013; Cockbain *et al.*, 2012; Fattore and Massa, 2018). Also, the consumption of dairy products with lower values of atherogenic and thrombogenic indices (AI and TI, respectively) leads to a decrease in the total cholesterol and the LDL-cholesterol in human blood plasma (Hirigoyen *et al.*, 2018; Poppitt *et al.*, 2002).

Dairy products assumed an important position in Mediterranean diet; however, they are often correlated with cardiovascular diseases. Although some information about physicochemical, textural, sensorial and nutritional characteristics of SEC can be found (Reis Lima *et al.*, 2019), knowledge about its variation among different producers, is rather scarce. It is therefore interesting, both for scientific and public health purposes, to evaluate nutritional aspects, regarding to fatty acid profile and its characterization considering the atherogenicity. Thus, the aim of the present study was to characterize the FA profile and the related health and nutritional lipid indices (AI, TI and $\Sigma(\omega-6)/\Sigma(\omega-3)$ values) of SEC from 6

producers located in 5 geographical different points of the PDO denomination and to overcome the lack of information between the moment of production on the desirable, short chain saturated and medium chain saturated fatty acids (DFA, SCFA and MCFA, respectively) relative abundances.

2. Materials and methods

2.1 Serra da Estrela cheese samples

Twenty-four SECs (approximately 1 kg), produced between November 2017 and March 2018, with approximately 45 days of maturation, were collected at selected certified producers and immediately transported, in refrigerated boxes, to the laboratory, being then split in different portions, which were frozen (-40°C) until analysis. The cheeses were produced with the milk, collected from ewes (“ChurraMondegueira” and “Bordaleira” autochthonous breeds) acquired in 6 certified cheese producers (coded as Producer 1 to 6) located in 5 municipalities within the delimited PDO region (*Celorico da Beira* – CB, *Gouveia* – G, *Nelas* – N, *Oliveira do Hospital* – OH and *Penalva do Castelo* - PC), belonging to 3 Portuguese districts, namely, Coimbra (OH, Producer 1), Guarda (CB, Producer 2 and G, Producer 5) and Viseu (N, Producer 6 and PC, Producers 3 and 4) districts (Portugal). In total, 48 independent samples were studied (two samples per cheese), according to: Producer 1 – 5 cheeses \times 2 collected in November 2017, December 2017, January 2018, February 2018 and March 2018; Producer 2 – 3 cheeses \times 2 collected in November 2017, February 2018 and March 2018; Producer 3 – 3 cheeses \times 2 collected in December 2017 and March 2018; Producer 4 – 4 cheeses \times 2 collected in December 2017, February 2018 and March 2018; Producer 5 – 5 cheeses \times 2 collected in November 2017, January 2018, February 2018 and March 2018; Producer 6 – 4 cheeses \times 2 collected in November 2017, December 2017, February 2018 and March 2018.

2.2 Moisture, total fat, total protein and salt contents of Serra da Estrela cheeses: sample preparation and NIR analysis

To expose the interior of the cheese, 1.5 cm of the rind was removed and a slice of approximately 100 g was placed in a flat-bottom glass cuvette and analyzed, in triplicate, on a NIRMasterTM spectrophotometer (Near Infrared Spectroscopy) from BuchiNIRSolutionsTM (Flawil, Switzerland). Spectra were recorded in this instrument which is equipped with a polarization interferometer with TeO₂ wedges, an extended range InGaAs detector (temperature controlled) working in diffuse reflectance with a spectral range of 800 – 2500 nm (resolution: 8 cm^{-1}) combined with NIRWareTM software package, also from BuchiNIRSolutionsTM. A blank signal was previously obtained with external reference Spectralon®. The internal background was measured with a gold plate reflector. Broad-based calibration was used in this study, which was previously adjusted with samples of SEC.

2.3 Fatty acids profiles of Serra da Estrela cheeses: sample preparation and gas chromatography analysis

Lipids were extracted following the International Standard Method described in ISO 14156:2001. Briefly, 0.5 g of each cheese sample (48 samples = 24 cheeses \times 2 independent samples) were weighed and placed into a 10 mL vial, to which 5 mL of *n*-hexane were added plus 1 mL of a KOH-methanol solution (5 mol/L). The mixture was then placed in an ultrasonic bath during 5 min, after which it was allowed to stand for 5 min more at ambient temperature. This procedure was repeated 3 times, in order to ensure the formation of 2 immiscible phases. Then 8 drops of glacial acetic acid were added and the mixture was

manually shaken during 1 min. Then, the *n*-hexane phase was removed from the vial and filtered through a nylon filter (0.2 μm from Millipore), and frozen until being analyzed by gas-chromatography (GC).

GC analysis of fatty acid methyl esters (FAME) was carried out in GC 1000 instrument from DANI equipped with a split/splitless injector, a flame ionization detector (FID) and a Zebron column (ZB-FAME from Phenomenex: (30 + 5) m \times 0.25 mm ID \times 0.20 μm). The oven temperature was programmed as follows: the initial temperature of the column was 100°C, held for 2 min, then a 10°C/min ramp was used until 140°C, followed by a 3°C/min ramp until 190°C, then a 30°C/min ramp until reaching 260°C and held for 2 min. The carrier gas (hydrogen) flow rate was 4.0 mL/min (0.61 bar), measured at 50°C. Split injection (1:59) was carried out at 250°C, being the detector at 260°C. A constant flow rate of 1 mL/min was used. For each analysis 1 μL of the sample was injected in GC equipment. The identification was carried out by comparing the relative retention times of the FAME to commercial standards (reference sample of FAME Mix Supelco 37 (C4–C24)). The quantification was achieved through CSW 1.7 (DataApex 1.7, Prague, Czech Republic). The results were expressed in relative percentage of each fatty acid.

