

# Propriedades Nutricionais, Químicas e Bioativas dos alimentos – o caso do Queijo da Serra da Estrela

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## 1. INTRODUCTION

**Serra da Estrela** is a Portuguese traditional cheese that bears a Protected Designation of Origin label, produced in a limited geographical region, using

- Raw ewe milk from “Churra Mondegueira” and “Bordaleira” autochthonous breeds
- Wild thistle flower (*Cynara cardunculus* L.)
- Salt



- ✓ Milk and dairy products are considered an important part of a balanced diet.
- ✓ The nutritional value of proteins present in cheese depends on its AA content.
- ✓ The fat content is the more quantitatively and qualitatively variable component of SEC depending on season, lactation, stage, breed, genotype and feeding.

**Table 1.** Nutritional composition of Serra Estrela (SE) cheese, for 100 g (Adapted from Queijos dos Frescos aos Curados, 2018 [11]).

<b>Energy</b>	333 kcal	
<b>Lipids</b>	27 g	
<b>Saturated fat</b>	14.2 g	
<b>Carbohydrates</b>	0.2 g	
<b>Sugars</b>	0.2 g	
<b>Proteins</b>	21 g	
<b>Salt</b>	1.78 g	
<b>Vitamin A</b>	240 $\mu$ g	800 $\mu$ g RDD
<b>Vitamin D</b>	0.2 $\mu$ g	25 $\mu$ g RDD
<b>Vitamin B2</b>	0.6 mg	1.6 mg RDD
<b>Vitamin B3</b>	5.6 mg	15 mg RDD
<b>Vitamin B6</b>	0.09 mg	1.4 mg RDD
<b>Vitamin B12</b>	1.4 mg	2.5 $\mu$ g RDD
<b>Folate</b>	31 mg	
<b>Potassium</b>	80 mg	
<b>Calcium</b>	700 mg	
<b>Phosphorus</b>	480 mg	
<b>Magnesium</b>	53 mg	
<b>Iron</b>	0.7 mg	
<b>Zinc</b>	2.8 mg	

