



Chemometric characterization of the fatty acid and trace element profiles of organic and conventional Galician cheeses

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ARTICLE INFO

Keywords:

Galician cheese
Fatty acids
Trace and toxic elements
Organic production
Health-related nutritional indices

ABSTRACT

The aim of this study was to evaluate the fatty acid profile and trace element content in three different Galician cow's milk cheeses (organic made from pasteurized milk, industrial made from pasteurized milk and artisanal made with raw milk). Twenty-five fatty acids and thirteen elements were determined by respectively gas liquid chromatography and inductively coupled plasma mass spectrometry, in thirty representative samples of the cheeses. The organic cheeses had significantly lower palmitic acid contents and significantly higher contents of odd and branched fatty acids, oleic acid, vaccenic acid, α -linolenic acid and conjugated linoleic acid than the other cheeses. The organic cheeses also had lower concentrations of trace and toxic element residues. Moreover, an association between medium chain fatty acids and palmitic acid and the presence of several mineral elements was detected by chemometric techniques. The nutritional evaluation of the organic cheeses was more favorable than for the other cheeses, as confirmed by the values of health-related indices.

1. Introduction

In the European Union (EU), production of cow's milk cheese increased in the last years. Spain is one of the principal cheese producing countries in the EU, and Galicia (which produces one-third-of the total amount of cheese produced in Spain) has a long tradition of cheese making. Most of the cheeses produced in Galicia are soft or semi-soft cheeses elaborated with cow's milk and matured for a short time. Traditionally, Galician cheeses were elaborated using raw milk from pasture-fed cattle (Rodríguez-Alonso et al., 2009), but nowadays they are almost entirely manufactured with pasteurized cow's milk from intensive production systems. However, artisan cheeses made from raw milk produced on small, pasture-based farms are still sold in local markets and gourmet stores. In line with sustainable development goals, the organic cheese production in Galicia has increased over the last

decade, maintaining traditional practices but incorporating organic dairy farming regulations (Regulation (EU), 2018). Consumers perceive that organic products are healthier and of better quality than conventional products; therefore, the number of organic producers and the production of organic cheeses with pasteurized milk has increased significantly in the last decade (Rodríguez-Bermúdez et al., 2020).

Cow's milk lipids contain over 400 different fatty acids (FA), with diverse number of carbon atoms and various degrees of saturation: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA). Some of these FA are of potential benefit to human health, including oleic acid (C18:1cis9), conjugated linoleic acid (CLA), mainly rumenic acid (C18:2cis9 trans11), odd and branched-chain fatty acids (OBCFAs), and vaccenic (C18:1trans11) and α -linolenic acids (C18:3n-3) (Paszczyk, 2022; Paszczyk et al., 2022). The FA composition of bovine milk is affected by many factors, particularly diet, but also by the breed,

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<https://doi.org/10.1016/j.afres.2024.100475>

Received 3 July 2024; Received in revised form 6 August 2024; Accepted 12 August 2024

Available online 13 August 2024

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genetic parameters, parity, geographical location, season, number of calvings, milk and fat yield, days in milk and metabolic state, among others (Ellis et al., 2006; Rodríguez-Bermúdez et al., 2023). The FA composition of milk will therefore depend on the type of farm production system involved (organic vs. conventional or grazing vs. intensive). Milk from cows fed pasture-based rations has higher concentrations of PUFA than milk from cattle reared in intensive, zero-grazing systems, which has higher concentrations of SFA (Butler et al., 2008; Hasanaliyeva et al., 2023; Rodríguez-Bermúdez et al., 2023). European regulations on organic dairy production require farms to adopt high grazing or forage consumption (>60 % dry matter), with reduced amounts of concentrated feed (Regulation (EU), 2018). Previous studies in the EU comparing FA profiles in conventional and organic cow's milk consistently point out that organic milk has significantly higher concentrations of human-health beneficial PUFA than milk produced in intensive systems (Adler et al., 2013; Capuano et al., 2015; Collomb et al., 2008; Ellis et al., 2006; Hanuš et al., 2018; Linehan et al., 2024; Liu et al., 2020; Ormston et al., 2023; Średnicka-Tober et al., 2016; Tzamaloukas et al., 2021). However, these studies usually involve liquid milk rather than processed dairy products (Średnicka-Tober et al., 2016), and data on cheese are scarcer and more variable. Recent research has shown that the effects of organic farming systems on the FA profiles of cheese were similar to those in the milk used to produce the cheese, and MUFA and PUFA concentrations were higher in organic cheese than in other cheeses (Tzamaloukas et al., 2021). By contrast, another recent study of Italian cheese found only minor differences in the FA composition of organic and conventional cheeses (Manuelian et al., 2023).