2.4 Lipid related health quality indices

From the fatty acids profiles of cheeses, the contents (in per cent) of desirable fatty acids (DFA), which allowed assessing the content of beneficial fatty acids for health (Barač *et al.*, 2018; Osmari *et al.*, 2011; Taboada *et al.*, 2015), short chain saturated fatty acids (SCFA) and medium chain saturated fatty acids (MCFA) were calculated as follows:

$$DFA = \Sigma MUFA + \Sigma PUFA + C_{18:0} \quad (1)$$

$$SCFA = C_{4:0} + C_{6:0} \quad (2)$$

$$MCFA = C_{8:0} + C_{10:0} + C_{11:0} + C_{12:0} + C_{13:0} + C_{14:0} + C_{15:0} \quad (3)$$

The atherogenic index (AI) and thrombogenic index (TI), linking fatty acid profile to cardiovascular risk, were calculated according to Ulbricht and Southgate (1991):

$$AI = \frac{4 \times C_{12:0} + C_{14:0} + C_{16:0}}{\Sigma MUFA + \Sigma PUFA} \quad (4)$$

$$TI = \frac{C_{14:0} + C_{16:0} + C_{18:0}}{0.5 \times \Sigma MUFA + 0.5 \times (PUFA - n6) + 3 \times (PUFA - n3) + \frac{PUFA - n3}{PUFA - n6}}$$

Finally, the $\Sigma(\omega-6)/\Sigma(\omega-3)$ ratio was also calculated, since a high intake of $\omega-6$ acids has been recognized to be undesirable, being a low ratio envisaged from a nutritional point of view (Aro *et al.*, 2005; Garaffo *et al.*, 2011; Simopoulos, 2002):

$$\frac{\Sigma(\omega-6)}{\Sigma(\omega-3)} = \frac{PUFA - n6}{PUFA - n3} = \frac{C_{18:2n6t} + C_{18:2n6c} + C_{20:4n6}}{C_{18:3n3}} \quad (6)$$

2.5 Statistical analysis

The effects of cheese producer, geographical origin and production season on Serra da Estrela fatty acids profiles as well as on health and nutritional indices (e.g. ω -6/ ω -3 ratio, atherogenic index, thrombogenic index, DFA, SCFA and MCFA) were evaluated through the one-way analysis of variance (one-way ANOVA), being the post-hoc multi-comparison Tukey's test further used if a significant statistical effect was found ($p < 0.05$). Furthermore, for the health and nutritional indices, the results were graphically evaluated using boxplots. All statistical analysis was performed using the *Subselect* (Cadima *et al.*, 2004, 2018) and *MASS* (Venables and Ripley, 2002) packages of the open source statistical R programme (version 2.15.1), at a 5 per cent significance level.

3. Results and discussion

3.1 Serra da Estrela cheese moisture, total fat, total protein and salt contents: changes with producer, location and moment of manufacturing

The mean values of moisture, total fat, total protein and salt contents per producer, geographical origin or moment of production are shown in Tables I to III. Results showed that, the producer, geographical origin and the moment of manufacturing significantly influenced the contents of the above mentioned chemical parameters ($p \leq 0.007$, one-way ANOVA). In brief, cheeses from Producer #3 and #6 had the highest moisture content and the lowest total fat, total protein and salt levels. On the other hand, cheeses produced in January 2018 showed the lowest moisture content and the highest total fat content. Oppositely, Guiné *et al.* (2016) did not found any significant effect of the producer neither of the geographical origin on the moisture content. Regarding the fat content, it was highly dependent on the geographical origin but not on the producer. For the protein content, those researchers found a significant effect of both producer and geographical origin. Salt contents significantly varied between producer and geographical origin. In fact, in the present study it was observed that, depending on the fixed effect under evaluation, moisture content varied in the range of 44-52 per cent, total fat from 20 to 30 per cent, total protein between 19-25 per cent and finally, salt content ranges from 0.9 to 1.8 per cent. The moisture contents were within the values legally established (DR 42/85 of 05th July that fixed a range between 61-69 per cent referred to fat free cheese), and similar to the contents reported by Macedo *et al.* (2004), Guiné *et al.* (2016) and Carochó *et al.* (2016b) for SECs. The fat contents found were similar to those determined by Macedo *et al.* (2004) and Carochó *et al.* (2016a). The total protein levels found in this work were higher compared to those reported by Macedo *et al.* (2004) but in the same order of values reported by Guiné *et al.* (2016) and Carochó *et al.* (2016a, 2016b). Finally, salt contents were similar of those reported by Guiné *et al.* (2016) and slightly higher than those observed by Macedo *et al.* (2004). These findings pointed out that, although the production region of this traditional ewe PDO cheese is limited and its production is legally regulated, different cheese compositions have been reported in the literature, showing the high chemical variability of SEC.

3.2 Effects of producer's location and moment of production in lipidic profile of Serra da Estrela cheese

Free fatty acids (FFA) play an important role in flavours of many varieties of ripened cheese. The two major sources for FFA in ripened cheese are:

- (1) the end products of carbohydrate and protein metabolism by bacteria; and
- (2) the direct breakdown products of milk fat by lipolysis.

Table I. Physicochemical data and main fatty acids found in SECs (mean \pm standard deviation) produced by 6 certified producers (producers 1 to 6) at 5 geographical origins located within the PDO region, from November 2017 to March 2018