RDD: Recommended daily portion (Portuguese Association

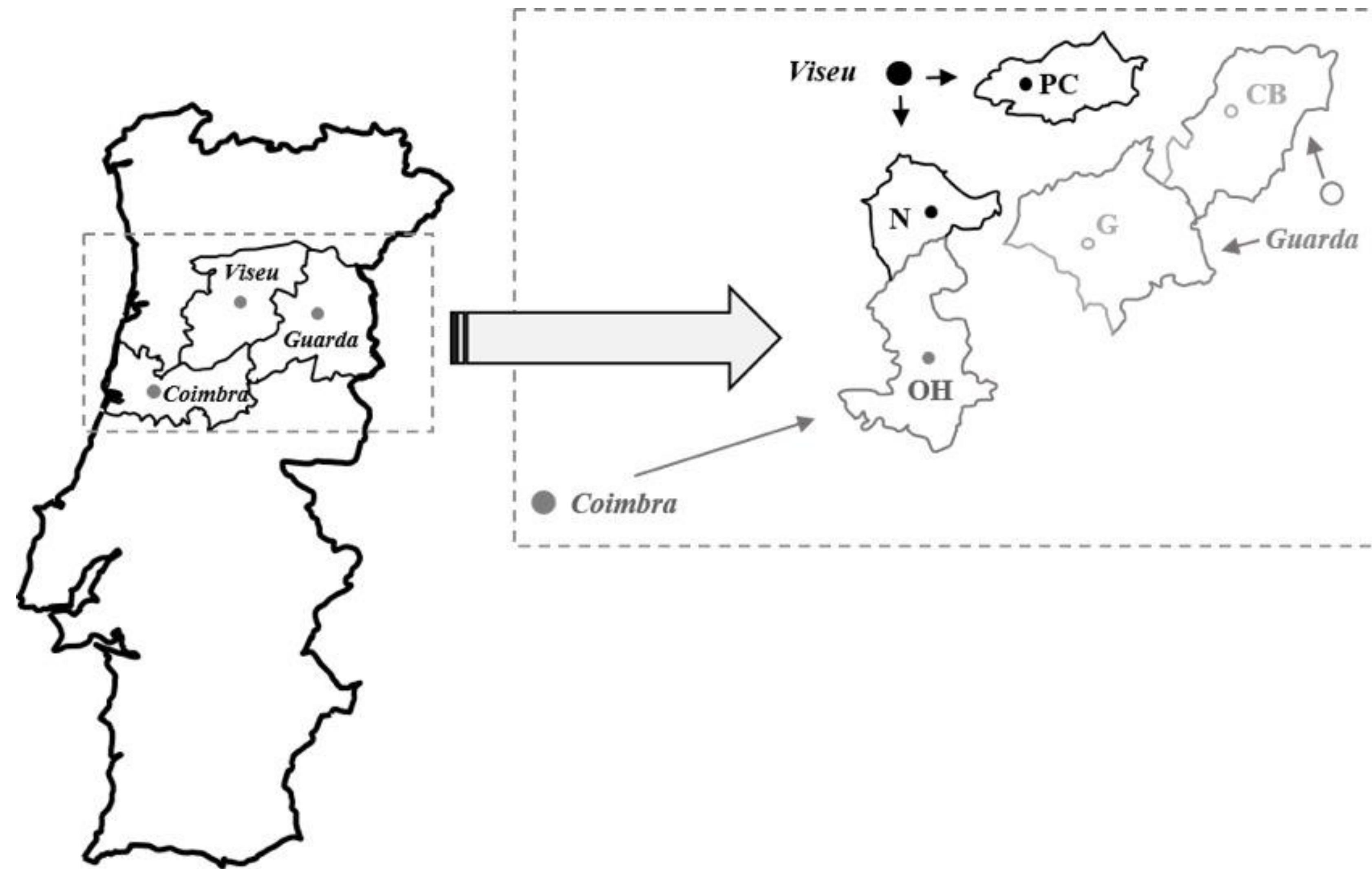


Figure 1. Geographical origin of the Serra da Estrela PDO cheese samples evaluated (CB – Celorico da Beira, Producer 2; G – Gouveia, Producer 5; N – Nelas, Producer 6; OH – Oliveira do Hospital, Producer 1; and, PC – Penalva do Castelo, Producers 3 and 4).

Cheeses were produced with the milk, collected from ewes acquired in 6 certified cheese producers located in 5 municipalities within the delimited PDO region.

## 2. MATERIAL AND METHODS

Twenty-four Serra da Estrela cheeses, 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 ( $-40\text{ }^{\circ}\text{C}$ ) until analysis.



## 2. 1. MATERIAL AND METHODS



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 × 0.25 mm ID × 0.20 μm). A constant flow rate of 1 mL/min was used. For each analysis 1 uL of the sample was injected in GC equipment.

The identification was carried out by comparing the relative retention times of the FuAME to the commercial standard.

## 2. 2. MATERIAL AND METHODS

### **Amino acids** of SEC: standard solutions and gas chromatography analysis

The internal standard (IS) method was used to calibrate the chromatographic system for the AA quantification, being the N-Acetyl-L-Tyrosine chosen as the IS because it was not detected in the protein fraction of the cheese samples analyzed. Peaks identification took into account the retention time, the quantification transition (m/z) and the confirmatory transition (m/z) data.

The chromatographic analysis was performed using an UPLC–MS/MS (Dionex Ultimate 3000 UPLC instrument from Thermo Scientific, USA) equipped with a diode-array detector and coupled to a mass detector Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) with an ESI source. A multistep gradient program was used at a flow rate of 0.40 mL/min, being the injection volume of 5  $\mu$ L. Each chromatographic assay comprised a 10 min run. Data acquisition was carried out with Xcalibur<sup>®</sup> data system (Thermofinnigan, San Jose, CA, USA).