Recent data on trace element composition and the toxic metal residues in Galician cheese according to the manufacturing processes (de Oliveira Filho et al., 2022) revealed that the element contents of industrial cheeses produced with milk from intensive dairy farms were different from those in artisan and organic cheeses (in which the values were similar). However, studies on the FA profile of organic cheeses produced in this region and on the relationship between the FA profile and trace element composition and the toxic element residues are scarce. It is possible that both the FA profile and element contents are affected by the type of diet and livestock management. Although pasteurization of the milk does not significantly affect the fatty acid profile of cheeses (Ioannidou et al., 2022), differences in the type of milk used and the manufacturing process could also influence the FA profile and the elemental composition of the cheeses. The aim of this study was to compare the FA profile in relation to the trace element composition and the toxic element residues in organic Galician cow's milk cheese made from pasteurized milk and in industrial pasteurized and artisan raw milk cheeses.

2. Materials and methods

2.1. Sample collection

Thirty representative samples of Galician cow's milk cheeses were analyzed in the present study. All samples were semi-soft cheeses made from pressed white curd and with a short ripening time (about ten days), following the Galician cheese-making tradition. Three different groups were identified in the total of 30 samples. The first group was composed of 10 organically produced cheeses (certified by CRAEGA, the Galician Organic Agriculture Regulatory Council, the official regulatory body responsible for authenticating organic products in Galicia). The organic milk were obtained from cows in small-scale farms that the feeding were based on maximum use of grazing pasturage according to European regulations (Regulation (EU), 2018) with daily rations based mainly on fresh pasture and no mineral supplementation on the diets. These samples, made from pasteurized milk, were classified as organic samples (O). The second group included 10 industrially-produced samples, also made from pasteurized milk. These industrial cheeses were manufactured by dairy industries with high cheese production that obtain the

milk from several farms with mostly with intensive production system and feeding based on concentrates. Samples in this group were classified as industrial pasteurized (IP) milk cheeses. The third group comprised 10 samples of cheeses made from raw milk by traditional artisanal producers with milk from their own small-scale farms and the feeding of the cows was probably very variable (concentrates, silage, pasture, etc.); they were classified as artisan raw (AR) milk cheeses. Organic and industrial cheeses were obtained from local supermarkets and gourmet stores, while artisan cheeses were purchased directly from the manufacturers in January and February 2019. All samples were labeled according to the type/origin and placed in polyethylene bags. The bags were then sealed, transported to the laboratory and stored at 4 °C for two or three days until analysis.

2.2. Lipid extraction and fatty acid analysis

Total lipids were extracted from the samples using the Weibull-Berntrop gravimetric method, ISO 8262-3:2005 (ISO, 2005), which consists of acid hydrolysis (using HCl) followed by fat extraction by the Soxhlet method. The fatty acid composition was determined by gas-liquid chromatography of methyl esters prepared in basic conditions (KOH: methanol) according to the International Organization for Standardization method (ISO, 2017). The gas chromatograph system (Agilent 7820 gas chromatograph, Agilent Technologies, Santa Clara, California, USA) was equipped with a flame ionization detector. Data acquisition and analysis were performed using chromatographic software (Agilent OpenLAB CDS ChemStation, Edition C.01.08, Agilent Technologies). The fatty acid methyl esters were separated on a capillary column HP-88 (88 % cyanopropyl) aryl-polysiloxane stationary phases, length 60 m, internal diameter 0.25 mm and film thickness 0.2 μm (Agilent Technologies). The split/splitless injector was used with Split 50:1, injection volume 1 μL, and the oven temperature was programmed from an initial temperature of 180 °C to 210 °C (at an increasing rate of 4 °C min⁻¹), with a final isothermic period (210 °C, 30 min). The injector and detector temperatures were both held at 250 °C.

The different fatty acid methyl esters were identified by comparison of the retention times with those of authentic standards (Sigma-Aldrich, St. Louis, MO, USA) and injecting a standard mixture of methyl esters (C-4:0 to C-22:6) (Larodan product number 90-1100; mixture ME 100; Larodan AB, Solna, Sweden). The individual fatty acids were calculated from the ratio of the peak area of each, relative to the total area for all studied fatty acids. The results obtained were expressed as a percentage of the total area of injected methyl esters. All analyses were conducted in duplicate.