Parameters	Cheese producer origin						<i>p</i> ¹
	Producer 1 (5 cheeses \times 2 independent samples)	Producer 2 (3 cheeses \times 2 independent samples)	Producer 3 (3 cheeses \times 2 independent samples)	Producer 4 (4 cheeses \times 2 independent samples)	Producer 5 (5 cheeses \times 2 independent samples)	Producer 6 (4 cheeses \times 2 independent samples)	
<i>Physicochemical data (%)²</i>							
Moisture	46.0 \pm 2.1 ^b	46.9 \pm 1.0 ^b	51.9 \pm 1.0 ^a	47.2 \pm 2.1 ^b	49.0 \pm 3.4 ^b	52.2 \pm 1.2 ^a	<0.001
Total fat	28.9 \pm 1.8 ^a	29.6 \pm 0.4 ^a	21.8 \pm 1.1 ^c	25.6 \pm 1.6 ^b	20.5 \pm 2.8 ^{c,d}	19.0 \pm 1.0 ^d	<0.001
Total protein	19.5 \pm 0.8 ^d	19.0 \pm 0.3 ^d	21.3 \pm 1.5 ^c	22.6 \pm 1.3 ^{b,c}	24.5 \pm 1.8 ^a	23.2 \pm 1.3 ^b	<0.001
Salt	1.13 \pm 0.12 ^{b,d}	0.9 \pm 0.1 ^c	1.0 \pm 0.2 ^{b,c}	1.0 \pm 0.2 ^{c,d}	1.8 \pm 0.3 ^a	1.2 \pm 0.2 ^b	<0.001
<i>Fatty acids profile (%)²</i>							
C4:0	4.5 \pm 1.2	3.7 \pm 0.9	4.4 \pm 1.7	4.6 \pm 1.0	4.2 \pm 1.1	4.2 \pm 2.3	0.841
C6:0	4.0 \pm 0.7	3.3 \pm 0.8	3.5 \pm 1.2	3.4 \pm 0.4	3.8 \pm 1.0	3.6 \pm 1.5	0.736
C8:0	3.6 \pm 1.0	2.8 \pm 1.0	3.3 \pm 1.2	3.0 \pm 0.5	3.2 \pm 0.8	3.4 \pm 1.0	0.531
C10:0	9.3 \pm 2.8	6.6 \pm 1.1	9.0 \pm 3.0	8.0 \pm 0.8	8.6 \pm 1.7	9.6 \pm 2.2	0.143
C12:0	5.8 \pm 0.9 ^a	3.8 \pm 0.4 ^b	5.8 \pm 1.3 ^a	5.0 \pm 0.5 ^{a,b}	4.9 \pm 0.6 ^{a,b}	5.0 \pm 1.4 ^a	0.001
C14:0	12.0 \pm 0.8 ^a	9.6 \pm 1.4 ^b	11.7 \pm 1.4 ^a	11.0 \pm 1.2 ^a	11.5 \pm 0.7 ^a	12.3 \pm 1.1 ^a	0.001
C14:1	0.3 \pm 0.1	0.4 \pm 0.1	0.3 \pm 0.2	0.3 \pm 0.1	0.3 \pm 0.2	0.3 \pm 0.1	0.931
C16:0	24.1 \pm 1.7 ^{a,b}	22.0 \pm 2.0 ^b	23.1 \pm 1.5 ^{a,b}	22.9 \pm 1.3 ^{a,b}	24.7 \pm 2.0 ^a	25.4 \pm 1.0 ^a	0.004
C16:1	0.84 \pm 0.09 ^{a,b}	0.70 \pm 0.10 ^{a,b}	0.90 \pm 0.08 ^{a,b}	0.88 \pm 0.09 ^{a,b}	0.68 \pm 0.27 ^b	0.91 \pm 0.09 ^a	0.007
C18:0	10.0 \pm 1.2 ^b	13.1 \pm 0.6 ^a	10.8 \pm 1.8 ^{a,b}	11.2 \pm 1.1 ^{a,b}	10.8 \pm 0.6 ^b	9.5 \pm 2.5 ^b	0.001
C18:1n6c	17.4 \pm 2.3 ^b	24.2 \pm 2.1 ^a	19.2 \pm 2.7 ^{a,b}	20.8 \pm 2.2 ^{a,b}	18.6 \pm 3.0 ^b	16.3 \pm 4.9 ^b	<0.001
C18:2n6t	1.1 \pm 0.4 ^b	1.9 \pm 0.4 ^a	1.0 \pm 0.4 ^b	1.7 \pm 0.2 ^a	1.5 \pm 0.4 ^{a,b}	1.1 \pm 0.3 ^b	<0.001
C18:2n6c	3.2 \pm 0.5 ^b	2.8 \pm 0.3 ^b	3.2 \pm 0.2 ^{a,b}	2.8 \pm 0.2 ^b	3.1 \pm 0.5 ^b	3.7 \pm 0.2 ^a	<0.001
C18:3n3	1.4 \pm 0.1 ^{b,c}	2.1 \pm 0.2 ^a	1.3 \pm 0.3 ^{b,c}	1.6 \pm 0.2 ^b	1.6 \pm 0.3 ^b	1.1 \pm 0.4 ^c	<0.001
C20:0	0.20 \pm 0.05 ^b	0.32 \pm 0.07 ^a	0.22 \pm 0.04 ^b	0.23 \pm 0.03 ^b	0.021 \pm 0.04 ^b	0.24 \pm 0.07 ^b	0.002
C20:1	0.09 \pm 0.08	0.07 \pm 0.01	0.06 \pm 0.01	0.08 \pm 0.05	0.13 \pm 0.07	0.11 \pm 0.05	0.147
C20:4n6	0.22 \pm 0.18	0.29 \pm 0.05	0.41 \pm 0.05	0.34 \pm 0.04	0.32 \pm 0.10	0.32 \pm 0.20	0.162
C22:0	0.07 \pm 0.06 ^b	0.17 \pm 0.04 ^a	0.12 \pm 0.07 ^{a,b}	0.13 \pm 0.02 ^{a,b}	0.09 \pm 0.05 ^b	0.10 \pm 0.04 ^{a,b}	0.002
Σ SFA ³	75.4 \pm 2.8 ^a	67.3 \pm 2.0 ^b	73.7 \pm 3.3 ^a	71.5 \pm 2.3 ^{a,b}	73.8 \pm 3.5 ^a	76.1 \pm 5.2 ^a	<0.001
Σ MUFA	18.6 \pm 2.3 ^b	25.3 \pm 2.2 ^a	20.4 \pm 2.8 ^{a,b}	22.1 \pm 2.2 ^{a,b}	19.7 \pm 3.1 ^b	17.6 \pm 5.0 ^b	0.001
Σ PUFA	5.9 \pm 0.8 ^b	7.1 \pm 0.4 ^a	5.8 \pm 0.9 ^b	6.4 \pm 0.2 ^{a,b}	6.5 \pm 0.5 ^{a,b}	6.2 \pm 0.6 ^{a,b}	0.009