**Table 2.**  
**Fatty acids profile (%), determined by GC-FID, of SEC (mean ± standard deviation) produced at 5 geographical origins was made**

Fatty acids	Serra da Estrela cheeses (n = 24 cheeses × 2 independent samples)	
	Mean content ± standard deviation (%)	Minimum-Maximum range (%)
C4:0 (butyric acid)	4.3 ± 1.4	0.5-6.4
C6:0 (caproic acid)	3.6 ± 1.0	1.1-6.2
C8:0 (caprilic acid)	3.2 ± 0.9	1.7-6.1
C10:0 (capric acid)	8.6 ± 2.2	4.1-14.9
C11:0 (undecanoic acid)	0.10 ± 0.04	0.03-0.24
C12:0 (lauric acid)	5.2 ± 1.1	3.3-7.9
C13:0 (tridecanoic acid)	0.09 ± 0.02	0.04-0.14
C14:0 (miristic acid)	11.4 ± 1.3	7.6-13.7
C14:1 (miristoleic acid)	0.30 ± 0.14	0.13-0.56
C15:0 (pentadecanoic acid)	1.0 ± 0.2	0.5-1.4
C16:0 (palmitic acid)	23.9 ± 1.9	18.7-26.8
C16:1 (palmitoleic acid)	0.8 ± 0.2	0.2-1.0
C17:0 (heptadecanoic acid)	0.7 ± 0.1	0.4-1.0
C18:0 (stearic acid)	10.8 ± 1.8	6.9-13.9
C18:1n9c (oleic acid)	19.1 ± 3.8	11.2-27.0
C18:2n6t (elaidic acid)	1.4 ± 0.5	0.4-2.3
C18:2n6c (linoleic acid)	3.1 ± 0.5	2.4-4.1
C18:3n3 (γ-linoleic acid)	1.5 ± 0.4	0.4-2.3
C20:0 (arachic acid)	0.23 ± 0.06	0.12-0.40
C20:1 (eucosenoic acid)	0.09 ± 0.05	0.02-0.30
C21:0 (hencosanoic acid)	0.07 ± 0.05	0.02-0.28
C20:4n6 (arachidonic acid)	0.3 ± 0.1	0.01-0.5
C22:0 (behenic acid)	0.11 ± 0.06	0.01-0.2
SSFA	73.4 ± 4.2	64.8-81.9
SMUFA	20.3 ± 3.8	12.4-28.1
SPUFA	6.3 ± 0.7	4.3-7.5