Some human health related indices were calculated on the basis of the fatty acid composition of the cheeses (according to Ormston et al. (2023): the atherogenicity index,

$$AI\ index = \frac{C12:0 + 4 \times C14:0 + C16:0}{MUFA + PUFA}$$

the thrombogenicity index,

$$TI\ index = \frac{C14:0 + C16:0 + C18:0}{0.5 \times MUFA + 0.5 \times (PUFA - 6) + 3 \times (PUFA - 3) + \frac{PUFA - 3}{PUFA - 6}}$$

the hypocholesterolemic to hypercholesterolemic ratio,

$$hH\ ratio = \frac{C18:1n-9 + PUFA}{C12:0 + C14:0 + C16:0}$$

and the n-6/n-3 ratio index,

$$\frac{n-6}{n-3}\ ratio = \frac{PUFA - 6}{PUFA - 3}$$

2.3. Analysis of essential and toxic elements

Essential and toxic elements were determined by ICP-MS. Subsamples of 1 g of each cheese sample were prepared by acid digestion in a microwave-assisted digestion system (Ethos Plus, Milestone, Sorisole, Italy) with 5 mL of 69 % concentrated Hiperpure nitric acid (PanReac, Spain) and 2 mL of 33 % w/v hydrogen peroxide (PanReac, Spain). The digested samples were diluted with ultrapure water (Milli-Q, Millipore Co., Bedford, MA, USA) to a final volume of 15 mL. Additional preparation was required for iodine, consisting of treatment of the sample by an alkaline extraction procedure (EN, 2007) at high temperature with a mixture of tetramethylammonium hydroxide 25% w/v (Sigma-Aldrich, Spain). A total of thirteen essential elements (Co, Cr, Cu, Fe, I, Mn, Mo, Se and Zn) and toxic elements (As, Cd, Hg and Pb) were determined by ICP-MS, in a mass spectrometer (Agilent 7700 ICP-MS, Agilent Technologies) equipped with collision/reaction cell interference reduction technology. Determinations were carried out according to the previously described method (de Oliveira Filho et al., 2022; Rey-Crespo et al., 2013; Rodríguez-Bermúdez et al., 2018). All samples were analyzed in triplicate, and the element concentrations were expressed as $\mu\text{g kg}^{-1}$ or mg kg^{-1} wet weight (w/w).

The analytical quality of the results was evaluated throughout the study by analyzing blank samples and certified reference material (CRM) (NIST SRM-1549, non-fat milk powder), along with the samples. The limits of detection were calculated as three times the standard deviation of the blanks, and they were found to be sufficient for determining the concentrations of all elements in the samples. The analytical recovery of the CRM analyzed ranged between 91 and 110 % for the different elements.

2.4. Statistical and chemometric analysis

A data matrix was constructed from the result of the analyses, in which the rows corresponded to the 30 cheese samples (O, IP and AR classes) and the columns included the concentrations of 38 compounds determined (twenty-five FAs plus thirteen essential and toxic elements) measured by GC and ICP-MS, respectively. Data normality was checked using the Kolmogorov–Smirnov test. When the data were not normally distributed, they were log-transformed before analysis. The potential differences in composition for O, IP and AR groups were evaluated by ANOVA and post-hoc Tukey tests.

The data were analyzed by chemometric techniques. In order to test for the potential influence of the cheese type (O, IP and AR) on the fatty acid and mineral composition, the relationships between samples, between variables and between variables and samples were assessed using two unsupervised chemometric techniques: Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). PCA was used to reveal the latent structure of the data. This technique allows examination of the n -dimensional composition matrix in a reduced dimension (where n is the number of variables to be studied), preserving the maximum data variance. HCA (often applied in conjunction with PCA) was used to identify any clusters of similar samples (or variables), calculated from the distance between samples (or between variables) as a measure of similarity. This technique yields a dendrogram, in which sample similarities in the n -dimensional space of the variables are mapped in a bidimensional plot (Massart & Kaufman, 1983). In both cases, data were autoscaled before PCA and HCA to avoid the potential influence of the different weightings of the variables (FAs and minerals) in the chemometric study. In this procedure, the original variables were substituted with new variables of the same size (with zero mean and unit variance). For this task, each value of an original variable was converted to the new value by subtracting the mean value of the variable and dividing the result by the standard deviation.

All statistical and chemometric analyses were carried out using the software packages IBM SPSS for Windows v.27 (IBM Corporation, Armonk, NY, USA) and Statgraphics Centurion XVIII v.18.1.12

Table 1

Fatty acid profiles (% of total fatty acids) and health-related indices (mean \pm SD) of organic pasteurized (O), industrial pasteurized (IP) and artisan raw milk (AR) cheeses.