Notes: ¹*p* values refer to one-way ANOVA at a 5% significance level; different lower-case letters mean significant statistical differences at a 5% significance level, for the post-hoc multi-comparison Tukey's test; ²Values are given as mean \pm SD; ³Values of Σ SFA include the mean contents found for odd-chain saturated fatty acids not listed in the table due to the low levels found, namely C11:0, C13:0, C15:0, C17:0 and C21:0

Parameters	Different locations of manufacture within the PDO region				p^1	
	CB (3 cheeses × 2 independent samples)	G (5 cheeses × 2 independent samples)	N (4 cheeses × 2 independent samples)	OH (5 cheeses × 2 independent samples)		PC (7 cheeses × 2 independent samples)
<i>Physicochemical data (%)²</i>						
Moisture	46.9 ± 1.0 ^{bc}	49.0 ± 3.4 ^b	52.2 ± 1.2 ^a	46.0 ± 2.1 ^c	49.3 ± 2.9 ^b	<0.001
Total fat	29.6 ± 0.4 ^a	20.5 ± 2.8 ^c	19.0 ± 1.0 ^c	28.9 ± 1.8 ^a	23.5 ± 2.3 ^b	<0.001
Total protein	19.0 ± 0.3 ^d	24.5 ± 1.8 ^a	23.2 ± 1.3 ^b	19.5 ± 0.8 ^d	22.0 ± 1.5 ^b	<0.001
Salt	0.9 ± 0.1 ^c	1.8 ± 0.3 ^a	1.2 ± 0.2 ^b	1.1 ± 0.1 ^b	1.0 ± 0.2 ^c	<0.001
<i>Fatty acids profile (%)²</i>						
C4:0	3.7 ± 0.9	4.2 ± 1.1	4.2 ± 2.3	4.5 ± 1.2	4.6 ± 1.3	0.735
C6:0	3.3 ± 0.8	3.8 ± 1.0	3.6 ± 1.5	4.0 ± 0.7	3.4 ± 0.8	0.606
C8:0	2.8 ± 0.9	3.2 ± 0.8	3.4 ± 1.0	3.6 ± 1.0	3.1 ± 0.8	0.439
C10:0	6.6 ± 1.1	8.6 ± 1.7	9.6 ± 2.2	9.3 ± 2.8	8.4 ± 2.0	0.106
C12:0	3.8 ± 0.4 ^b	4.9 ± 0.6 ^{ab}	6.0 ± 1.4 ^a	5.8 ± 0.9 ^a	5.3 ± 1.0 ^a	0.001
C14:0	9.6 ± 1.4 ^b	11.5 ± 0.7 ^a	12.3 ± 1.1 ^a	12.0 ± 0.8 ^a	11.3 ± 1.3 ^a	0.001
C14:1	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.852
C16:0	22.0 ± 2.0 ^c	24.7 ± 2.0 ^{ab}	25.4 ± 1.0 ^a	24.1 ± 1.7 ^{ac}	23.0 ± 1.3 ^{bc}	0.001
C16:1	0.70 ± 0.10 ^{ab}	0.68 ± 0.27 ^b	0.91 ± 0.09 ^a	0.84 ± 0.09 ^{ab}	0.89 ± 0.08 ^a	0.003
C18:0	13.1 ± 0.6 ^a	10.8 ± 0.6 ^b	9.5 ± 2.5 ^b	10.0 ± 1.2 ^b	11.0 ± 1.4 ^b	0.001
C18:1n9c	24.1 ± 2.1 ^a	18.6 ± 3.0 ^b	16.3 ± 4.9 ^b	17.4 ± 2.3 ^b	20.1 ± 2.5 ^{ab}	<0.001
C18:2n6t	1.9 ± 0.4 ^a	1.5 ± 0.4 ^{ab}	1.1 ± 0.3 ^b	1.1 ± 0.4 ^b	1.4 ± 0.5 ^{ab}	0.005
C18:2n6c	2.8 ± 0.3 ^b	3.1 ± 0.5 ^b	3.7 ± 0.2 ^a	3.2 ± 0.5 ^b	3.0 ± 0.3 ^b	<0.001
C18:3n3	2.0 ± 0.2 ^a	1.6 ± 0.3 ^b	1.1 ± 0.4 ^c	1.4 ± 0.1 ^{bc}	1.5 ± 0.3 ^{bc}	<0.001
C20:0	0.32 ± 0.07 ^a	0.21 ± 0.07 ^a	0.24 ± 0.07 ^b	0.20 ± 0.05 ^b	0.22 ± 0.03 ^b	0.001
C20:1	0.07 ± 0.00	0.13 ± 0.07	0.11 ± 0.05	0.09 ± 0.08	0.07 ± 0.04	0.098
C20:4n6	0.29 ± 0.05	0.32 ± 0.10	0.32 ± 0.20	0.22 ± 0.18	0.37 ± 0.06	0.136
C22:0	0.17 ± 0.04 ^a	0.09 ± 0.05 ^{bc}	0.10 ± 0.04 ^{bc}	0.06 ± 0.06 ^c	0.13 ± 0.04 ^{ab}	0.001
ΣSFA ³	67.6 ± 2.0 ^b	73.8 ± 3.5 ^a	76.1 ± 5.2 ^a	75.4 ± 2.8 ^a	72.4 ± 2.8 ^a	<0.001
ΣMUFA	25.3 ± 2.2 ^a	19.7 ± 3.1 ^b	17.6 ± 5.0 ^b	18.6 ± 2.3 ^b	21.4 ± 2.5 ^{ab}	<0.001
ΣPUFA	7.1 ± 0.4 ^a	6.5 ± 0.5 ^{ab}	6.3 ± 0.6 ^{ab}	5.9 ± 0.8 ^b	6.2 ± 0.7 ^b	0.012

Notes: ¹ p values refer to one-way ANOVA at a 5% significance level; different lower-case letters mean significant statistical differences at a 5% significance level, for the post-hoc multi-comparison Tukey's test; ²Values are given as mean ± SD; ³Values of ΣSFA include the mean contents found for odd-chain saturated fatty acids not listed in the table due to the low levels found, namely C11:0, C13:0, C15:0, C17:0 and C21:0

