Food Chemistry Advances 2 (2023) 100212

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

Nutritional and quality evaluation of hyperbaric stored fresh cheeses

Ricardo V. Duarte[a,b], Susana Casal[c], Ana M. Gomes[b], Ivonne Delgadillo[a], Jorge A. Saraiva[a,*]

[a] LAQV-REQUIMTE, Departamento de Química, Universidade de Aveiro, Aveiro 3810-193, Portugal
[b] CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto 4169-005, Portugal
[c] LAQV-REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Portugal

ARTICLE INFO

Keywords:
Volatile organic compounds
Protein digestibility
Hyperbaric storage
Fatty acids
Free amino acids

ABSTRACT

Cow's and goat's fresh cheese (FC), two highly perishable dairy products, were stored under hyperbaric storage at room temperature (HS/RT, 50–100 MPa) and compared with refrigeration (RF, 4 °C), for 60 days.

Under HS/RT (≥75 MPa), FCs presented a more stable volatile organic and fatty acid profiles, and reduced lipid oxidation rate, particularly for cow's FC, resembling more to FC prior storage, contrarily to refrigerated cheeses. No changes were observed for total protein, but free amino acids increased over time after 60 days at 75/RT of 13- and 16-fold, and at 100/RT of 14- and 8-fold, respectively in cow's and goat's FC, which may have contributed to increased protein digestibility (5.2%) observed for goat's FC after 60 days at 100/RT.

In general, the results indicate the overall increased preservation performance achieved by HS/RT for cow's and goat's FC when compared with RF, slowing down FC matrix degradation, possibly leading to considerable shelf-life extension.

1. Introduction

In the past decade, a new preservation methodology has arisen, based on storage under moderate pressure (between 25 and 150 MPa), hyperbaric storage (HS), which relies mainly on microbial growth slowdown/inhibition, comparable to conventional refrigeration (RF) (Segovia-Bravo, Guignon, Bermejo-Prada, Sanz & Otero, 2012). Initially, the first studies regarding HS emphasized the combination of sub-zero or low temperatures with low pressure (Charm, Longmaid & Carver, 1977; Mitsuda, 1972). However, when HS is applied at room temperature (RT), it arises as an environmentally friendlier food preservation methodology compared to RF, with substantial potential to extend foods shelf-life and increased microbial safety (Fidalgo et al., 2014; Santos, Castro, Delgadillo & Saraiva, 2020). During storage at uncontrolled variable RT, energy employed to maintain the temperature is null, being only applied during the compression/decompression of the storage vessel, resulting in up to 26-fold lower energy used by HS/RT, comparatively to RF (Bermejo-Prada, Colmant, Otero & Guignon, 2017).

The possible effect of HS at and above RT was evaluated firstly in fruit juices, from more acid ones (strawberry juice) to low acidity juices, more perishable (watermelon and melon juice) (Fidalgo et al., 2014; Queirós et al., 2014; Segovia-Bravo et al., 2012), with the results pinpointing to a possible shelf-life extension, caused by microbial inhibition/inactivation during HS, with reduced physicochemical changes observed. Some non-liquid highly perishable foods were also studied at HS/RT as a case study, initially for short storage periods with favourable outcomes (Duarte et al., 2014; Fernandes et al., 2015). The Portuguese whey cheese was one of them, which only has a couple of weeks of shelf-life at RF, presenting after 8 h at 100/RT no pronounced changes in colour, pH, and water activity, showing a slight rise in lipid oxidation values, with a pronounced microbial inactivation in all microbiological groups even at and above RT (25–37 °C) (Duarte et al., 2014). In a second study, this product presented improved stability under 100 MPa during longer storage periods (10 days) at variable RT, maintaining the pH, water activity, and fatty acid profile, while showing fewer colour losses compared to RF, with a pronounced microbial inactivation effect to undetectable counts (1 logCFU/g) in all the studied microbiological groups, from the 3rd day of storage onwards (Duarte et al., 2017).

Other fresh dairy products, such as fresh cheeses (FC) are also extremely perishable, characterized by short shelf-life (a few weeks at RF), mainly due to their high water activity and rich nutritional profile which promote microbial spoilage, leading to increased synaeresis, pH decrease, lipolysis, proteolysis, oxidation, and off-flavour formation, that crucially limits its shelf-life. In a previous study, FC stored under HS/RT conditions (75–100 MPa) resulted in increased microbial control, leading to an increased microbial shelf-life, while also maintaining most of its basic physicochemical parameters (pH, whey loss, moisture content, and colour) at levels comparable to cheeses prior to storage (*unpublished results*). Also, in that study a significant hyperbaric inactivation was observed, gradually reducing total aerobic mesophiles counts (from

* Corresponding author.
E-mail address: jorgesaraiva@ua.pt (J.A. Saraiva).

https://doi.org/10.1016/j.focha.2023.100212
Received 21 July 2022; Received in revised form 7 January 2023; Accepted 14 February 2023

~7 logCFU/g) more than 5 log units throughout the 60 days of storage, reaching progressively values below the quantification and detection limits (2 and 1 logCFU/g) (*unpublished results*). In the present study, the effect of HS/RT (50–100 MPa) for 60 days was studied on two FC (from pasteurized cow's and goat's milk), with a more in-depth approach on nutritional and physicochemical parameters evaluation, regarding total protein, free amino acids, in vitro protein digestibility, lipid oxidation, fatty acids, and volatile organic profiles and compared with RF under atmospheric pressure (AP).

2. Materials and methods

2.1. Preparation and storage of fresh cheeses

From a local supermarket, two commercial kinds of fresh cheese (FC) were acquired, one made from pasteurized cow's milk and the other from pasteurized goat's milk. Cheeses were produced through enzymatic coagulation, with each type of cheese bought from the same company and lot with similar remaining shelf-life. During transportation temperature was kept low (3–8 °C), being later packaged into low permeability polyamide-polyethylene bags (90 μm, IdeiaPack, Comércio de Embalagens, LDA, Abraveses, Viseu, Portugal), previously sterilized with UV-light, under aseptic conditions inside a laminar flow cabinet (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain) and heat-sealed individually.

The experiments under HS (50, 75, and 100 MPa) at room temperature (RT), were performed in a high-pressure equipment SFP FPG13900 Model (Stansted Fluid Power, Stansted, UK), with a mixture of (40:60) propylene glycol and water used as the pressurizing fluid. Sampling occurred at 3, 7, 14, 28, 42, and 60 days of storage, and as a control, cheeses were also stored under atmospheric pressure (0.1 MPa) at RT (15–22 °C) and refrigeration (4 °C), during 3 and 7 days, respectively, since samples were microbiologically unacceptable on these periods (*accepted in Journal of Food Science*), and thus those storage experiment were interrupted. Cheeses at AP/RF and AP/RT were kept immersed in the same pressurizing fluid and in the dark, to mimic as much as possible the same environment of HS samples inside the HP vessel, except for pressure.

2.2. Total nitrogen, free amino acids, and protein digestibility

The protein profile determination was assessed based on the evaluation of total nitrogen through the Kjeldahl method, free amino acids (FAA) employing the EZ:Faast Amino Acid Analysis Kit available for GC-FID and also by in vitro protein digestibility. Micro-Kjeldahl procedure was performed with a Kjeltec system 1002 Distilling unit (Tecator, Sweden) and the crude protein content calculated by multiplying the total nitrogen content by 6.38 (AOAC Official Method 2001.14, 2002).

As for the determination and quantification of FAA, cheese samples were homogenised in the same volume of 0.01 M HCl, centrifuged (17,000 × g at 4 °C for 5 min), and the supernatant was collected and centrifuged again. 100μL of the second supernatant was used for the analysis of FAA using the EZ:Faast Amino Acid Analysis Kit (GC-FID) (Badawy, Morgan & Turner, 2008) with the individual FAA results expressed in nmol/g of cheese. Protein digestibility was evaluated based on the method developed by Arte et al. (2015) with minor adjustments. FC samples were incubated with 1.5 mg of pepsin in 15 mL of 0.1 M HCl at 37 °C, at 150 rpm for 3 h, then neutralized with 2 M NaOH, 4 mg of pancreatin in 7.5 mL of phosphate buffer (pH 8.0) and 1 mL of toluene were added, followed by incubation for 24 h at 37 °C, at 150 rpm. To inactivate pancreatin, 10 mL of trichloroacetic acid (10%, wt/vol) was added, followed by centrifugation (5000 × g at RT for 20 min) to separate undigested protein. The supernatant was used for nitrogen determination, by micro-Kjeldahl method. This analysis was performed in cow's and goat's FC stored under 100/RT for 60 days, and compared with the respective FC prior to storage, and expressed in% (Eq. (1)).

$$\text{Protein digestibility (\%)} = \frac{N\ Digested\ protein}{Total\ N} \times 100 \quad (1)$$

2.3. Fatty acids profile

A method similar to the one performed by Sobral, Casal, Faria, Cunha and Ferreira (2020) was used for fatty acids profile determination. In sum, after cheeses fatty acids extraction and derivatization, determination was carried out by gas chromatography, as fatty acids methyl esters (FAMEs). FAMEs profile was analysed using a GC (Chrompack CP-9001 model, Netherlands) with flame ionization detection (FID). The identification of fatty acids and FID calibration was attained with a certified reference standard mixture (TraceCert – Supelco 37 component FAME mix, USA) and the results were expressed in relative percentages of their FAMEs.