Fatty acid	O	IP	AR	P
C6:0	1.12 \pm 0.16	1.33 \pm 0.21	1.25 \pm 0.22	0.0783
C8:0	0.87 \pm 0.11	1.00 \pm 0.18	0.93 \pm 0.12	0.1418
C10:0	2.17 \pm 0.32	2.41 \pm 0.21	2.41 \pm 0.27	0.0845
C10:1	0.25 \pm 0.03 ^a	0.31 \pm 0.03 ^b	0.30 \pm 0.03 ^b	0.0003
C11:0	0.15 \pm 0.71 ^a	0.17 \pm 0.01 ^{ab}	0.19 \pm 0.03 ^b	0.0111
C12:0	2.78 \pm 0.36 ^a	3.02 \pm 0.17 ^{ab}	3.19 \pm 0.36 ^b	0.0192
C14:0	10.80 \pm 0.76	11.06 \pm 0.52	11.50 \pm 0.56	0.0548
C14:1	0.72 \pm 0.10 ^a	0.99 \pm 0.12 ^b	0.93 \pm 0.09 ^b	0.0000
C15:0iso	0.31 \pm 0.08 ^b	0.24 \pm 0.03 ^a	0.25 \pm 0.04 ^a	0.0288
C15:0anteiso	0.53 \pm 0.13 ^b	0.41 \pm 0.06 ^a	0.42 \pm 0.06 ^a	0.0079
C15:0	1.41 \pm 0.35 ^b	1.17 \pm 0.06 ^a	1.20 \pm 0.13 ^a	0.0461
C16:0	29.49 \pm 1.53 ^a	32.89 \pm 1.47 ^b	32.92 \pm 2.66 ^b	0.0005
C16:1	2.30 \pm 0.17	2.26 \pm 0.18	2.17 \pm 0.17	0.2041
C17:0iso	0.45 \pm 0.08 ^b	0.31 \pm 0.05 ^a	0.36 \pm 0.07 ^a	0.0006
C17:0anteiso	0.62 \pm 0.10 ^b	0.48 \pm 0.11 ^a	0.49 \pm 0.07 ^a	0.0077
C17:0	0.89 \pm 0.11 ^b	0.71 \pm 0.05 ^a	0.71 \pm 0.07 ^a	0.0000
C17:1	0.38 \pm 0.07 ^b	0.29 \pm 0.03 ^a	0.29 \pm 0.05 ^a	0.0010
C18:0	12.08 \pm 0.79	11.24 \pm 0.56	11.50 \pm 1.12	0.0988
C18:1cis9	24.84 \pm 1.22 ^b	22.98 \pm 1.46 ^a	22.43 \pm 1.52 ^a	0.0018
C18:1trans11	3.22 \pm 0.53 ^b	2.77 \pm 0.40 ^{ab}	2.48 \pm 0.62 ^a	0.0153
Other C18:1	0.54 \pm 0.14	0.56 \pm 0.09	0.55 \pm 0.07	0.8811
C18:2n-6	1.93 \pm 0.21	1.97 \pm 0.27	2.14 \pm 0.32	0.2100
C18:3n-3	1.02 \pm 0.31 ^b	0.59 \pm 0.13 ^a	0.61 \pm 0.15 ^a	0.0001
C18:2cis9 trans11	0.99 \pm 0.38	0.72 \pm 0.23	0.64 \pm 0.32	0.0516
C20:1	0.22 \pm 0.04 ^b	0.18 \pm 0.02 ^a	0.19 \pm 0.03 ^a	0.0202
SFA	63.67 \pm 1.57 ^a	66.44 \pm 1.87 ^b	67.35 \pm 2.30 ^b	0.0006
MUFA	32.47 \pm 1.13 ^b	30.35 \pm 1.65 ^a	29.34 \pm 1.88 ^a	0.0005
PUFA	3.93 \pm 0.75	3.28 \pm 0.51	3.39 \pm 0.55	0.0555
AI	2.08 \pm 0.19 ^a	2.40 \pm 0.24 ^a	2.53 \pm 0.29 ^b	0.0011
TI	2.41 \pm 0.17 ^a	2.76 \pm 0.20 ^b	2.86 \pm 0.26 ^b	0.0002
hH	0.67 \pm 0.06 ^b	0.56 \pm 0.06 ^a	0.55 \pm 0.08 ^a	0.0004
n-6/n-3	2.05 \pm 0.59 ^a	3.49 \pm 0.75 ^b	3.76 \pm 1.27 ^b	0.0005

Different superscript letters (a, b, c) in the same row indicate significant differences ($P < 0.05$).

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

AI: atherogenicity index; TI: thrombogenicity index; hH: hypocholesterolemic to hypercholesterolemic ratio; n-6/n-3: n-6/n-3 ratio.

(Statistical Graphics Corporation, Rockville, MD, USA).