Fatty acids profile

Table II. Physicochemical data and main fatty acids found in SECs (mean ± standard deviation) produced at 5 geographical origins (CB – *celorico da beira*, G – *gouveia*, N – *nelas*, OH – *oliveira do hospital* and PC – *penalva do castelo*) located within the PDO region, by 6 different certified producers from November 2017 to March 2018

Table III. Physicochemical data and main fatty acids found in SECs (mean \pm standard deviation) produced at 5 different dates (2017: November and december; 2018: January, february and march) in 5 geographical origins located within the PDO region, by 6 different local certified producers

Parameters	Cheese production date					p^1
	November 2017 (4 cheeses \times 2 independent samples)	December 2017 (3 cheeses \times 2 independent samples)	January 2018 (2 cheeses \times 2 independent samples)	February 2018 (6 cheeses \times 2 independent samples)	March 2018 (9 cheeses \times 2 independent samples)	
<i>Physicochemical data (%)²</i>						
Moisture	49.1 \pm 3.1 ^{ab}	50.6 \pm 2.2 ^a	43.8 \pm 0.4 ^c	47.8 \pm 2.8 ^b	49.2 \pm 3.1 ^{ab}	<0.001
Total fat	25.9 \pm 4.9 ^{ab}	22.9 \pm 2.6 ^{ab}	28.2 \pm 3.4 ^a	24.4 \pm 4.4 ^{ab}	22.5 \pm 4.5 ^b	0.007
Total protein	19.8 \pm 1.5 ^b	21.5 \pm 1.2 ^{ab}	22.0 \pm 2.2 ^{ab}	22.4 \pm 2.4 ^a	22.8 \pm 2.5 ^a	<0.001
Salt	1.1 \pm 0.1 ^{b,c}	0.9 \pm 0.2 ^c	1.7 \pm 0.6 ^a	1.3 \pm 0.3 ^b	1.3 \pm 0.4 ^b	<0.001
<i>Fatty acids profile (%)²</i>						
C4:0	4.0 \pm 1.2 ^a	2.1 \pm 1.3 ^b	3.7 \pm 1.2 ^{ab}	4.8 \pm 0.7 ^a	4.9 \pm 1.0 ^a	<0.001
C6:0	3.8 \pm 0.6	2.7 \pm 1.2	4.2 \pm 1.0	3.8 \pm 0.9	3.6 \pm 0.9	0.111
C8:0	3.4 \pm 0.7 ^{ab}	3.3 \pm 1.2 ^{ab}	4.7 \pm 1.0 ^a	3.1 \pm 0.6 ^b	2.9 \pm 0.8 ^b	0.005
C10:0	8.2 \pm 1.3 ^b	9.0 \pm 2.4 ^{ab}	12.0 \pm 2.1 ^a	8.6 \pm 1.7 ^b	7.8 \pm 2.2 ^b	0.008
C12:0	4.5 \pm 0.7	5.6 \pm 1.4	6.3 \pm 1.0	5.4 \pm 1.1	5.1 \pm 1.0	0.065
C14:0	10.3 \pm 1.5	11.5 \pm 1.9	11.9 \pm 1.3	11.8 \pm 1.3	11.5 \pm 0.8	0.115
C14:1	0.28 \pm 0.13 ^b	0.49 \pm 0.04 ^a	0.49 \pm 0.05 ^a	0.28 \pm 0.03 ^b	0.23 \pm 0.10 ^b	<0.001
C16:0	23.9 \pm 2.6 ^{ab}	23.4 \pm 2.8 ^{ab}	21.2 \pm 1.5 ^b	24.2 \pm 1.5 ^a	24.4 \pm 1.1 ^a	0.039
C16:1	0.63 \pm 0.29 ^b	0.89 \pm 0.12 ^a	0.71 \pm 0.12 ^{ab}	0.85 \pm 0.10 ^a	0.87 \pm 0.08 ^a	0.003
C18:0	11.7 \pm 1.2	11.5 \pm 1.4	9.5 \pm 1.5	10.4 \pm 1.9	10.6 \pm 1.9	0.212
C18:1n9c	21.0 \pm 3.3	21.3 \pm 3.1	17.5 \pm 4.0	17.8 \pm 4.5	18.7 \pm 3.2	0.166
C18:2n6t	0.89 \pm 0.31 ^b	0.95 \pm 0.44 ^b	1.14 \pm 0.68 ^{ab}	1.62 \pm 0.34 ^a	1.61 \pm 0.38 ^a	<0.001
C18:2n6c	3.5 \pm 0.4 ^a	3.4 \pm 0.6 ^{ab}	2.7 \pm 0.2 ^b	3.0 \pm 0.4 ^{ab}	3.1 \pm 0.4 ^{ab}	0.023
C18:3n3	1.4 \pm 0.5	1.3 \pm 0.3	1.6 \pm 0.4	1.5 \pm 0.2	1.6 \pm 0.4	0.535
C20:0	0.29 \pm 0.07 ^a	0.25 \pm 0.06 ^{ab}	0.17 \pm 0.03 ^b	0.22 \pm 0.05 ^{ab}	0.22 \pm 0.05 ^{ab}	0.012
C20:1	0.08 \pm 0.02	0.05 \pm 0.01	0.05 \pm 0.00	0.12 \pm 0.07	0.10 \pm 0.07	0.075
C20:4n6	0.43 \pm 0.08 ^a	0.40 \pm 0.07 ^{ab}	0.22 \pm 0.05 ^{b,c}	0.21 \pm 0.15 ^c	0.31 \pm 0.12 ^a	0.001
C22:0	0.14 \pm 0.05	0.11 \pm 0.03	0.07 \pm 0.02	0.08 \pm 0.06	0.12 \pm 0.06	0.060
Σ SFA ³	71.8 \pm 3.8	71.2 \pm 4.0	75.5 \pm 5.1	74.6 \pm 4.8	73.4 \pm 3.5	0.300
Σ MUFA	22.0 \pm 3.4	22.8 \pm 3.0	18.8 \pm 3.9	19.0 \pm 4.5	19.9 \pm 3.3	0.171
Σ PUFA	6.2 \pm 0.5	6.0 \pm 1.0	5.7 \pm 1.2	6.3 \pm 0.5	6.6 \pm 0.5	0.070