2.4. Lipid oxidation by-products

Malondialdehyde (MDA) quantification was used to assess secondary lipid oxidation state, based on 2-thiobarbituric acid reactive substances (TBARS) method adapted from King (1962). Originally 1 g of fresh cheese was crushed into smaller pieces and mixed with 3 mL 7.5% trichloroacetic acid, followed by centrifugation at 4000 × g at 4 °C for 20 min (Universal 320-R, Hettich Group, Tuttlingen, Germany). The extract was then filtered (Whatman n°1) and the same volume of 46 mM 2-thiobarbituric acid was added, vortexed and immersed in boiling water for 40 min, and then cooled down in cold water. Using a micro-plate spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Scientific, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 532 nm triplicates were measured. Standard solutions of MDA in 7.5% trichloroacetic acid were prepared from 1,1,3,3-tetramethoxypropane and a calibration curve was prepared at a concentration ranging from 0.2 to 10 μg/L. TBARS results were expressed as μg of malondialdehyde per g of cheese.

2.5. Volatile organic compounds

Yue et al. (2015) method was used for the volatile organic compounds (VOC) profile determination, through headspace solid-phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry (GC–MS). Initially, 2 g of cheese and 50μL of cyclohexanone (25 μg/mL, internal standard) were added into the vial, then straight away sealed with a polypropylene cap with silicon septum. During 30 min at 50 °C the compounds were released, then the SPME fibre (DVB/CAR/PDMS; 50/30 μm; Supelco Inc.) was inserted inside the vial, and exposed for further 30 min at the same temperature, to allow volatiles adsorption into the fibre, and then it was inserted in the injection port of the GC equipment, Agilent GC-7890 gas chromatographer equipped with a mass spectrometer Agilent 5977B, and a DB-5 MS Capillary GC column (30 m × 0.25 mm I.D. × 0.25 μm film thickness, Agilent, USA). Thermal desorption was achieved at 260 °C in splitless mode, with helium at a linear velocity 1 mL/min. Initially, the temperature of the oven was set at 35 °C during 5 min, increasing to 100 °C at a rate of 4 °C/min, followed by an increase of 10 °C/min until 225 °C and maintained for 0.25 min (total 33.5 min). The transfer line was maintained at 280 °C and the ion source at 230 °C, with an ionization energy of 70 eV. Scanning of the mass spectra was performed from 20 to 350 m/z in full scan mode. Reference mass spectra library (NIST 11), retention times, retention index computer matching and individual standards (when available), were used for volatile compounds identification. The VOC profile semi-quantitative determination was calculated using cyclohexanone as an internal standard equivalents basis, and the results were expressed in μg/100 g of cheese.

2.6. Statistical analysis

The storage experiments were all carried out in triplicate and all analyses were done at least in triplicate. Analysis of Variance (ANOVA) was performed, followed by a multiple comparison post hoc test, Tukey's HSD test, at a 5% level of significance. In order to identify statistical patterns from the VOC data set, principal component analysis (PCA) was conducted for both cheeses.

3. Results and discussion

3.1. Protein profile

In Tables 1 and 2**,** the initial total protein concentration of cow's and goat's FC can be observed, with the first one presenting a slightly lower concentration when compared to the other, 15.11±0.49 g/100 g and 16.99±0.50 g/100 g, respectively, similar to what is found in the literature (Sant'Ana et al., 2013; Van Hekken, Tunick, Farkye & Tomasula, 2013). In general, both types of FC presented a comparable behaviour under the same storage conditions, with no considerable variation ($p>0.05$) observed in all storage conditions, with storage at 75 and 100/RT retaining the protein content constant throughout the storage ($p>0.05$), in the two kinds of FC even after 60 days, similarly to what was observed when raw milk was stored under the same HS conditions (75/100 MPa) at variable RT for 60 days (Duarte, Pinto, Gomes, Delgadillo & Saraiva, 2022).

Initially, cow's cheese was high in glutamic acid, followed by aspartic acid, ornithine, leucine, and glycine with a total FAA of 1.1 ± 0.1 μmol/g (Table 1), while goat's FC had a total FAA of 0.9 ± 0.1 μmol/g initially (Table 2), mainly composed by glycine, followed by ornithine, glutamine, glutamic acid, valine, and aspartic acid. The initial FAA compositions in this study were also comparable to cheeses made with cow's and goat's milk reported in the literature (Atanasova et al., 2021; Teter et al., 2020).

In the first days of storage (day 3), no variations were observed concerning individual FAA ($p>0.05$) of cow's FC stored at AP/RF, when compared to the initial ones, while cheeses at AP/RT displayed ($p<0.05$) a 12-fold rise in alanine and a 3-fold decline in ornithine, while also several amino acids were now undetected such as glycine, isoleucine, threonine, proline and histidine that were present initially, which could have been employed in microbial metabolism (Hoskisson, Sharples & Hobbs, 2003). Even with these small variations in the concentration of individual FAA, total FAA content remained similar ($p>0.05$) to the initial ones for both storage conditions.

At day 3 of storage under 50/RT, changes were only observed in ornithine (decrease of 0.5-fold) without pronounced variations for all the other individual and total FAA ($p>0.05$). Nevertheless, on the following storage periods a considerable increase in the majority of FAA was detected ($p<0.05$), with increases of 100-, 48-, 27- and 21-fold, for alanine, histidine, threonine, and valine, respectively, after 28 days of storage. Also, on the 28th day FAA were majorly composed of alanine, leucine, serine, glutamic acid, valine, and lysine (altogether representing 66% of total FAA), characterized additionally by the presence of serine, phenylalanine, cystine and threonine that were initially undetected, contributing to an overall increase of 7-fold in total FAA. Such increment might be caused by residual activity of the enzymatic coagulant used for FC production, or plasmin residual activity, initially present in the pasteurized milk, that hydrolyse caseins into intermediate-sized peptides (Enright, Patricia Bland, Needs & Kelly, 1999). Additionally, these smaller peptides can be hydrolysed into amino acids by the microbial flora present in the FC, as high microbial loads were observed throughout the storage at 50/RT (around 6.6 and 6.4 logCFU, for total aerobic mesophiles (TAM) and lactic acid bacteria (LAB), respectively, *unpublished data*), or by extracellular proteinases and peptidases released from that microflora (Abellán et al., 2012).

Under the higher storage pressures, 75 and 100/RT, this proteolytic effect was lower ($p<0.05$), comparatively to storage at 50/RT, with an increase rate of FAA per day of 93.66, 85.12 and 254.52 nmol/g, respectively (*supplementary material Figure S1*), resulting in rises in total FAA of 5.9 and 5.7-fold, under 75 and 100/RT respectively, at day 60 of storage. After 28 days under 50/RT, the 100-fold increase observed in alanine was much higher than the ones observed for storage under 75 and 100/RT after 60 days, of 13 and 14-fold, respectively, which was associated by Eugster, Fuchsmann, Schlichtherle-Cerny, Bütikofer and Irmler (2019) with the microbial activity of added starter cultures in cheese ripening. Initially, undetected FAA as serine, phenylalanine, cystine and threonine were present in all three HS conditions, with samples stored under 75 and 100/RT showing a higher abundancy in leucine, glutamic acid, valine, and asparagine, reaching a comparable total FAA after 60 days of storage of 6.3 ± 0.8 and 6.1 ± 0.5 μmol/g ($p>0.05$), respectively. Interestingly, both storage conditions were able to gradually inactivate the microbial load present in FC samples, at a quicker rate for 100/RT (with D_p-values for TAM of 17.8 and 13.4 days, for 75 and 100/RT, respectively, *unpublished data*), which could potentially justify partially at least the results of lower FAA increase.

As for goat's FC, on the 3rd day of storage no significant ($p>0.05$) oscillations were observed in individual or total FAA at AP/RF, while a high proteolytic activity ($p<0.05$) of cheeses stored at AP/RT was observed, causing increments, particularly in valine, leucine, glutamic acid, proline and serine, contributing for an overall increase of 20-fold in total FAA ($p<0.05$), relatively to the initial cheese, despite the significant reduction ($p<0.05$) in glycine (similar to what was reported for cow's FC under AP/RT).

As for storage under pressure, globally goat's FC displayed signs of proteolysis throughout the storage, yet at different rates under the different pressures. At the lowest storage pressure (50 MPa) an estimated rise of 641.63 nmol/g FAA per day occurred (*supplementary material Figure S2*), with substantial increases ($p<0.05$) observed in almost all FAA, except for glycine, aspartic acid, ornithine, and glutamine that remained in similar concentrations ($p>0.05$) as the initial ones. Alanine, leucine, valine, glutamic acid, and lysine presented a more prominent abundance ($p<0.05$), with leucine, histidine, methionine and valine showing a higher abundance after 28 days of storage, with increments of 380-, 208-, 65- and 61-fold, respectively. Goat's FC had an initial high microbial load (close to 6 logCFU/g, for LAB, *unpublished data*), which grew under 50/RT (reaching almost 8 logCFU/g after the 7th day of storage), possibly contributing to increased FAA as LAB are well known to promote proteolysis in cheeses (Abellán et al., 2012), resulting in an increase of 30-fold in total FAA after 28 days.