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

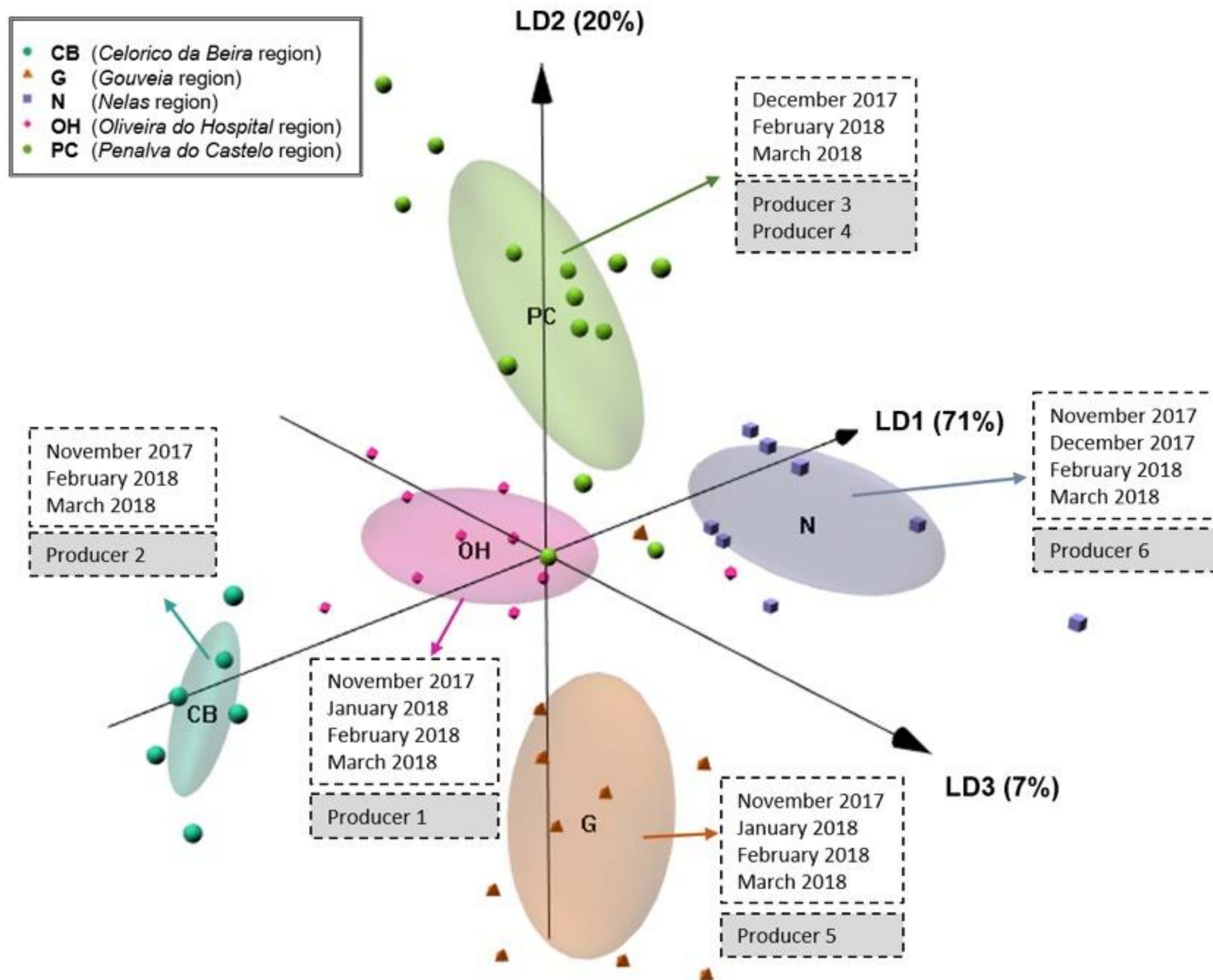


Figure 2. LDA-SA model classification of Serra da Estrela PDO cheeses (24 cheeses × 2 independent samples) according to the production geographical origin 3D plots of the first 3 most significant discriminant functions based on relative abundance of 12 fatty acids (C6:0, C8:0, C11:0, C12:0, C15:0, C16:0, C16:1, C17:0, C18:1n9c, C18:2n6t, C21:0 and C20:4n6) and the MUFA, selected using the SA algorithm.

REVIEW ARTICLE

Nutrition and Atherosclerosis

5 Nimbe Torres, Martha Guevara-Cruz, Laura A. Velázquez-Villegas, and Armando R. Tovar

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Cardiovascular disease (CVD) is a universal problem in modern society. Atherosclerosis is the leading cause of CVD resulting in high rate of mortality in the population. Nutrition science has focused on the role of essential nutrients in preventing deficiencies, at the present time, the nutritional strategies are crucial to promote health and intervene with these global noncommunicable diseases. In many cases, diet is a major driving force, which is much easier to change and follow than other factors. It is important to establish that the first strategy to treat atherosclerosis is to modify lifestyle habits, focusing on the beneficial properties of specific nutrients. In the last decades, epidemiological, clinical and experimental studies have demonstrated that diet plays a central role in the prevention of atherosclerosis. In this review we will focus on the effect of specific foods, nutrients and bioactive compounds, including epidemiological facts, potential mechanisms of action and dietary recommendations to reduce the risk of atherosclerosis. In particular, we include information about fiber, plant sterols and stanols, niacin, taurine, olive oil, omega 3 fatty acids, antioxidants, minerals, methyl nutrients and soy. In addition, we also show that dysbiosis of the intestinal microbiota associated with a consumption of certain animal food sources can generate some metabolites that are involved in the development of atherosclerosis and its consequences on CVD. According to the epidemiological, clinical and experimental studies we suggest a recommendation for some dietary foods, nutrients and bioactive compounds to support the complementary clinical management of patients with atherosclerosis. © 2015 IMSS. Published by Elsevier Inc.