Notes: ¹ p values refer to one-way ANOVA at a 5% significance level; different lower-case letters mean significant statistical differences at a 5% significance level, for the post-hoc multi-comparison Tukey's test; ² χ^2 Values are given as mean \pm SD; ³ χ^2 Values of Σ SFA include the mean contents found for odd-chain saturated fatty acids not listed in the table due to the low levels found, namely C11:0, C13:0, C15:0, C17:0 and C21:0

In all Serra da Estrela PDO cheeses evaluated (24 cheeses \times 2 independent samples) 23 fatty acids were quantified, independently of the cheese producer, geographical origin and moment of production, being the mean contents shown in [Tables I-III](#), with the exception of the 5 odd-chain saturated fatty acids, which were detected in low levels ($C_{11:0}$, $C_{13:0}$, $C_{15:0}$, $C_{17:0}$ and $C_{21:0}$). The FFA profiles were in line with those previously established for this traditional cheese by other authors ([Carocho et al., 2016a, 2016b](#); [Macedo and Malcata, 1996](#); [Partidário et al., 1998](#)). Overall, 15 saturated fatty acids ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, $C_{13:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, $C_{20:0}$, $C_{21:0}$ and $C_{22:0}$), 4 monounsaturated fatty acids (MUFA) ($C_{14:1}$, $C_{16:1}$, $C_{18:1n9c}$ and $C_{20:1}$) and 4 polyunsaturated fatty acids (PUFA) ($C_{18:2n6c}$, $C_{18:2n6c}$, $C_{18:3n3}$ and $C_{20:4n6}$) were identified. Among the fatty acids classes, the saturated fatty acids were predominant and palmitic acid ($C_{16:0}$) contributed most to the profile of saturated fatty acids ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$). Also, oleic acid ($C_{18:1n9cis}$) was the fatty acid that contributed most to the profile of unsaturated fatty acids. Similarly, the PUFA levels were predominantly constituted by linoleic acid ($C_{18:2n6b}$, $C_{18:2n6c}$) and linolenic acid ($C_{18:3n3}$). The quantified levels of FFA are in accordance with those determined by [Carocho et al. \(2015, 2016a, 2016b\)](#) for this type of PDO cheese. Also, SEC fatty acids profiles were within the same order of magnitude of those found in semi-hard uncooked Italian cheese ([Cabiddu et al., 2006](#)), Manchego Spanish cheese ([Gómez-Cortés et al., 2009](#)), Chilean, French and Spanish commercial cheeses ([Aguilar et al., 2014](#)), Serbian white brined cheeses ([Barac et al., 2018](#)), Portuguese Terrincho ewe cheese ([Pinho et al., 2003](#)) or of hard ewe milk cheeses ([Hernández-Ramos et al., 2018](#)).

Fatty acids profiles of SECs ([Tables I-III](#)) were significantly influenced by cheese producer, geographical origin or moment of production, probably due to the difference in the milk used, since the FFA composition of the milk fat depends on the animals' feed, the season and the stage of lactation ([Cabiddu et al., 2006](#); [Jaramillo et al., 2008](#); [Vera et al., 2009](#); [Gómez-Cortés et al., 2015](#); [Giorgio et al., 2019](#); [Nájera et al., 2017](#)). Furthermore, the effect of the possibly distinct handling of the curds during manufacture should be considered, since SEC is handmade ([Estrada et al., 2019](#); [Poveda et al., 2000](#)). Cheese producer and geographical origin mostly affected the relative contents of some high chain saturated fatty acids, as well as unsaturated fatty acids (in general, $p \leq 0.007$, for one-way ANOVA). Also, total SFA, MUFA and PUFA relative abundances were also significantly influenced by cheese producer and geographical origin ($p \leq 0.012$, for one-way ANOVA), which confirms that, although the production of this high-value PDO traditional cheese is legally regulated, differences of fatty acids contents emerged according to the cheese's producer/geographical origin. On the contrary, the moment of production seems to mostly affect the relative abundance of short, medium and high chain saturated fatty acids as well as of some unsaturated fatty acids ($p < 0.044$, for one-way ANOVA) but did not influenced the relative abundance of total SFA, MUFA and PUFA ($0.070 \leq p \leq 0.300$, for one-way ANOVA).

Finally, it should be remarked that, SECs showed a relative high abundance of SFA (ranging from 67 to 76 per cent), followed by a medium content of MUFA (relative contents of 17-25 per cent) and by a low level of PUFA (varying in the range 5-7 per cent).

3.3 Lipid quality indices: influence of origin and moment of production

The availability of data concerning to lipid quality indices may allow having a clearer and informative knowledge regarding the nutritional and/or health impact of the consumption of dairy products. In this context, some researchers have been focussing their attention on the determination of some lipid health-related quality indices (e.g. DFA, SCFA and MCFA relative abundance as well as AI, TI and $\Sigma(\omega-6)/\Sigma(\omega-3)$ values)

besides the fatty acids' composition of cheeses. Indeed, low values of AI, TI and $\Sigma(\omega-6)/\Sigma(\omega-3)$ are highly desirable as well as higher values of DFA, SCFA and MCFA. Several researchers reported the AI values for different types of ewe cheese (0.84 for supplemented diet to 5.57 for no supplemented diets) showing that sheep diet supplementation allowed a decrease of the AI values (Cabiddu *et al.*, 2006; Gómez-Cortés *et al.*, 2009; Vargas-Bello-Pérez *et al.*, 2013a, 2013b). For commercial ewe cheese, Aguilar *et al.* (2014) found mean AI values of 2.33-2.63, TI values varying within 2.81-3.10 and, $\Sigma(\omega-6)/\Sigma(\omega-3)$ ratios ranging between 3.79 to 6.45, which were similar to those previously reported in the literature for ewe cheeses. Despite the relevance of lipid quality indices, namely from a health point of view, no information could be found in the literature regarding Serra da Estrela PDO cheeses. The results presented in this study provide a nutritional evaluation of the fatty acids components and their health-related lipid indices. All indices evaluated showed a wide variability (Figures 1-3).