3. Results & discussion

3.1. Fatty acid profile

The results of the univariate analysis of the FA concentrations in Galician organic pasteurized (O), industrial pasteurized (IP) and artisan raw milk (AR) cheeses are presented in Table 1. Some statistically significant differences between the O cheeses and the IP and AR cheeses were observed. The total saturated fatty acid (SFA) content was significantly lower in the O samples than in the other samples (Table 1). This was due to the significantly lower concentration of palmitic acid (C16:0), the main SFA, in the organic cheese than in the other two cheeses. The C12:0 (lauric acid) content was also significantly lower in the O samples than in the other samples. The stearic acid (C18:0) content was higher in the O samples than in the other samples, but the differences were not statistically significant (see Table 1). In a study on cheese made in Cyprus, Tzamaloukas et al. (2021) also observed lower concentrations of medium chain FA (such as lauric acid), palmitic acid and total SFAs and higher concentrations of stearic, oleic, vaccenic and α -linolenic acids, CLA and of total MUFA and PUFA in organic cheeses than in conventional cheeses. Nevertheless, in a comparison of conventional and organic milk produced in England, Ormston et al. (2023) observed a significantly higher content of total SFAs in organic milk than in conventional milk due to the significantly higher contents of C-4:0, C-6:0, C-8:0 and C-14:0. However, these researchers did not find any

difference in the palmitic acid content of organic and conventional milk. The authors indicate that the different SFA content is due to different proportions of pasture, total forage, whole crop and grass silages in the cattle diets. Lower intake of grass and pasture and higher proportions of whole crop and maize silage by dairy cattle lead to an increase in the levels of SFA in milk. The high proportion of total pasture in the diet of the herds that produce the milk used to make organic cheeses in Galicia explains why the total SFA content was lowest in the O samples in the present study. The results for milk can be compared with those obtained in cheeses as organic farming does not have different effects on the FA composition of milk and fresh cheese (Tzamaloukas et al., 2021). We assumed that this finding also applies to soft cheeses with a short maturation time, such as Galician cheeses.

Another notable result was the significantly higher content of all the odd and branched chain fatty acids (OBCFA) with 15 or 17 atoms of carbon (C15:0iso, C15:0anteiso, C15:0, C17:0iso, C17:0anteiso, C17:0, C17:1) in the O samples than in the RA and IP samples (see Table 1). In contrast to our findings, Tzamaloukas et al. (2021) noted that organic cheese made in Cyprus had a lower OBCFA content and higher a higher linoleic content than conventional cheese. OBCFAs are mainly formed by ruminal bacteria and transferred to the milk via this route; only a small proportion of OBCFAs are produced in mammary glands. Dietary factors influence the OBCFA content in milk, and consequently, in cheeses. However, the effect of diet in these FA is uncertain. Feeding dairy cattle a high proportion of forage or grass silage increases the proportion of OBCFA; by contrast, diets including corn silage or supplemented with PUFA can lead to a decrease in the OBCFA content of milk (Abdoul-Aziz et al., 2021). This study also indicated that OBCFAs are markers of ruminal bacterial colonization following the consumption of fresh pasture. The inclusion of a high proportion of pasture in the diets of dairy cows in organic production systems can explain the high OBCFA content in Galician organic cheeses.

In addition, the total MUFA content was significantly higher in the O samples than in the other samples (see Table 1), due to the significantly higher concentration of oleic acid (C18:1n-9), the main MUFA. The vaccenic acid (C-18:1trans 11) and C18:3n-3 (α -linolenic acid) contents were also significantly higher in the organic than in the other cheeses. The concentration of the main CLA (rumenic acid, C18:2cis9 trans11) was higher in the O samples than in the other samples, although the difference was not statistically significant. The high levels of linolenic and rumenic acids in the O cheeses led to the high total PUFA content (Table 1). There was no significant difference between groups in relation to the linoleic acid content (C 18:2n-6). These findings are consistent with those of Tzamaloukas et al. (2021). Nevertheless, in relation to oleic acid, some contradictory results have been found regarding organic milk and cheeses. Thus, some studies have reported low oleic acid (and total MUFA) contents in organic milk (Ellis et al., 2006; Ormston et al., 2023), with some researchers suggesting that grazing may cause a decrease in oleic acid and even that the inclusion of concentrates may reduce biohydrogenation and lead to higher MUFA concentrations in milk produced in intensive systems (Butler et al., 2008; Ormston et al., 2023). However, the results of the present study are not consistent with these previous findings or with those of other studies of organic cheeses made with milk from organic production system in which the cows are not fed concentrates (Tzamaloukas et al., 2021). These results may be influenced by differences in types of feeding in different organic production systems.