After 60 days of storage, the proteolysis rate was almost 2-fold slower under 75 and 100/RT, resulting in rises in total FAA of 16- and 8-fold, respectively, with increases of 151.57 and 71.73 nmol/g FAA per day (*supplementary material Figure S2*), respectively, reaching at day 60th of storage total FAA values of 9.5 ± 0.9 and 4.8 ± 0.4 μmol/g, respectively. Interestingly, TAM and LAB counts were greatly inactivated under those conditions, but the inactivation rate under 100 MPa was almost 3-fold faster (D_p-values for TAM of 9.9 and 3.4 days, and for Lab of 6.3 and 1.9 days, under 75 and 100/RT, respectively, *unpublished data*), and thus, residual proteolytic activity from microbial proteases seem to be the main reason accountable for the proteolysis observed. In general, under 75 and 100/RT cheeses presented almost half concentration in most FAA between the two storage conditions, however, both presented a greater abundance ($p>0.05$) in leucine, valine, aspartic and glutamic acid. Also, likewise with storage at 50/RT new amino acids were now detected, such as isoleucine, phenylalanine, serine, and tryptophan. Even under prolonged FC refrigerated storage, increased proteolysis is to be expected, as reported by Sant'Ana et al. (2013), who observed increased proteolysis in FC stored at AP/RF after 21 days, assigned mainly to the action of LAB, extracellular proteases, and to a lesser degree to plasmin.

Storage of cheeses under HS at 75 and 100/RT generally resulted over time in an increased concentration in FAA, even though even af-

Table 1
- Total protein (g/100 g), protein digestibility (%), free amino acids (nmol/g) of cow's fresh cheese prior storage (Initial) and stored under the different conditions (AP/RT, AP/RF and 50, 75 and 100/RT). Different letters (a–g) indicate significant differences ($p<0.05$) between the different storage conditions. Standard deviation is at least below 10% of the mean value, and thus is not displayed in the Table.

Condition	Initial	AP/RT	AP/RF	50 MPa/RT			75 MPa/RT				100 MPa/RT			
Days	0	3	3	3	14	28	3	14	28	60	3	14	28	60
Total Protein (g/100 g)	15.11a	13.09a	1.46a	14.08a	14.47a	12.92a	14.57a	13.49a	15.39a	13.78a	15.32a	14.53a	14.85a	14.74a
Digestibility (%)	81.2a	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	81.4a
FAA (nmol/g)														
Alanine	16.7a	207.6bc	24.1a	19.9a	789.7d	1770.5e	24.7a	49.0ab	164.3abc	218.1c	34.0ab	98.2abc	256.3c	227.1c
Glycine	69.6a	*ND*	83.0a	116.9abc	154.4bcd	247.9e	106.7abc	156.9bcd	188.1de	172.1cd	89.0ab	162.8cd	203.0de	158.5cd
Valine*	23.3a	43.0a	11.1a	35.2a	176.5b	486.6e	25.1a	111.6ab	310.5cd	784.0 g	26.5a	219.1bc	382.3de	668.7f
3-Aminoisobutyric acid	18.8ab	*ND*	*ND*	12.6ab	39.0abc	69.4abcde	15.0ab	56.4abcd	116.6def	142.7f	12.0a	141.1ef	88.8bcde	108.0de
Leucine*	85.6ab	303.8b	50.6a	66.4a	431.7bc	1333.1e	86.1ab	378.7abc	969.4d	1511.1e	145.8ab	650.7cd	1538.2e	1525.0e
Isoleucine*	4.8a	*ND*	*ND*	*ND*	33.7ab	96.2c	*ND*	31.7ab	104.0c	278.9e	*ND*	67.7bc	165.7d	297.2e
Threonine	5.0a	*ND*	*ND*	*ND*	30.2ab	136.6c	*ND*	*ND*	46.0abc	63.3abc	*ND*	*nd*	121.3bc	105.4abc
Serine	*ND*	20.2 a	*ND*	*ND*	166.4 bcd	605.9 e	*ND*	39.1 ab	137.9 abc	182.1 bcd	*ND*	66.5 ab	288.3 d	255.0 cd
Proline	19.8a	69.7abc	*ND*	*ND*	41.8abc	76.5abc	*ND*	104.2bcde	166.3de	185.9e	*ND*	36.5ab	117.1cde	87.9abcd
Asparagine	13.7ab	36.4abcd	11.7ab	21.6abc	26.4abcd	29.7abcd	10.2a	24.9abcd	48.1cd	57.3d	15.1ab	98.1e	41.5bcd	32.1abcd
Aspartic acid	181.8abc	56.8ab	37.4a	94.6ab	350.9abcde	415.7cde	128.0ab	363.0bcde	568.0e	471.9cde	113.7ab	182.8bcd	473.7de	488.6de
Methionine*	10.9a	38.0a	14.1a	*ND*	42.7a	108.1b	19.6a	43.1a	72.7ab	51.1ab	19.9a	111.6b	114.7b	62.6ab
Hydroxyproline	12.3ab	24.1ab	9.0a	9.6ab	30.3abc	41.3abcd	11.2ab	80.4e	65.8cde	74.1de	14.6ab	39.9abcd	42.5bcd	35.2abcd
Glutamic acid	292.3bcd	224.4ab	209.8abc	58.4a	173.6ab	496.6de	167.5ab	207.3ab	439.8cd	502.4de	255.8abc	481.9de	899.5f	653.8e
Phenylalanine*	*ND*	19.7a	*ND*	*ND*	34.8a	495.7e	*ND*	60.0ab	219.8bc	269.9cd	*ND*	92.4ab	269.9cd	390.9de
Glutamine	174.6abcd	69.4ab	58.3a	74.5ab	294.8bde	286.6bcde	117.7abc	320.0de	607.2f	392.2ef	101.6ab	175.8abcd	406.2e	413.5ef
Ornithine	167.5b	54.3a	201.2b	84.9a	40.5a	75.6a	54.5a	53.2a	42.8a	72.8a	45.1a	45.1a	49.5a	48.6a
Lysine*	14.1a	14.2a	15.1a	32.4ab	166.0bc	493.9e	30.7ab	155.8b	298.6cd	348.0de	33.6ab	121.1ab	164.0bc	94.4ab
Histidine*	5.7a	*ND*	*ND*	*ND*	94.8bc	272.2d	*ND*	57.1ab	144.7c	307.6d	10.1a	110.5bc	265.6d	300.7d
Tyrosine*	2.3a	2.0a	3.3a	4.4ab	6.6ab	4.7ab	2.9a	12.2ab	15.4ab	32.5cd	10.1ab	49.3d	21.3bc	11.7ab
Tryptophan*	*ND*	33.4ab	4.0a	*ND*	65.6ab	309.3e	*ND*	43.4ab	109.2bc	240.2de	*ND*	63.8ab	170.2cd	270.3e
Cystine	*ND*	*ND*	*ND*	*ND*	39.7a	168.4c	*ND*	11.3a	101.1ab	73.3a	*ND*	*ND*	48.6a	80.3a
Total FAA (μmol/g)	1.1a	1.2a	0.8a	0.6a	3.2b	7.8c	0.8a	2.3b	4.4c	6.3d	0.9a	2.9b	6.1d	6.1d

ND and *NP* - stands for not detected and not performed, respectively.
* - essential amino acids.

Table 2
Total protein (g/100 g), protein digestibility (%), free amino acids (nmol/g) of goat's fresh cheese prior storage (Initial) and under the different conditions (AP/RT, AP/RF and 50, 75 and 100/RT). Different letters (a–i) indicate significant differences ($p<0.05$) between the different storage conditions. Standard deviation is at least below 10% of the mean value, and thus is not displayed in the Table.