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! Key Words: ■ ■ ■.



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# Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study

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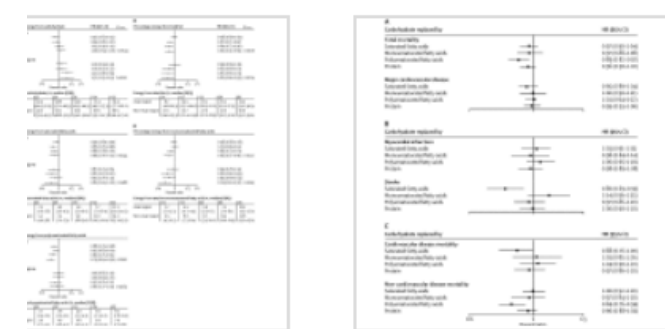
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The Lancet, Volume 390, Issue 10107, 4–10 November 2017, Pages 2018–2019  
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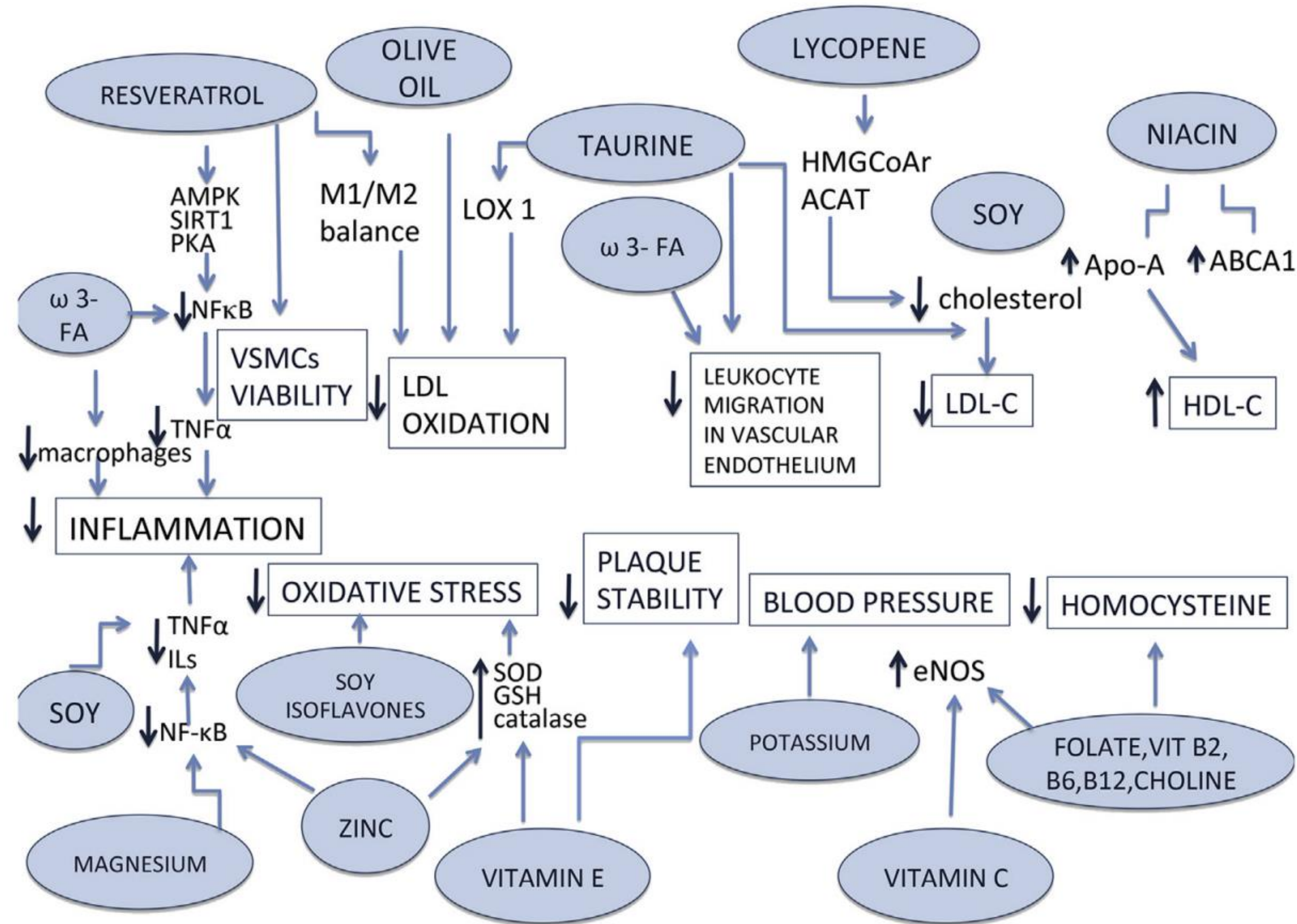
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/ appendix