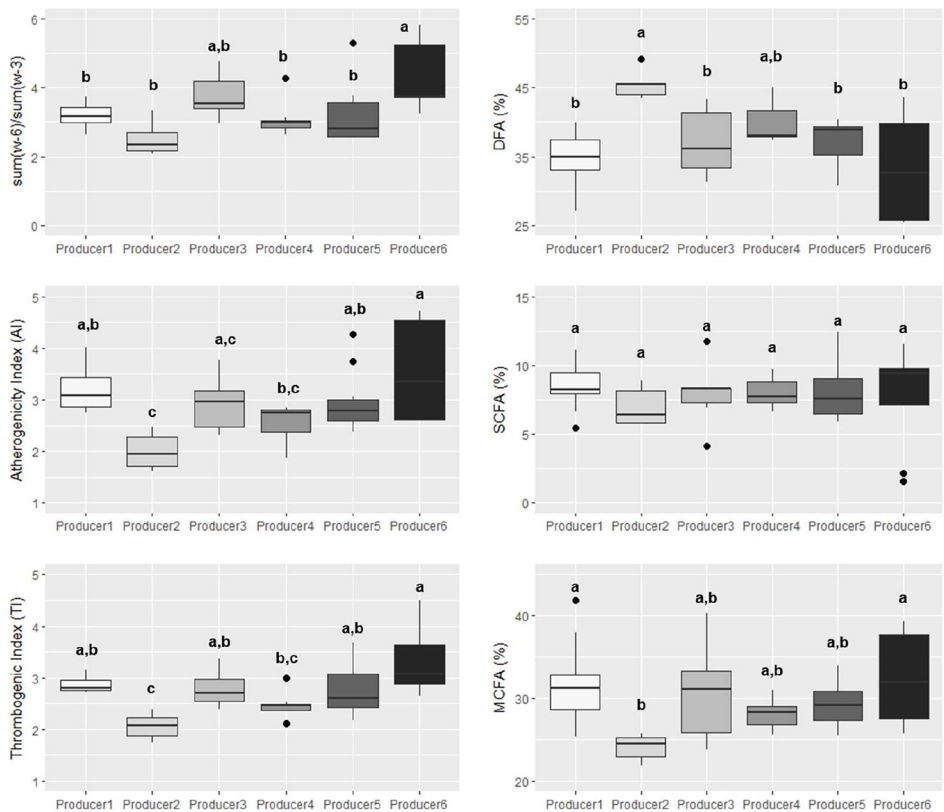


Figure 1. Boxplots for lipid quality indices as influenced by Serra da Estrela PDO cheese producer (Producers 1 to 6)

Notes: AI: atherogenic index; TI: thrombogenic index; $\omega6/\omega3$ ratio: $\Sigma(\omega-6) / \Sigma(\omega-3)$ ratio; MCFA: medium saturated chain fatty acids in %; SCFA: short chain saturated fatty acids in %; and, DFA: desirable fatty acids in %. Different lower-case letters mean significant statistical differences at a 5% significance level, for the post-hoc multi-comparison Tukey's test

Fatty acids profile

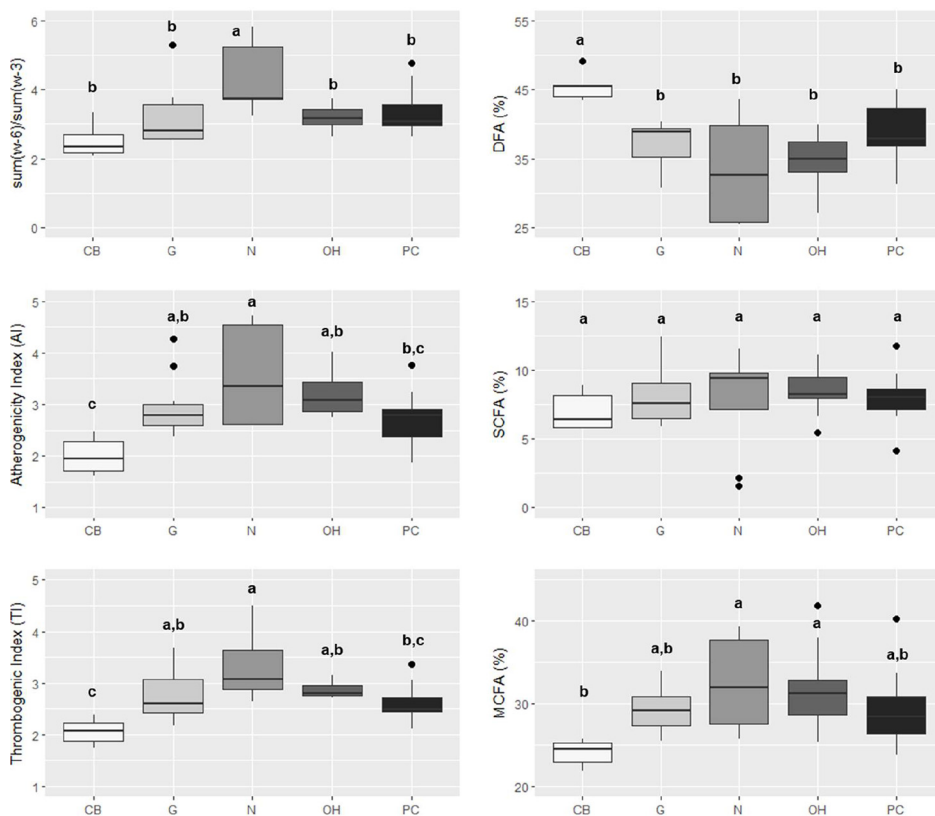


Figure 2. Boxplots for lipid quality indices as influenced by Serra da Estrela PDO cheese geographical origin (municipalities of Celorico da Beira – CB, Gouveia – G, Nelas – N, Oliveira do Hospital – OH, and Penalva do Castelo – PC)