Condition	Initial	AP/RT	AP/RF	50 MPa/RT			75 MPa/RT				100 MPa/RT			
Days	0	3	3	3	14	28	3	14	28	60	3	14	28	60
Total Protein (g/100 g)	16.99abc	15.36a	17.02abc	18.84c	18.33bc	15.35a	18.30bc	16.43ab	16.83abc	15.96a	18.19bc	16.75abc	17.13abc	15.81a
Digestibility (%)	75.8a	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	81.0b
FAA (nmol/g)														
Alanine	41.4 ab	486.1 g	12.1 a	183.4 cde	841.3 h	1011.1 i	25.1 ab	134.5 abcd	200.4 def	323.0 f	55.9 abc	107.8 abcd	151.0 bcde	265.5 ef
Glycine	202.6 bcde	8.6 a	133.0 b	136.7 bc	225.4 def	276.5 ef	189.2 bcd	285.4 ef	277.0 ef	289.0 f	217.2 cdef	241.2 def	203.8 bcde	215.6 cdef
Valine*	54.4a	1296.1d	17.1a	218.5a	2426.1e	3383.8f	70.0a	733.9bc	1282.0d	2469.5e	98.8a	325.7ab	719.9bc	1168.0cd
3-Aminoisobutyric acid	5.6a	89.2b	*ND*	16.8a	*ND*	*ND*	9.3a	*ND*	82.0b	195.1c	21.4a	*ND*	*ND*	*ND*
Leucine*	12.6a	1572.3e	14.5a	332.1ab	3175.8f	4799.8 g	50.6a	776.9bc	1308.7de	2839.9f	84.1a	455.4ab	1023.1cd	1610.6e
Isoleucine*	*ND*	149.4bc	*ND*	28.4a	263.7d	398.0f	*ND*	86.2abc	118.8bc	355.4ef	*ND*	58.7ab	137.9bc	276.7de
Threonine	*ND*	95.4b	*ND*	*ND*	10.7a	*ND*	*ND*	12.5a	5.6a	*ND*	*ND*	5.5a	*ND*	*ND*
Serine	*ND*	1644.7d	*ND*	*ND*	566.9c	462.6bc	*ND*	31.4a	93.4a	219.7ab	*ND*	16.1a	27.8a	201.1ab
Proline	*ND*	1488.9c	*ND*	179.0a	547.9b	724.5b	*ND*	14.8a	29.7a	85.5a	*ND*	8.2a	*ND*	18.4a
Asparagine	13.0a	102.9e	12.6a	32.4abc	68.1d	51.7cd	16.1ab	54.6cd	76.7cd	173.7f	35.1abc	20.2abc	31.8abc	47.2bcd
Aspartic acid	34.6a	77.2a	42.2a	36.9a	51.5a	40.0a	38.9a	100.0a	193.0b	494.6c	93.9a	33.1a	35.0a	62.4a
Methionine*	6.0a	384.7c	*ND*	28.5ab	341.0c	389.6c	*ND*	35.6ab	52.0ab	84.5b	*ND*	24.1b	21.9ab	68.7ab
Glutamic acid	65.7a	1823.3b	*ND*	171.2a	2329.1bc	2889.7c	*ND*	86.2a	222.6a	489.7a	42.4a	109.5a	210.9a	456.1a
Phenylalanine*	*ND*	500.4d	*ND*	52.6a	831.4e	1108.0f	*ND*	60.5a	133.6ab	274.1c	*ND*	26.8a	75.0a	210.4bc
Glutamine	56.3a	176.1bc	50.7a	46.0a	104.8ab	81.4a	48.3a	112.0abc	178.2c	431.6d	85.2a	67.2a	63.1a	81.3a
Ornithine	83.6bcd	178.3e	101.3cd	39.8ab	58.5abc	110.0d	34.9a	43.3ab	36.7ab	72.1bcd	41.6ab	50.5ab	56.0abc	68.9bcd
Lysine*	23.1a	575.2c	24.1a	35.1a	999.6d	1208.3d	27.8a	235.3ab	303.6b	250.5ab	48.6a	103.0ab	106.3ab	164.0ab
Histidine*	2.6a	292.7b	*ND*	8.9a	576.9c	529.8c	*ND*	75.8a	57.3a	93.1a	*ND*	89.5a	132.7ab	282.0b
Tyrosine*	1.3a	324.4c	*ND*	11.0a	157.9b	71.3a	*ND*	22.9a	4.0a	3.0a	*ND*	5.3a	2.4a	2.3a
Tryptophan*	*ND*	367.0cd	*ND*	81.4ab	467.2d	340.9cd	*ND*	124.2ab	112.5ab	454.8d	*ND*	55.8a	77.2a	240.9bc
Cystine	*ND*	*ND*	*ND*	*ND*	*ND*	127.3b	*ND*	27.5a	32.8a	73.4ab	*ND*	*ND*	*ND*	56.2a
Total FAA (μmol/g)	0.6a	11.6de	0.4a	1.6ab	13.9e	17.4f	0.5a	3.1bc	4.8c	9.5d	0.8ab	1.7ab	3.0bc	4.8c

ND and *NP* - stands for not detected and not performed, respectively.
* - essential amino acids.

ter 60 days, values were significantly lower than the ones reported by Abellán et al. (2012) for goat cheese at day 1 of maturation. However, the possible impact of these increases should be further evaluated in the sensory properties of HS cheeses.

Concerning protein digestibility, prior to storage cow's (Table 1) and goat's (Table 2) FC presented values of 81.2 ± 2.1 and 75.8 ± 1.1%, respectively. After 60 days at variable RR under 100 MPa, no substantial variations were detected for cow's FC (81.4 ± 2.7%), while an increase ($p<0.05$) to 81.0 ± 1.8% was observed for goat's FC. These results could be related to the increase in FAA of 8-fold observed for goat's FC, after 60 days of storage, at 100/RT, indicating a higher proteolysis in goat's FC, which could be responsible for the increased protein digestibility.

3.2. Fatty acids profile

The fatty acid profile of cow's and goat's FC are presented in *supplementary material Table S1* and *S2*, respectively. Both cheeses had in general a similar fatty acid composition, with minor variations, presenting a higher abundance in saturated fatty acids (SFA), followed by monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), overall comparable to the profiles described by Van Nieuwenhove, Oliszewski and González (2009). Initially, the fatty acids content of cow's FC had a total SFA, MUFA and PUFA of 63.98±0.52%, 31.12±0.38% and 4.42±0.12%, respectively, as for goat's FC had initially a total SFA, MUFA and PUFA content of 66.16±0.93%, 27.94±0.83% and 5.09±0.18%, respectively. Cow's and goat's cheeses SFA content was composed mainly by palmitic acid (C16:0, 32.01±0.11% and 27.00±0.35%, respectively), myristic acid (C14:0, 11.49±0.29%, 10.60±0.19%, respectively), stearic acid (C18:0, 10.35±0.19%, 9.37±0.30%, respectively), with the major variance related with a higher capric acid (C10:0) percentage detected for goat's FC (9.28±0.82%) relatively to cow's FC (2.83±0.20%), comparable to what is found in the literature (Sant'Ana et al., 2013). Regarding cow's and goat's cheeses MUFA content, the most abundant fatty acids were oleic acid (C18:1*c*, 22.80±0.36% and 20.98±0.71%, respectively) and elaidic acid (C18:1*t*, 2.72±0.07% and 2.77±0.12%, respectively), as for PUFA the most representative was linoleic acid (C18:2*c*, 2.44±0.05% and 3.42±0.13%, respectively).

Cow's FC fatty acid profile presented some variations under the different storage conditions when compared to the profile prior to storage. Generally, longer HS periods tended to present higher ($p>0.05$) SFA values, with storage at 75 and 100/RT achieving values of 65.46±1.04% and 64.96±0.65%, respectively. Under 75/RT especially, this tendency was more noticeable, exhibiting a tendency for a higher percentage of palmitic acid ($p<0.05$), stearic acid and myristic acid ($p<0.05$). MUFA and PUFA content tended to present lower values under HS, with the major differences ($p<0.05$) being related to storage at 100/RT after 7 and 14 days, displaying values similar to the initial ones on the following storage periods ($p>0.05$). Despite the oscillations ($p<0.05$) identified regarding oleic, linoleic, and α-linolenic acids (C18:3c6,c9,c12), overall, the majority of MUFA and PUFA content was not impacted during HS ($p>0.05$).

Even though some variability in few individual fatty acids of goat's FC were observed during the different storage conditions, under HS no significant changes ($p>0.05$) were detected even after 60 days, comparatively to the initial cheese. Although the same tendency was observed comparatively to cow's FC storage, with cheeses at 75 and 100/RT presenting higher percentages ($p>0.05$) regarding SFA, accompanied by a reduction ($p>0.05$) in MUFA and PUFA content. Comparable outcomes were also found in HS of raw milk for 60 days, with a more noticeable increase in SFA content ($p<0.05$) being reported especially for storage at 75/RT, while MUFA and PUFA contents decreased throughout the storage (Duarte et al., 2022).

Generally, the fatty acid profile of both cow's and goat's cheeses were successfully maintained during storage under 75 and 100/RT, throughout the duration of the study.

3.3. Secondary lipid oxidation by-products

Throughout storage under the different storage conditions, an overall raise of TBARS values from 1.20±0.11 μg MDA/g to a maximum of 2.78±0.48 μg MDA/g and from 0.67 ± 0.07 to 2.13±0.11 μg MDA/g, for cows' and goats' FC was observed, respectively (*supplementary material Table S3*).

Such changes were more noticeable ($p<0.05$) in cows' FC stored under 50/RT after 14 days of storage, reaching 2.78 μg MDA/g. As for storage at 75 and 100/RT, lipid oxidation increased slowly up to 2.21±0.16 (1.8-fold) and 1.90±0.11 μg MDA/g (1.6-fold) at the 60th day of storage, respectively, but with values similar ($p>0.05$) to the ones detected at the 3rd day for each of these two storage conditions.

Lipid oxidation was overall more stable in goats' FC under most of the storage conditions, while at 75/RT a strong rise ($p<0.05$) in TBARS values was observed mainly from the 42nd day of storage, reaching 2.13±0. 32 μg MDA/g (3.2-fold) at the 60th day of storage. A substantial slower lipid oxidation rate was attained under 100/RT throughout storage, reaching 1.15±0.05 μg MDA/g (1.7-fold) after 60 days of storage, which was comparable to the one observed on the 7th day of storage (0.86±0.11 μg MDA/g).

Several factors can impact lipid oxidation, such as the presence of light, oxygen or enzymes, promoting the formation of several volatile compounds, giving rise to off-flavours, with a rising rate over the storage period (Van Hekken et al., 2013). In fact, increase in lipid oxidation by products in cows' FC stored under AP/RF was described by Zamora, Juan and Trujillo (2015), reporting increases of 2.5-fold after 13 days, while Ercan, Soysal and Bozkurt (2019) reported increases around 3.4-fold after 21 days, both greater than the ones described in the present work for both cows' and goats' FC even after 60 days under 100/RT, of 1.6 and 1.7-fold increase, respectively. Equivalent results to the ones observed for FCs were also obtained in raw milk under HS (Duarte et al., 2022), reporting a tendency to a more pronounce increase ($p>0.05$) in TBARS values under 50–75/RT, while storage at 100/RT delayed lipid oxidation throughout the entire storage ($p>0.05$).