In conclusion, we found that a high carbohydrate intake was associated with an adverse impact on total mortality, whereas fats including saturated and unsaturated fatty acids were associated with lower risk of total mortality and stroke. We did not observe any detrimental effect of fat intakes on cardiovascular disease events. Global dietary guidelines should be reconsidered in light of the consistency of findings from the present study, with the conclusions from meta-analyses of other observational studies<sup>8, 10, 54</sup> and the results of recent randomised controlled trials.<sup>36</sup>



**Figure 1.** Effects of some nutrients and bioactive compounds on biological factors involved in the atherosclerotic process. ABCA1, ATP-binding cassette A1; AMPK, 5' AMP-activated protein kinase; ACAT, acyl-coenzyme A:cholesterol acyltransferase; eNOS, endothelial nitric oxide synthase; FA, fatty acid; GSH, glutathione; HDL-C, high-density lipoprotein cholesterol; HMGCoAr, 3-hydroxy-3-methyl glutaryl-CoA reductase; ILs, interleukins; LDL-C, low density lipoprotein cholesterol; LOX1, oxidized LDL receptor 1; M1/M2, M1/M2 macrophages; NF-κB, necrosis factor κB; PKA, protein kinase A; SIRT1, sirtuin 1; SOD, superoxide dismutase; TNFα, tumor necrosis factor α; VSMCs, vascular smooth muscle cells.

**Table** Amino acids profile (mg/100 g cheese of dry matter), determined by UPLC-DAS-MS/MS, of Serra da Estrela cheeses (mean  $\pm$  standard deviation) produced by 6 certified producers (Producers 1 to 6) located in 5 geographical origins (CB – Celorico da Beira, G – Gouveia, N – Nelas, OH – Oliveira do Hospital and PC – Penalva do Castelo) during November 2017 to March 2018. Different lower case letters mean significant statistical differences at a 5% significance level (one-way ANOVA followed by the Tukey’s multi-comparison test). Each independent cheese sample was injected in triplicate.