Other studies have also reported high concentrations of vaccenic, α -linolenic and CLA in organic milk (Ormston et al., 2023) and in organic cheeses such as Grana Padano (Prandini et al., 2009), Mozzarella (Bergamo et al., 2003) and Halloumi (Tzamaloukas et al., 2021). The greater contribution of grazing in the diet of organically reared cattle also explains the high contents of α -linolenic acid (C-18:3n-3) and of trans FAs, such as vaccenic acid, and CLA, such as rumenic acid. Lipids from fresh grass have a high content of α -linolenic acid, which influences the content in milk, and consequently, the organic cheeses have higher

Table 2

Concentrations of essential trace and toxic elements of organic pasteurized (O), industrial pasteurized (IP) and artisan raw (AR) milk cheeses (all concentrations are expressed as $\mu\text{g kg}^{-1}$ wet weight, except Fe and Zn, which are expressed as mg kg^{-1} wet weight).

Element	O	IP	AR	P
Co	4.38 \pm 1.58	4.96 \pm 2.54	4.56 \pm 3.08	0.8707
Cr	51.6 \pm 36.3	154.7 \pm 74.2	142.7 \pm 220.1	0.1969
Cu	384 \pm 279	669 \pm 519	500 \pm 243	0.2382
Fe	2.94 \pm 1.04 ^a	6.29 \pm 4.59 ^{ab}	12.5 \pm 13.7 ^b	0.0483
I	220 \pm 217	216 \pm 122	240 \pm 211	0.9539
Mn	424 \pm 156 ^a	600 \pm 245 ^{ab}	641 \pm 172 ^b	0.0437
Mo	206 \pm 45 ^b	149 \pm 39.0 ^a	274 \pm 88 ^c	0.0005
Se	288 \pm 153 ^a	508 \pm 147 ^b	383 \pm 201 ^{ab}	0.0247
Zn	49.8 \pm 6.86 ^a	58.7 \pm 9.23 ^b	59.0 \pm 9.98 ^b	0.0424
As	4.06 \pm 1.73	4.89 \pm 2.15	4.42 \pm 4.34	0.8216
Cd	2.17 \pm 0.77	1.79 \pm 1.69	2.69 \pm 1.73	0.3998
Hg	0.88 \pm 0.13	0.94 \pm 0.06	2.67 \pm 3.53	0.0993
Pb	14.83 \pm 7.06 ^a	11.98 \pm 5.18 ^a	46.83 \pm 40.40 ^b	0.0047

levels of α -linolenic acid and total PUFA. Biohydrogenation of α -linolenic acid in the rumen produces trans fatty acids such as vaccenic acid, which accounts for the higher concentrations of this FA in organic cheeses. Vaccenic acid is precursor of CLA in the rumen, and the high content of α -linolenic acid in the diet also explains the high levels of CLA. In organic production systems in which cows do not have access to fresh pasture, the levels of vaccenic and CLA are not significantly increased in milk (Ellis et al., 2006). Regarding the linoleic acid content, no significant difference was observed between the Galician organic cheeses and the other cheeses. However, other studies have reported significant increases in this FA in organic milk (Tzamaloukas et al., 2021). The increased concentration of linoleic acid in organic milk has been related to higher proportions of corn silage in cattle feed (Shingfield et al., 2005). In Galicia, corn silage is not used in a high proportion in the diets of cows in organic production systems and therefore no significant differences were observed in this case. Significantly lower concentrations of linoleic acid have been detected in organically produced Grana Padano (Prandini et al., 2009) and Mozzarella cheeses (Bergamo et al., 2003) than in their conventionally produced counterparts. However, regarding different cheeses from Italy, Manuelian et al. (2023) reported little difference in the FA composition of organic cheese and conventional cheeses as in both cases the cows were fed similar diets based on corn meal or corn silage without pasture.

The differences in FA composition of organic cheeses in relation to the other groups were also reflected in the health-related indices calculated as described by Ormston et al. (2023). The O cheeses yielded lower values of AI, TI, n-6/n-3 indices and higher values of h/H index than the other cheeses (Table 1). For all four indices, there were statistically significant differences ($P < 0.05$) between the O cheese and both of the other types of cheese (IP and AR) (Table 1). The n-6/n-3 ratio in the present work was lower in O cheeses than in the other cheeses (See Table 1), being closer to the suggested optimum n-6/n-3 ratio of 1:1 in the human diet (Simopoulos, 2002). This result is similar to those obtained for organic milk in England, which also yielded lower values of the n-6/n-3 ratio (Ormston et al., 2023). However, Tzamaloukas et al. (2021) reported non-significant differences for the n-6/n-3 index in organic Halloumi cheeses, explaining this result for the highest values of linoleic acid in organic cheeses. In relation to human health-related indices, Galician organic cheeses yielded higher values of all of the health-related indices studied than the other cheeses: the lowest values of AI and TI and the highest value of hH (Table 1). Other studies of organic milk products (Ormston et al., 2023; Tzamaloukas et al., 2021) have indicated the beneficial health effects of consuming organic cheeses, although the results are somewhat different from those observed in the present study. Lower values of AI were also observed in organic samples of Halloumi cheese. Organic milk from England also yielded low TI values; however, the values of AI and hH were not