3.4. Volatile organic compounds

A total of eighteen volatile organic compounds (VOC) were detected (Table 3) initially in cow's FC, consisting essentially of free fatty acids (FFA), esters, ketones, and aldehydes, without alcohol compounds, similar to what is described for this kind of dairy product (Tunick, Iandola & Van Hekken, 2013). Butanoic, hexanoic, octanoic and decanoic acids were the more abundant FFA, with sorbic acid ((2E,4E)-hexa-2,4-dienoic acid) also being identified, added in the form of potassium sorbate as a preservative by the producer, as stated in the product label. The main esters present were ethyl butanoate and hexanoate, as for ketones, pentan-2-one and heptan-2-one were the most abundant compounds, and nonanal was the main aldehyde, which was only detected in the cheese prior to storage.

No changes in the VOC profile ($p>0.05$) of cheeses on the 3rd day of storage under AP/RF were detected, although a slight increase ($p>0.05$) in most FFA, aldehydes, esters and a decrease in ketones occurred, with alcohol compounds such as pentan-2-ol, cyclohexanol and hexan-1-ol being now found. A clear distinguishable VOC profile was detected in cheeses kept under AP/RT during 3 days, with increased concentrations ($p<0.05$) of FFA, aldehydes, esters, and alcohols. In practically all FFA, an increase of up to 10-fold was observed and on their corresponding ethyl esters after 3 days, with acetic and nonanoic acid, ethyl octanoate and dodecanoate being now detected. 2-methylbut-2-enal and hexan-1-ol contributed mainly to the increase in aldehydes and alcohols, respectively, with no considerable differences observed concerning ketones ($p>0.05$). These changes could result from high microbial or/and enzymatic activity, promoting lipolysis and the release of FFA, as well as lactose and amino acids degradation, with ethyl esters created by esterification of the FFA, and alcohols resulting probably from the reduc-

Table 3
- Volatile organic compounds (μg internal standard equivalents/100 g) of cow's FC prior storage (Initial) and under the different conditions (AP/RT, AP/RF and 50, 75 and 100/RT). Different letters (a–e) indicate significant differences ($p<0.05$) between the different conditions. Standard deviation is at least below 10% of the mean value, and thus is not displayed in the Table.

Condition	Initial	AP/RT	AP/RF	50 MPa/RT		75 MPa/RT				100 MPa/RT			
Days	0	3	3	7	28	7	14	28	60	7	14	28	60
Free fatty acids	182.10a	2076.29f	318.40ab	602.05d	1654.27e	177.03a	301.29ab	223.26ab	169.55a	252.42ab	247.83ab	402.03bc	572.93cd
acetic acid	*nd*	284.66d	4.69a	28.67b	137.33c	1.32a	0.69a	0.50a	1.19a	*nd*	*nd*	*nd*	*nd*
butanoic acid	19.01a	229.34d	32.79a	78.00bc	275.23e	20.73a	43.02a	29.82a	19.37a	39.71a	49.74ab	56.54abc	89.14c
hexanoic acid	28.90a	370.52c	56.60a	153.49b	494.08d	31.04a	86.10ab	56.84a	52.48a	62.86a	92.15ab	105.66ab	169.61b
(2E,4E)-hexa-2,4-dienoic acid	69.19ab	773.42e	115.25bc	135.18c	277.05d	46.35a	46.42a	32.38a	47.12a	48.40a	43.00a	43.57a	59.53a
octanoic acid	47.88ab	315.17e	65.27ab	132.93cd	357.54e	49.36ab	86.07abc	59.90ab	26.93a	60.71ab	82.21abc	120.13bcd	168.53d
nonanoic acid	*nd*	13.64c	*nd*	6.56ab	9.57bc	2.70ab	4.11ab	4.87ab	3.17ab	4.14ab	2.64a	2.87ab	3.51ab
decanoic acid	20.53a	96.63d	39.49abc	65.95bcd	102.98d	26.84ab	35.06abc	40.42abc	19.61a	36.65abc	51.14abc	76.06cd	68.75bcd
dodecanoic acid	*nd*	6.54bcd	4.31ab	7.82cd	10.07d	3.33ab	4.71abc	3.56ab	2.40a	4.09ab	4.28ab	5.05abc	4.82ab
Esters	26.60a	339.46c	63.34a	137.31b	617.04d	23.85a	34.64a	32.07a	22.51a	34.84a	45.17a	39.80a	35.51a
ethyl acetate	4.84ab	58.49d	8.77b	21.45c	19.47c	5.78ab	3.91a	3.45a	3.78a	6.33ab	4.72ab	3.45a	4.63ab
ethyl butanoate	9.97a	121.69c	17.56ab	32.71b	138.47c	7.21a	6.61a	6.18a	3.20a	11.32a	9.20a	8.10a	8.94a
ethyl hexanoate	11.68a	70.85b	16.62a	21.55a	119.34c	8.16a	8.46a	10.95a	5.86a	10.61a	15.08a	12.84a	13.94a
ethyl octanoate	*nd*	51.88c	14.17ab	37.44ab	128.71d	*nd*	8.58a	8.45a	9.80a	*nd*	10.03a	11.18ab	6.64a
ethyl nonanoate	*nd*	*nd*	*nd*	*nd*	3.13	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
ethyl decanoate	2.58a	34.65c	7.93a	22.64bc	200.89d	2.70a	5.81a	3.05a	1.15a	6.10a	6.13a	4.41a	3.23a
ethyl dodecanoate	*nd*	1.90a	0.81a	1.52a	7.04b	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Alcohols	*nd*	12.84c	8.24b	8.95bc	20.05d	1.04a	ND	1.16a	2.30a	*nd*	*nd*	0.50a	0.80a
pentan-2-ol	*nd*	*nd*	2.63	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
butane-2,3-diol	*nd*	*nd*	*nd*	5.02a	6.04a	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
hexan-1-ol	*nd*	12.84d	1.42ab	3.93c	ND	1.04ab	ND	1.16ab	2.30b	ND	ND	0.50a	0.80ab
cyclohexanol	*nd*	*nd*	4.19	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
heptan-2-ol	*nd*	*nd*	*nd*	*nd*	14.00	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Aldehydes	14.57abc	127.40e	16.26bc	98.85d	19.10c	3.76ab	3.74ab	3.74ab	5.98abc	6.27abc	3.13a	3.39b	5.86abc
3-methylbutanal	*nd*	12.16b	2.42a	8.95b	*nd*	*nd*	*0.83a*	*nd*	1.06a	*nd*	*nd*	*nd*	1.28a
2-methylbut-2-enal	*nd*	90.90c	6.90a	82.63c	19.10b	1.85a	1.70a	2.35a	2.27a	1.19a	1.44a	0.61a	1.13a
3-methylbut-2-enal	3.01a	10.14b	3.13a	7.28ab	*nd*	*nd*	*nd*	*nd*	*nd*	2.84a	*nd*	*nd*	*nd*
hexanal	2.07a	*nd*	2.05a	*nd*	*nd*	1.91a	1.21a	1.39a	2.14a	2.24a	1.70a	2.14a	2.44a
heptanal	0.83a	14.20b	2.80a	*nd*	*nd*	*nd*	*nd*	*nd*	0.67a	*nd*	*nd*	0.84a	1.01a
nonanal	8.97	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Ketones	15.51a	16.47ab	7.97a	12.42a	6.23a	10.02a	16.85a	16.04a	12.92a	19.05abc	30.60c	17.08ab	27.96bc
pentan-2-one	4.11a	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	4.11a	6.45a	6.56a	*nd*
heptan-2-one	8.46a	9.51a	4.86a	7.07a	*nd*	7.46a	12.01ab	11.39ab	9.23a	11.57ab	18.40bc	8.00a	21.20c
nonan-2-one	2.94ab	6.96bc	3.11abc	5.35abc	6.23abc	2.56a	4.85abc	4.64abc	3.69abc	3.61abc	5.76abc	2.51ab	6.76c
Others	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
heptane	25.24c	42.67d	2.43a	13.25b	*nd*	3.16ab	7.94ab	9.99ab	12.00b	10.31ab	7.34ab	5.61ab	9.34ab
toluene	3.88b	11.86c	2.60ab	2.75ab	*nd*	2.16ab	1.11a	1.84a	2.23ab	2.60ab	1.75a	1.77a	1.72a

nd – stands for not detected.