Amino acids (AA)	Cheese producer			
	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)
<b><i>Essential amino acids (EAA)</i></b>				
Histidine*	0.49 $\pm$ 0.08a	0.30 $\pm$ 0.02a,b	0.46 $\pm$ 0.18a,c	0.20 $\pm$ 0.02b
Leucine+Isoleucine*.#	42 $\pm$ 14d	70 $\pm$ 13c,d	121 $\pm$ 11a	64 $\pm$ 10c,d
Lysine*	0.55 $\pm$ 0.35	0.32 $\pm$ 0.13	0.57 $\pm$ 0.16	0.83 $\pm$ 0.26
Methionine*	1.6 $\pm$ 1.3a,b	2.1 $\pm$ 1.4a,b	3.7 $\pm$ 0.5a	1.1 $\pm$ 0.6b
Phenylalanine*	22 $\pm$ 7b	46 $\pm$ 9a	68 $\pm$ 6a	34 $\pm$ 3a,b
Threonine*	0.38 $\pm$ 0.23b,c	0.28 $\pm$ 0.08c	0.91 $\pm$ 0.22a	0.29 $\pm$ 0.07c
Tryptophan*	3.9 $\pm$ 3.0a,b	4.1 $\pm$ 2.7a,b	1.5 $\pm$ 0.6b	2.7 $\pm$ 1.7a,b
Valine*.#	9 $\pm$ 7c	23 $\pm$ 6a,b	30 $\pm$ 4a	12 $\pm$ 2b,c
<b><i>Non-essential amino acids</i></b>				
Alanine	N.D.	N.D.	N.D.	N.D.
Arginine	0.26 $\pm$ 0.06 <sup>a,b</sup>	0.13 $\pm$ 0.02b	0.14 $\pm$ 0.01b	0.14 $\pm$ 0.02b
Asparagine	23 $\pm$ 10b	38 $\pm$ 8b	70 $\pm$ 6a	41 $\pm$ 8b
Aspartic acid	0.6 $\pm$ 0.2b	0.9 $\pm$ 0.3b	2.7 $\pm$ 0.3a	0.9 $\pm$ 0.2b
Cysteine	104 $\pm$ 39c	215 $\pm$ 34a	164 $\pm$ 8a,c	122 $\pm$ 48b,c
Cystine	N.D.	N.D.	N.D.	N.D.
Glutamic acid	0.9 $\pm$ 0.7b	3.0 $\pm$ 0.6a,b	9.0 $\pm$ 11.4a	4.4 $\pm$ 1.8a,b
Glutamine	0.48 $\pm$ 0.40	0.24 $\pm$ 0.13	0.55 $\pm$ 0.18	0.80 $\pm$ 0.28
Glycine	N.D.	N.D.	N.D.	N.D.
Proline	37 $\pm$ 12c	57 $\pm$ 6b,c	100 $\pm$ 7a	57 $\pm$ 9b,c
Serine	1.4 $\pm$ 0.6a	0.67 $\pm$ 0.16b,c	0.47 $\pm$ 0.06c	0.69 $\pm$ 0.18b,c
Tyrosine	1.1 $\pm$ 1.3b	0.4 $\pm$ 0.2b	0.6 $\pm$ 0.2b	5.4 $\pm$ 4.5a
SAA	247 $\pm$ 88c	461 $\pm$ 66a,b	574 $\pm$ 33a	347 $\pm$ 56b,c
SEAA	79 $\pm$ 29c	146 $\pm$ 21b	226 $\pm$ 14a	116 $\pm$ 14b,c

# Focus on Health profile of SEC, a particular interesting food type

Nutrients have emerged as an important research topic since they seem to be able to modulate the inflammatory status of human being, namely:

Preventing low-grade **systemic inflammation**

**Coadjuvants** in conventional therapies **Immunity**

Gut-systemic inflammatory associations

**Inverse** association with **alergies** Biopeptides that lower **blood pressure** **Antimicrobial** peptides

Proteins with **high biological** value with **easy digestibility** containing all the **essential aa**

Improvement of blood glucose levels

Helps **muscle recovery**

## 4. CONCLUSIONS

This study demonstrated some nutritional characteristics of cheese may be used as chemical biomarkers for assessing the origin and production date of Serra da Estrela PDO cheeses, which is of major importance for producers and consumers.