Notes: AI: atherogenic index; TI: thrombogenic index; $\omega 6/\omega 3$ ratio: $\Sigma(\omega-6) / \Sigma(\omega-3)$ ratio; MCFA: medium saturated chain fatty acids in %; SCFA: short chain saturated fatty acids in %; and, DFA: desirable fatty acids in %. Different lower-case letters mean significant statistical differences at a 5% significance level, for the post-hoc multi-comparison Tukey's test

Besides, from the 3 main effects evaluated, the moment of production was the one that, from a statistical point of view, had a lower influence on the studied lipid quality indices. The relative abundances of DFA varied from 25.4 to 49.1 per cent, of SCFA ranged between 1.6 and 12.4 per cent, and of MCFA within 21.9 and 41.8 per cent. From the results (Figures 1-3) emerged that cheeses from Producer 2/CB region possessed significantly greater DFA values and significantly lower MCFA than the other 5 certified producers under study although regarding the total samples tested this producer presented the highest fat content, which could be related to animals' fed and/or different manufacturing practices. The AI, TI and $\Sigma(\omega-6)/\Sigma(\omega-3)$ values found for SECs also showed a wide variability, varying in mean values in 2.92 ± 0.73 , 2.73 ± 0.52 and 3.33 ± 0.86 , respectively. No guidelines currently exist regarding the AI and TI of dairy products, and lower indices are thought to be better for human health (Bentes *et al.*, 2009). According to Turan *et al.* (2007), lower AI and TI translate into more anti-

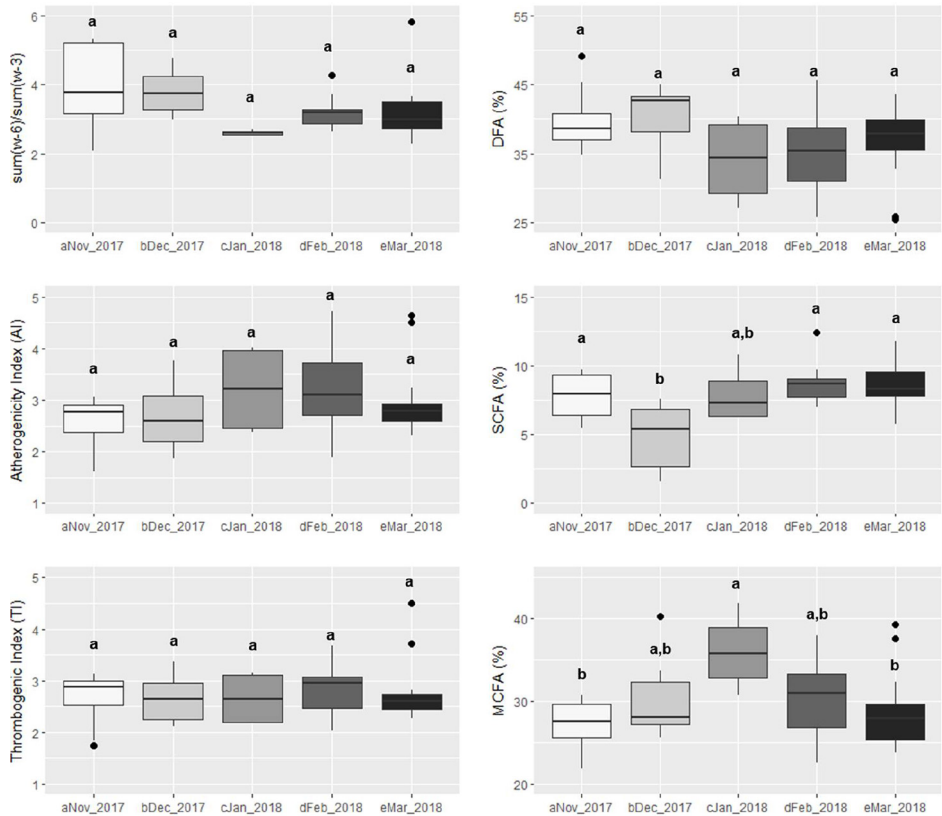


Figure 3. Boxplots for lipid quality indices as influenced by Serra da Estrela PDO cheese production date (November 2017 to March 2018)

Notes: AI: atherogenic index; TI: thrombogenic index; $\omega 6/\omega 3$ ratio: $\Sigma(\omega-6) / \Sigma(\omega-3)$ ratio; MCFA: medium saturated chain fatty acids in %; SCFA: short chain saturated fatty acids in %; and, DFA: desirable fatty acids in %. Different lower-case letters mean significant statistical differences at a 5% significance level, for the post-hoc multi-comparison Tukey's test

atherogenic FAs and better disease prevention profiles. Nevertheless, it should be remarked that the determined levels are in accordance with the ranges previously reported by other researchers for ewe, goat and cow cheeses (Aguilar *et al.*, 2014; Cabiddu *et al.*, 2006; Gómez-Cortés *et al.*, 2009; Oliveira *et al.*, 2015; Rodrigues *et al.*, 2012; Taboada *et al.*, 2015; Vargas-Bello-Pérez *et al.*, 2013a, 2013b).

Moment of manufacturing presented low AI and TI and the $\Sigma(\omega-6)/\Sigma(\omega-3)$ values were below recommended level (less than 4.0) thus differences observed might be attributed to differences in animal's feed (location in de region) and to the producer. As we previously considered the ewes' maintenance and the feed quality might directly influence the milk chemical and microbiological characteristics and consequently the final cheese composition (Balthazar *et al.*, 2017; Jaramillo *et al.*, 2008; Sanz Sampelayo *et al.*, 2007). Also, the natural and heterogeneous microflora associated with the processing and maturation contributed to the different profiles of SEC.

4. Conclusions

This work consists in a detailed study on the fatty acids composition and the related lipid health indices of SEC. SECs presented relative high abundance of SFA followed by a medium content of MUFA. We also tried to overcome the paradigm that these kind of cheeses may constitute a problem to consumers by calculating its lipid indices. The statistical significant differences found regarding the fatty acid profiles of the studied cheeses could foresee their future use to differentiate the cheese producer in different locations inside geographical origin, which may give some insights into animal feed and production management. Finally, the data from this study could be used to develop benchmark tools and strategies aiming at improving the nutritional characteristics of sheep cheese.

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