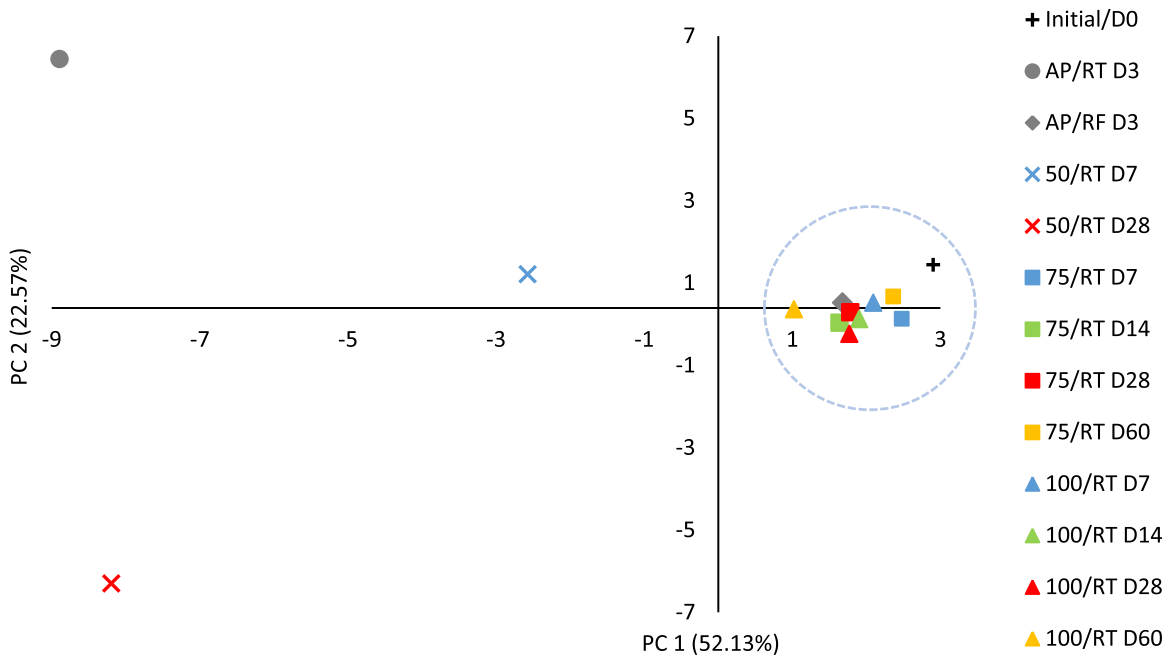

Fig. 1. - Principal component analysis score plot of the volatile compounds of cow's FC prior storage (Initial) and under the different conditions (AP/RT, AP/RF and 50, 75 and 100/RT). Same storage periods have the same colour, and same storage conditions the same symbol.

tion of aldehydes formed by amino acids degradation (Muñoz, Ortigosa, Torre & Izco, 2003; Toso, Procida & Stefanon, 2002).

Under 50/RT after 7 days, cheese VOC profile showed in general an evolution similar to storage at AP/RF concerning esters, alcohols, and FFA, that continuously arose over storage ($p<0.05$), while ketones and aldehydes concentration decreased on the 28th day. All FFA concentrations increased, especially for hexanoic and octanoic acids, with the now detected acetic and nonanoic acids, contributing to an estimated increase of 52.01 μg/100 g of FFA per day (*supplementary material, Figure S3*). Esters also increased, to around 21.49 μg/100 g per day, largely due to increases observed in ethyl decanoate, butanoate and hexanoate, and from ethyl octanoate and dodecanoate that were initially undetected. An estimated reduction over time of 0.32 μg/100 g per day in ketones occurred, probably due to its reduction to alcohols, which increased around 0.67 μg/100 g per day (*supplementary material Figure S3*), mainly from the formation of heptan-2-ol and butane-2,3-diol. Even with the initial increase in total aldehydes at the 7th day of storage, since these are transitory oxidation compounds, quick conversion into acids or alcohols can occur (Bezerra et al., 2017), resulting in considerable content reduction after 28 days of storage ($p<0.05$). The distinguishable VOC profile of cheeses kept at 50/RT can be assigned to the high microbial growth observed (above 6 and 5 log units for TAM and LAB, respectively, *unpublished data*), resulting in an overall quality loss of cheeses. On the other hand, chesses stored under 75–100 MPa preserved total aldehydes, esters, ketones and alcohols at constants levels ($p>0.05$) throughout the storage, with the exception for FFA at 100/RT, presenting an estimated increase of 6.54 μg/100 g per day (*supplementary material Figure S3*), which was more noticeable on the 42nd day of storage on forward. In general, it was observed that these storage conditions preserved generally the cheese VOC profile, resembling also more the initial one prior to storage.

With the VOC data from all the storage conditions, a PCA was conducted, presented in Fig. 1, which resulted from multivariate statistical analyses of cow's cheeses VOC throughout the storage. It can be seen in Fig. 1 the score plots of the different variables, with PC 1 and PC 2 accounting for 52.13% and 22.57% of total variability, respectively. On the positive PC 1, cheeses from storage at AP/RF, 75 and 100/RT at all storage periods are closer to the cheese prior storage. While on the negative PC 1, cheeses stored under 50/RT are further apart as the storage period increased to the cheese prior to storage, and the most distant ones were cheeses under AP/RT. From the loadings of the two principal components (*supplementary material Table S4*), compounds more connected with cow's FC prior to storage, mainly ketones and aldehydes like pentan-2-one, heptan-2-one, hexanal and nonanal are scored on the positive loadings on PC 1, while the negative PC 1 is related to compounds associated with cheese deterioration, particularly higher concentrations of FFA, esters and some alcohols.

A total of 24 compounds were identified (Table 4) initially in goat's FC, belonging to FFA ($n = 6$), followed by esters ($n = 5$), alcohols ($n = 5$), ketones ($n = 4$), and aldehydes ($n = 1$), close the ones described by Quintanilla, Hettinga, Beltrán, Escriche and Molina (2020).

VOC concentration tended to increase in AP/RT cheeses, with the exception of ketones and aldehydes that can be easily transformed into acids or alcohols. Alcohols, FFA and ethyl esters increased almost up to 10-fold ($p<0.05$), resulting in a substantial rise in 3-methylbutan-1-ol, butane-2,3-diol, acetic, butanoic and octanoic acids, and in ethyl butanoate and hexanoate. This evolution in AP/RF cheeses VOC profile was not so pronounced, despite the considerable increases ($p<0.05$) observed in acetic and nonanoic acids, ethyl esters remained within the values initially reported ($p>0.05$), but with a higher alcohol concentration, mostly from 3-methylbutan-1-ol ($p<0.05$), while ketones ($p<0.05$) and aldehydes concentration decreased after 3 days.

As for cheeses stored under pressure, 50/RT promoted noteworthy changes in cheese VOC profile, with an accentuated development ($p<0.05$) of FFA, ethyl esters and alcohols, while ketones and aldehydes were undetected just after 7 days of storage. In the following storage periods, a rise of all FFA, esters and alcohols were noticeable, contributing to a differentiated VOC profile relatively to cheeses prior to storage ($p<0.05$). On the other hand, 75 and 100/RT cheeses maintained a more stable VOC profile over storage, with a decrease in ketones content slower, while aldehydes increased slightly only after 60 days under 100/RT ($p<0.05$). In the first 14th days a greater alcohol formation was observed ($p<0.05$), reaching values closer to the initial ones on the following storage periods, whereas FFA remained within the same concentrations from the 7th day on forward, with no substantial changes ($p>0.05$) detected for esters.

Table 4
- Volatile organic compounds (μg internal standard equivalents/100 g) of goat's FC prior storage (Initial) and under the different conditions (AP/RT, AP/RF and 50, 75 and 100/RT). Different letters (a–f) indicate significant differences ($p<0.05$) between the different conditions. Standard deviation is at least below 10% of the mean value, and thus is not displayed in the Table.