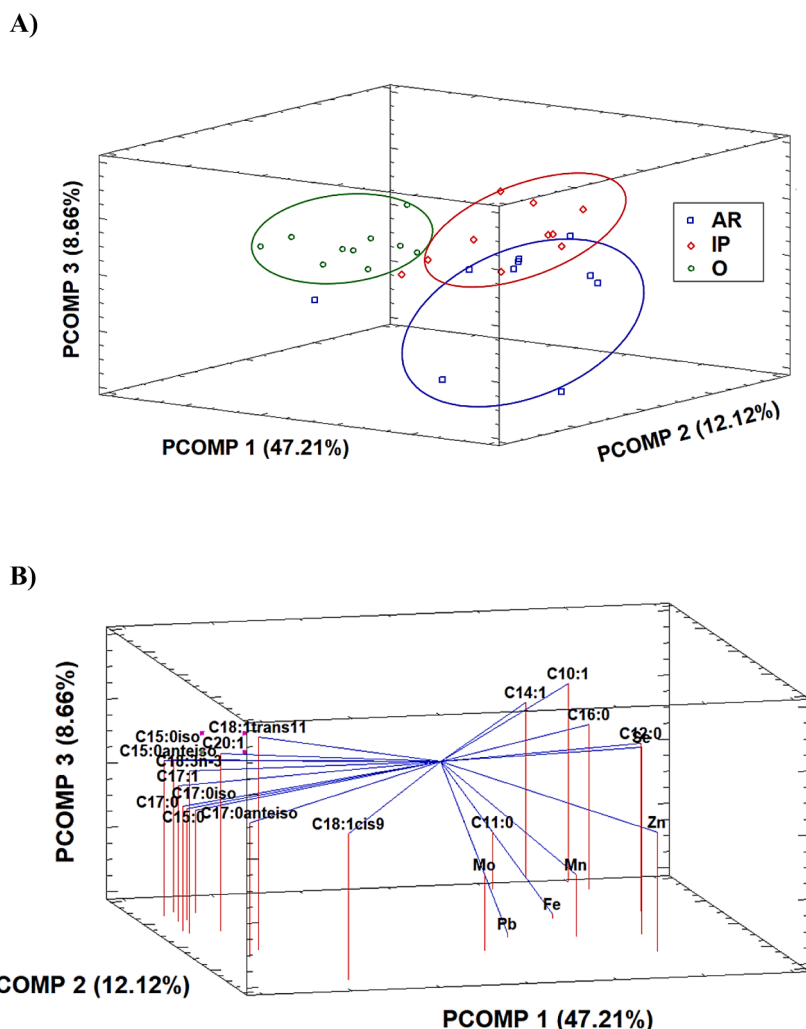


Fig. 1. Score plot of the samples (A) and loading plot of the variables (B) in the space of the first three principal components representing 67.99 % of the total data variance.

improved due mainly to the lower content of oleic acid and no decrease in palmitic acid (Ormston et al., 2023).

3.2. Trace and toxic elements profile

The essential and toxic trace element concentrations in the different cheeses are summarized in Table 2. In general, the O samples had lower levels of trace elements and toxic residues than the other types of cheeses, and these differences were statistically significant for Se, Zn, Pb, Mn and Fe. Artisan cheeses contained significantly higher concentrations of Pb, Fe, Mn and Mo than the O and IP cheeses. In fact, in 60 % of AR cheeses (6/10) the Pb content was above the limit established by the EU ($20 \mu\text{g kg}^{-1}$) for milk for the manufacture of milk-based products (Commission Regulation (EU) No 1881/2006, 2006), whereas in the other two groups no samples were below this limit. Overall, the levels of essential trace elements were normal and toxic element residues were low and similar to those reported for unpolluted areas (except for Pb in AR cheeses) (de Oliveira Filho et al., 2022; Moreno-Rojas et al., 2010; Zwierchowski & Ametaj, 2019). As in the present study, milk used to make organic cheese has generally been reported to contain lower amounts of minerals than conventionally produced milk (mainly Se, Zn, Cu). This difference can be attributed to regulations for organic production and to the limited mineral supplementation for organically managed cattle (Rey-Crespo et al., 2013; Rodríguez-Bermúdez et al., 2018; Średnicka-Tober et al., 2016). Other studies in different areas