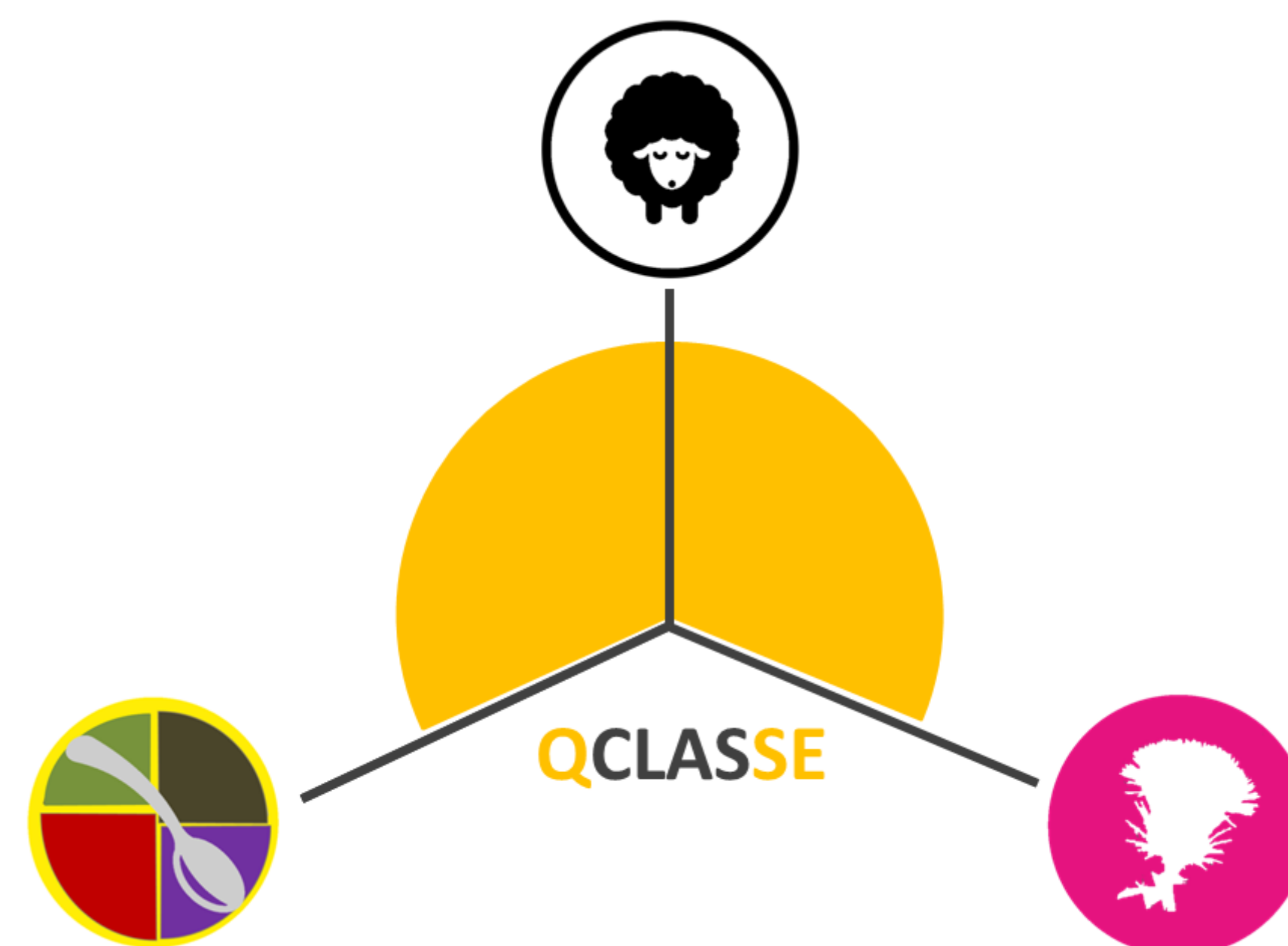
In fact, less abundant fatty acids (e.g.,  $C_{15:0}$ ,  $C_{16:1}$ ,  $C_{17:0}$ ,  $C_{20:1}$ ,  $C_{21:0}$  and  $C_{20:4n6}$ ) seem to play an important role as cheese origin biomarkers.





## 4. CONCLUSIONS

SEC contains some **bioactive compounds** that present unique characteristics and their nutritional value, as well as their potential health effects, must continue to be object of investigation.



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