Condition	Initial	AP/RT	AP/RF	50 MPa/RT		75 MPa/RT					100 MPa/RT		
Days	0	3	3	7	28	7	14	28	60	7	14	28	60
Free fatty acids	166.38a	3170.90c	1017.31ab	3282.07c	4239.54c	1007.95ab	1264.32b	1121.78ab	1286.54b	799.95ab	1255.01b	700.41ab	1576.32b
acetic acid	21.64a	422.79d	179.00bc	173.23c	319.21d	74.99abc	71.31abc	46.02abc	48.22ab	56.39abc	56.84abc	40.51ab	37.81a
butanoic acid	14.34a	571.82e	107.32abc	344.72d	535.75e	94.99abc	124.43bc	96.47abc	181.96c	68.04ab	82.43abc	82.85abc	162.53bc
hexanoic acid	27.06a	1013.36c	223.33ab	1029.30c	1294.91c	254.32ab	380.46b	301.62ab	454.01b	193.04ab	267.10ab	260.93ab	470.72b
octanoic acid	61.41a	736.95cd	254.26ab	1062.65de	1198.72e	340.61ab	389.41abc	307.57ab	372.90abc	284.01ab	423.42abc	199.49ab	550.83bc
nonanoic acid	8.81ab	10.24abc	26.39d	21.55cd	17.44bcd	8.39ab	6.69a	8.71ab	5.25a	3.91a	7.88ab	8.62ab	13.65abc
decanoic acid	44.19a	396.63b	168.37ab	710.73c	690.81c	233.13ab	298.83ab	359.02b	122.28ab	164.69ab	336.46b	108.12ab	343.57ab
dodecanoic acid	2.71a	27.18c	7.07a	13.34ab	18.94bc	9.90ab	11.09ab	11.08ab	3.35a	7.77a	12.83ab	6.57a	10.86ab
Esters	55.03a	156.58bc	46.71a	225.24c	563.92d	29.50a	119.02abc	110.92abc	101.84ab	24.83a	107.46abc	64.91ab	117.70abc
ethyl acetate	10.16ab	26.43c	15.21b	9.56ab	10.00ab	8.91ab	7.83ab	5.00a	3.40a	10.14ab	10.96ab	4.88a	4.67a
ethyl butanoate	5.52a	52.20d	12.02ab	27.71bc	75.04e	11.45ab	18.60abc	20.04abc	28.80c	11.50ab	13.30ab	18.82abc	16.29abc
ethyl hexanoate	8.98a	30.22a	7.12a	40.56a	138.36b	8.82a	22.03a	17.46a	20.94a	7.02a	11.93a	12.90a	23.17a
ethyl octanoate	24.94a	41.05a	*nd*	40.56a	138.36b	*nd*	18.85a	13.83a	14.80a	*nd*	14.23a	10.98a	18.52a
ethyl decanoate	3.55a	10.02abc	8.43ab	95.92d	172.44e	*nd*	51.71c	54.58cd	33.89abc	*nd*	52.50cd	23.61abc	55.45cd
ethyl dodecanoate	*nd*	*nd*	*nd*	*nd*	4.43	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Alcohols	93.97a	834.88f	364.48d	649.46e	768.53ef	350.06d	329.98cd	175.64ab	166.17a	343.01bcd	362.75d	160.06a	156.15ab
2-methylpropan-1-ol	11.05ab	39.41e	18.99bcd	29.16de	24.53cd	12.78ab	11.48ab	8.77a	8.95a	16.05abc	13.73ab	8.39a	8.63a
3-methylbutan-1-ol	44.09a	482.63c	197.42b	424.03c	555.42c	239.00b	245.46b	103.44ab	105.37ab	212.59b	244.21b	99.50ab	87.33ab
2-methylbutan-1-ol	5.76a	40.86de	14.80ab	30.89cd	54.08e	23.28bc	24.69bc	17.54abc	17.84abc	23.09bc	24.87bc	13.92ab	12.70ab
butane-2,3-diol	29.87a	253.64b	79.79a	81.56a	80.82a	75.00a	48.35a	45.89a	34.01a	104.07a	77.42a	38.26a	56.26a
cyclohexanol	1.73a	15.24b	14.01b	*nd*	18.69b	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
heptan-2-ol	*nd*	4.66ab	3.66a	7.63ab	8.53b	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Ketones	98.39b	16.12a	27.54a	*nd*	*nd*	37.49a	15.67a	11.77a	19.17a	28.80a	29.96a	9.83a	14.59a
butane-2,3-dione	42.55	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
3-hydroxybutan-2-one	59.97c	16.12ab	23.71ab	*nd*	*nd*	37.49bc	4.90a	3.92a	7.25ab	28.80ab	16.36ab	2.75a	*nd*
heptan-2-one	7.99a	*nd*	*nd*	*nd*	*nd*	*nd*	10.92ab	7.85a	11.20ab	*nd*	13.60b	7.86a	14.59b
nonan-2-one	5.00a	*nd*	*nd*	*nd*	*nd*	8.25abc	12.05bc	4.45a	5.47a	4.38a	7.10ab	3.71a	12.96c
Aldehydes													
3-methylbutanal	4.75a	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	3.87a	5.28ab	4.05a	3.30a	7.72ab	10.52b
Others	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
heptane	20.51abc	38.16bcd	24.55abc	51.21de	5.40a	44.97cde	4.63a	17.93abc	14.70abc	62.12e	10.01ab	6.30ab	42.90bcd
toluene	1.04a	5.85c	*nd*	5.20bc	5.26bc	2.83abc	1.91ab	3.82abc	1.42ab	3.05abc	4.85bc	3.61abc	2.66abc

nd – stands for not detected.

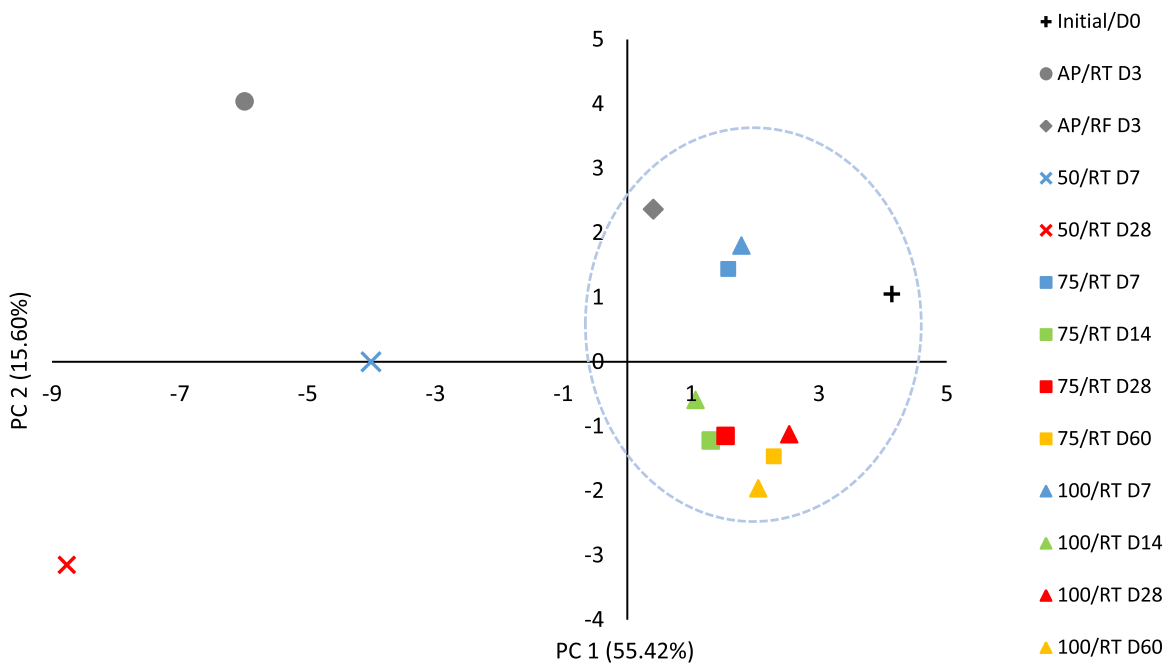

Fig. 2. - Principal component analysis score plot of the volatile compounds of goat's FC prior storage (Initial) and under the different conditions (AP/RT, AP/RF and 50, 75 and 100/RT). Same storage periods have the same colour, and same storage conditions the same symbol.

A second PCA was elaborated based on goat's cheeses VOC profile under the different storage conditions, which could explain 71.02% of total variance (Fig. 2), with 55.42% and 15.60% corresponding from PC 1 and PC 2, respectively. It can be observed that ketone and aldehyde compounds scored on the positive PC 1 (*supplementary material Table S5*), which are associated with unspoilt goat's FC like 3-methylbutanal, butane-2,3-dione, 3-hydroxybutan-2-one, heptan-2-one and nonan-2-one, while FFA, alcohols and esters compounds were more present in spoiled samples, with negative loadings on PC 1, such as heptan-2-ol, octanoic acid and ethyl butanoate (*supplementary material Table S5*).

In general, independent of the cheese type, HS (75–100/RT) preserved better the VOC profile throughout the storage, resembling more the VOC profile of cheeses prior to storage, even after 60 days at RT, with a better preservation of FFA, esters and alcohols over storage, comparatively with the other storage conditions. It is noteworthy, that even only after 3 days at low temperature (AP/RF), cheeses stored under HS (75–100/RT) after 60 days presented globally a more resembling VOC profile relatively to cheeses prior to storage.

4. Conclusions

Generally, a much better preservation of both cow's and goat's FC was achieved under HS, 75/100 MPa at RT, for a considerably longer storage period, comparatively to RF. From the two storage pressures, 100/RT cheeses exhibited a slower lipid oxidation rate and a more stable fatty acid profile and total protein throughout storage. Furthermore, mainly for the longest storage periods an increased FAA abundance in HS cheeses was observed, which should be further evaluated. However, storage under 75–100/RT, maintained cheeses volatile organic profile closer to cheeses prior to storage, without noticeable development of undesirable compounds connected with cheese spoilage, even after 60 days at uncontrolled variable RT, which was corroborated by the conducted PCAs.

It can be concluded that a much longer shelf-life can be attained under HS/RT (up to at least 60 days), launching new business opportunities, as FC has short shelf-life. These findings in addition with the low carbon footprint associated with HS, may further support its suitability for implementation in the food sector, although much technological development and research are required.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

CRediT authorship contribution statement

Ricardo V. Duarte: Conceptualization, Investigation, Writing – original draft. **Susana Casal:** Methodology, Resources. **Ana M. Gomes:** Supervision, Resources. **Ivonne Delgadillo:** Supervision, Resources. **Jorge A. Saraiva:** Supervision, Resources, Writing – review & editing.

Data availability

Data will be made available on request.

Acknowledgements

This work received support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020 and UIDP/50006/2020. We would also like to thank the CBQF scientific collaboration under the FCT projects UIDB/50016/2020, UIDB/50006/2020 and UIDP/50006/2020. Ricardo V. Duarte would like to thank also FCT/MCT for the PhD grant SFRH/BD/121727/2016.

Funding

This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects UIDB/50006/2020 and UIDP/50006/2020, and through CBQF, under the FCT project UIDB/50016/2020. Additionally, Ricardo V. Duarte was also financially supported by FCT/MCT in a PhD grant (SFRH/BD/121727/2016).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100212.

References

Abellán, A., Cayuela, J. M., Pino, A., Martínez-Cachá, A., Salazar, E., & Tejada, L. (2012). Free amino acid content of goat's milk cheese made with animal rennet and plant coagulant. *Journal of the Science of Food and Agriculture, 92*(8), 1657–1664. doi:10.1002/jsfa.5528.