have obtained similar results. Manuelian et al. (2023) reported lower levels of Zn, but higher Fe content in Italian organic cheese than in conventional cheeses, whereas Qin et al. (2021) reported that organic milk from Southern England contained, on average, lower contents of Cu, Fe, Mn and Zn than conventional milk. A previous study in Spain demonstrated that industrially produced Galician cheese contained significantly higher levels of the essential trace elements (Se, Cu and Zn) that are routinely supplemented in intensive dairy farming (de Oliveira Filho et al., 2022).

Milk and dairy products may contain toxic elements that can have harmful effects even at low concentrations (Linehan et al., 2024). Several studies have reported that higher levels of heavy metals in conventionally produced milk (Qin et al., 2021; Zwierchowski & Ametaj, 2019) primarily enter agricultural systems through fertilizers. In the case at hand, high Pb levels were only detected in AR samples. Industrial pasteurized cheeses contain low levels of this metal. Thus, the significantly higher Pb levels in AR cheeses (made on farms) may be related to possible contamination from the equipment during the manufacturing process (Enb et al., 2009; Pšenková et al., 2020). In general, the regulations for industrial production do not apply to farm-based production, and stainless-steel equipment is often not available on farms. Levels of Mo, Mn and Fe were also significantly higher in the AR cheeses. Similar results were recently reported by de Oliveira Filho et al. (2022) and may be attributed to the cows ingesting soil during grazing (Rodríguez-Bermúdez et al., 2018). However, the

The chemometric approach demonstrated that the organic samples comprise a separate group in the space of variables, while the IP and AR groups partly overlapped. The observed relationship between the metal elements and SCFA in the IP and AR samples indicates the potential influence of the type of feeding and management of livestock in intensive systems. The diet is highly standardized and usually comprises total mixed rations that include a high proportion of mineral supplemented concentrate feed (Orjales et al., 2018) and a high proportion of corn silage (Rodríguez-Bermúdez et al., 2023). The different management and feeding practices used in organic farm systems are reflected in the FA profile and toxic element composition of the cheese. In fact, the distribution of O samples clearly indicates that the composition of O cheeses is different than that of the other two types of cheese. In addition, the FA profile of the O cheese was healthier, as it was rich in unsaturated fatty acids (previously discussed) and contained low levels of metal residues. This finding was confirmed by the association between OBCFA and the O samples revealed by PCA and HCA. Notwithstanding the limited number of cheese samples analyzed (10 from each type of cheese), the results of this study indicate that Galician organic cheeses could have beneficial effects for human health as OBCFA have anticarcinogenic effects and protect against cardiovascular diseases, type II diabetes, Alzheimer's disease and metabolic syndrome (Abdoul-Aziz et al., 2021; Rodríguez-Alcalá et al., 2017).

4. Conclusions

Despite the small number of samples included in this study (only 10 samples of each type of cheese), some valuable conclusions were reached in relation to the fatty acid profile and metal content of the organic Galician cheese in comparison to artisan raw milk cheeses and cheeses made from industrial pasteurized milk. The Galician organic cheese is clearly different from the other cheeses, having a much healthier fatty acid profile and containing lower levels of potentially toxic elements. These findings can be related to the production system, the type of diet and the management of the cows producing the milk from which the cheese is made. The findings must be confirmed by analysis of larger numbers of samples and considering detailed information on the livestock feed. However, the potential health benefits of consuming organic cheeses are clearly indicated.

Ethical statement-studies in human and animals

The authors declare that no animal or human trials were conducted for this study.

CRediT authorship contribution statement

Angel Cobos: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Emanuel Felipe de Oliveira Filho:** Writing – original draft, Methodology, Investigation. **Marta Miranda:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Tania Ferreira:** Validation, Methodology, Investigation. **Marta López-Alonso:** Writing – review & editing, Funding acquisition, Conceptualization. **Olga Díaz:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition. **Carlos Herrero-Latorre:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to the Research infrastructures (Instrumental Analysis) of the Universidade de Santiago de Compostela, for analytical facilities. Special thanks are given to José Luis Rodríguez-Otero (Honorary Professor, USC), a pioneer in research on Galician milk and cheeses. This research was funded by Xunta de Galicia, grant number GPC ED431B 2019/13. Marta Miranda and Marta López-Alonso belong to Grupo de Potencial Crecimiento financed by GAIN (Axencia Galega de Innovación; grant number ED431B 2023/008).

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