Arte, E., Rizzello, C. G., Verni, M., Nordlund, E., Katina, K., & Coda, R. (2015). Impact of enzymatic and microbial bioprocessing on protein modification and nutritional properties of wheat bran. *Journal of Agricultural and Food Chemistry, 63*(39), 8685–8693. doi:10.1021/acs.jafc.5b03495.

Atanasova, J., Dalgalarrondo, M., Iliev, I., Moncheva, P., Todorov, S. D., & Ivanova, I. V. (2021). Formation of free amino acids and bioactive peptides during the ripening of bulgarian white brined cheeses. *Probiotics and antimicrobial proteins, 13*(1), 261–272. doi:10.1007/s12602-020-09669-0.

Badawy, A. A. B., Morgan, C. J., & Turner, J. A. (2008). Application of the Phenomenex EZ:FAasttrade mark amino acid analysis kit for rapid gas-chromatographic determination of concentrations of plasma tryptophan and its brain uptake competitors. *Amino acids, 34*(4), 587–596. doi:10.1007/s00726-007-0012-7.

Bermejo-Prada, A., Colmant, A., Otero, L., & Guignon, B. (2017). Industrial viability of the hyperbaric method to store perishable foods at room temperature. *Journal of food engineering, 193*, 76–85. doi:10.1016/j.jfoodeng.2016.08.014.

Bezerra, T. K. A., Arcanjo, N. M. d. O., Araújo, A. R. R. d., Queiroz, A. L. M. d., Oliveira, M. E. G. d., Gomes, A. M. P., et al., (2017). Volatile profile in goat coalho cheese supplemented with probiotic lactic acid bacteria. *LWT - Food Science and Technology, 76*, 209–215. doi:10.1016/j.lwt.2016.03.041.

Charm, S. E., Longmaid, H. E., & Carver, J. (1977). A simple system for extending refrigerated, nonfrozen preservation of biological material using pressure. *Cryobiology, 14*(5), 625–636. doi:10.1016/0011-2240(77)90174-2.

Duarte, R. V., Pinto, C. A., Gomes, A. M., Delgadillo, I., & Saraiva, J. A. (2022). A microbiological perspective of raw milk preserved at room temperature using hyperbaric storage compared to refrigerated storage. *Innovative Food Science & Emerging Technologies*, Article 103019. doi:10.1016/j.ifset.2022.103019.

Duarte, R., Moreira, S., Fernandes, P., Fidalgo, L., Santos, M., Queirós, R., et al., (2014). Preservation under pressure (hyperbaric storage) at 25°C, 30°C and 37°C of a highly perishable dairy food and comparison with refrigeration. *CyTA-Journal of Food*, 1–8.

Duarte, R., Moreira, S., Fernandes, P. A., Santos, D., Inácio, R., Alves, S., et al., (2017). Whey cheese longer shelf-life achievement at variable uncontrolled room temperature and comparison to refrigeration. *Journal of Food Processing and Preservation, 41*(6), e13307. doi:10.1111/jfpp.13307.

Enright, E., Patricia Bland, A., Needs, E. C., & Kelly, A. L. (1999). Proteolysis and physicochemical changes in milk on storage as affected by UHT treatment, plasmin activity and KIO3 addition. *International Dairy Journal, 9*(9), 581–591. doi:10.1016/S0958-6946(99)00128-4.

Ercan, S. Ş., Soysal, Ç., & Bozkurt, H. (2019). Biogenic amine contents of fresh and mature kashar cheeses during refrigerated storage. *Food and Health, 5*(1), 19–29. doi:10.3153/FH19003.

Eugster, E., Fuchsmann, P., Schlichtherle-Cerny, H., Bütikofer, U., & Irmler, S. (2019). Formation of alanine, α-aminobutyrate, acetate, and 2-butanol during cheese ripening by Pediococcus acidilactici FAM18098. *International Dairy Journal, 96*, 21–28. doi:10.1016/j.idairyj.2019.04.001.

Fernandes, P., Moreira, S., Duarte, R., Santos, D., Queirós, R., Fidalgo, L., et al., (2015). Preservation of sliced cooked ham at 25, 30 and 37°C under moderated pressure (hyperbaric storage) and comparison with refrigerated storage. *Food and Bioproducts Processing, 95*, 200–207. doi:10.1016/j.fbp.2015.05.004.

Fidalgo, L., Santos, M., Queirós, R., Inácio, R., Mota, M., Lopes, M., et al., (2014). Hyperbaric storage at and above room temperature of a highly perishable food. *Food and Bioprocess Technology, 7*(7), 2028–2037. doi:10.1007/s11947-013-1201-x.

Hoskisson, P. A., Sharples, G. P., & Hobbs, G. (2003). The importance of amino acids as carbon sources for Micromonospora echinospora (ATCC 15837). *Letters in applied microbiology, 36*(5), 268–271. doi:10.1046/j.1472-765X.2003.01306.x.

King, R. L. (1962). Oxidation of milk fat globule membrane material. I. Thiobarbituric acid reaction as a measure of oxidized flavor in milk and model systems. *Journal of Dairy Science, 45*(10), 1165–1171.

Mitsuda, H. (1972). Under water and underground storage of cereal grain. *Food technology, 26*, 50–56.

Muñoz, N., Ortigosa, M. a., Torre, P., & Izco, J. M. (2003). Free amino acids and volatile compounds in an ewe's milk cheese as affected by seasonal and cheese-making plant variations. *Food Chemistry, 83*(3), 329–338. doi:10.1016/S0308-8146(03)00133-X.

Queirós, R. P., Santos, M. D., Fidalgo, L. G., Mota, M. J., Lopes, R. P., Inácio, R. S., et al., (2014). Hyperbaric storage of melon juice at and above room temperature and comparison with storage at atmospheric pressure and refrigeration. *Food Chemistry, 147*, 209–214.

Quintanilla, P., Hettinga, K. A., Beltrán, M. C., Escriche, I., & Molina, M. P. (2020). Short communication: Volatile profile of matured Tronchón cheese affected by oxytetracycline in raw goat milk. *Journal of Dairy Science, 103*(7), 6015–6021. doi:10.3168/jds.2019-16510.

Sant'Ana, A. M. S., Bezerril, F. F., Madruga, M. S., Batista, A. S. M., Magnani, M., Souza, E. L., et al., (2013). Nutritional and sensory characteristics of Minas fresh cheese made with goat milk, cow milk, or a mixture of both. *Journal of Dairy Science, 96*(12), 7442–7453. doi:10.3168/jds.2013-6915.

Santos, M., Castro, R., Delgadillo, I., & Saraiva, J. A. (2020). Improvement of the refrigerated preservation technology by hyperbaric storage for raw fresh meat. *Journal of the Science of Food and Agriculture*. doi:10.1002/jsfa.10083.

Segovia-Bravo, K. A., Guignon, B., Bermejo-Prada, A., Sanz, P. D., & Otero, L. (2012). Hyperbaric storage at room temperature for food preservation: A study in strawberry juice. *Innovative Food Science & Emerging Technologies, 15*, 14–22. doi:10.1016/j.ifset.2012.02.005.

Sobral, M. M. C., Casal, S., Faria, M. A., Cunha, S. C., & Ferreira, I. M. P. L. V. O. (2020). Influence of culinary practices on protein and lipid oxidation of chicken meat burgers during cooking and in vitro gastrointestinal digestion. *Food and Chemical Toxicology, 141*, Article 111401. doi:10.1016/j.fct.2020.111401.

Teter, A., Barłowska, J., Król, J., Brodziak, A., Rutkowska, J., & Litwińczuk, Z. (2020). Volatile compounds and amino acid profile of short-ripened farmhouse cheese manufactured from the milk of the White-Backed native cow breed. *LWT, 129*, Article 109602. doi:10.1016/j.lwt.2020.109602.

Toso, B., Procida, G., & Stefanon, B. (2002). Determination of volatile compounds in cows' milk using headspace GC-MS. *Journal of Dairy Research, 69*(4), 569–577. doi:10.1017/S0022029902005782.

Tunick, M. H., Iandola, S. K., & Van Hekken, D. L. (2013). Comparison of SPME methods for determining volatile compounds in milk, cheese, and whey powder. *Foods (Basel, Switzerland), 2*(4), 534–543. doi:10.3390/foods2040534.

Van Hekken, D. L., Tunick, M. H., Farkye, N. Y., & Tomasula, P. M. (2013). Effect of hydrostatic high-pressure processing on the chemical, functional, and rheological properties of starter-free Queso Fresco. *Journal of Dairy Science, 96*(10), 6147–6160. doi:10.3168/jds.2012-6212.

Van Nieuwenhove, C. P., Oliszewski, R., & González, S. N. (2009). Fatty acid composition and conjugated linoleic acid content of cow and goat cheeses from northwest Argentina. *Journal of Food Quality, 32*(3), 303–314. doi:10.1111/j.1745-4557.2009.00258.x.

Yue, J., Zheng, Y., Liu, Z., Deng, Y., Jing, Y., Luo, Y., et al., (2015). Characterization of volatile compounds in microfiltered pasteurized milk using solid-phase microextraction and GC×GC-TOFMS. *International Journal of Food Properties, 18*(10), 2193–2212. doi:10.1080/10942912.2014.966389.

Zamora, A., Juan, B., & Trujillo, A. J. (2015). Compositional and biochemical changes during cold storage of starter-free fresh cheeses made from ultra-high-pressure homogenised milk. *Food Chemistry, 176*, 433–440. doi:10.1016/j.foodchem.2014.12.070.