Evaluation of cow's and goat's fresh cheese preservation under hyperbaric storage at room temperature up to 60 days versus refrigeration regarding nutritional and physicochemical parameters

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Abstract

Two highly perishable dairy products (cow's and goat's fresh cheese (FC)), were stored under hyperbaric storage at room temperature (HS/RT, 50-100MPa) and compared with RF (4ºC), for 60 days.

HS/RT (≥75MPa) lead to a more stable volatile organic and fatty acid profiles, reducing lipid oxidation rate, particularly for cow's FC, resembling more to FC prior storage, contrarily to refrigerated cheeses. No variation was observed for total protein, while 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.

These 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

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

The feasibility of HS at and above RT was studied initially in fruit juices as case study, in 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 outcomes pinpointing to a possible shelf-life extension, due to microbial inhibition/inactivation during HS, with minimal physicochemical changes reported. Other non-liquid highly perishable food products were also evaluated under HS/RT as a case study, initially for short storage periods with promising results (Duarte et al., 2014, Fernandes et al., 2015). One of these products was whey cheese, which has only a couple weeks of shelf-life at RF, presenting after 8 hours at 100/RT no pronounced changes in colour, pH, and water activity, showing a slightly increased in lipid oxidation values, with a clear microbial growth inactivation in all microbiological groups even at and above RT (25-37 ºC) (Duarte et al., 2014). One a second study, this product presented higher stability under HS (100 MPa) during longer storage periods (10 days) at variable RT, retaining the pH, water activity, and fatty acid profile, while presenting fewer colour losses comparatively to RF, with an additional microbial inactivation effect to undetectable counts (1 log CFU/g) in all the studied microbiological groups (from the 3rd day of storage onwards) (Duarte et al., 2017). Indeed, in some of the works described just above that were conducted by our research group, pointed to an additional hyperbaric inactivation (HI) effect along storage in various microbiological populations present in different foods, leading in most cases to constant undetectable counts (below the detection limit) until the end of the storage period. This HI effect, reached for several cases over 5 log units for vegetative bacteria, opening the possibility of achieving pasteurization during HS, a possibility that is now being intentionally and systematically studied in our research group.

Fresh cheeses (FC) are highly perishable dairy products, characterized by short shelf-life (a few weeks at RF), mainly due to their high water activity and rich nutritional profile that promote microbial spoilage, leading to increased syneresis, decrease in the pH, 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*). During that study significant HI was observed, gradually reducing total aerobic mesophiles counts more than 5 log units throughout the 60 days of storage, (initial counts of ~7 log CFU/g), reaching progressively values below the quantification and detection limits (2 and 1 log CFU/g), depending on pressure level and storage time (*unpublished results*). In the present study, the effect of HS/RT (50-100 MPa) during 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 in vitro protein digestibility, total protein, free amino acids, lipid oxidation, fatty acids and volatile organic profiles and compared with RF under atmospheric pressure (AP).

2. Materials And Methods

2.1 Fresh cheese samples preparation and storage

Two commercially fresh cheeses (FC) one made from pasteurized cow's milk and the other from pasteurized goat's milk, were acquired from a local supermarket. These cheeses were produced through enzymatic coagulation, with each kind of cheeses bought from the same company and lot with similar remaining shelf-life. Temperature was kept low (3-8 ºC) during FC transportation being after packaged into low permeability polyamide-polyethylene bags (90 micron, 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.

Fresh cheese samples were stored under different conditions, with the experiments performed under HS (50,75 and 100 MPa) at room temperature (RT) and sampling for analysis at 3, 7, 14, 28, 42 and 60 days, 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. As a control, cheeses were also stored under atmospheric pressure (0.1 MPa) at RT (15 - 22 ºC) and refrigeration (4 ºC), during 3 and 7 days, respectively, since samples were microbiologically unacceptable on those periods (*unpublished data*), and

thus those storage experiment were interrupted. Cheeses at AP/RF and AP/RT were kept immerse 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 Protein profile analysis and digestibility

Protein profile was assessed based on the determination 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 determined by multiplying the total nitrogen content by 6.38 (AOAC Official Method 2001.14, 2002). For FAA determination and quantification, cheese samples were homogenised in the same volume of 0.01 M HCl, centrifuged (17000 × g at 4 ºC for 5 min), and the supernatant was collected and centrifuged again. Then, 100 µL of the second supernatant was used for the analysis of free amino acids using the EZ:Faast Amino Acid Analysis Kit (GC-FID) (Badawy, Morgan, & Turner, 2008) being the results for individual FAA expressed in nmol per g of cheese. Digestibility was performed based on the method developed by Arte et al. (2015) with some modifications. Cheese 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. The enzyme was inactivated with 10 mL of trichloroacetic acid (10%, wt/vol) and centrifuged (5000 × g at RT for 20 min) to separate undigested protein. Nitrogen in the supernatant was determined by micro-Kjeldahl method. Digestibility 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).

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

2.3 Fatty Acids Profile

For the fatty acids profile determination, a similar method to the one described by Sobral, Casal, Faria, Cunha, and Ferreira (2020) was performed. Briefly, 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). Fatty acids identification and FID calibration was accomplished 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 Secondary lipid oxidation by-products

Lipid oxidation state was assessed by malondialdehyde (MDA) quantification, using 2-thiobarbituric acid reactive substances (TBARS) method adapted from King (1962). Initially 1 g of fresh cheese was crumbled into smaller pieces and homogenised with 3 mL 7.5% trichloroacetic acid, followed by centrifugation at 4000 × g at 4 ºC for 20 min (Universal 320-R, Hettich Group, Tuttlingen, Germany). The resulting extract was filtered (Whatman nº1) 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. Triplicates were measured using a micro-plate spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Scientific, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 532 nm. 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

Volatile organic compounds (VOC) profile determination was based on the method described by Yue et al. (2015), through headspace solid-phase microextraction (HS-SPME) followed by gas chromatography-mass spectrometry (GC-MS). 2 g of cheese and 50 µL of cyclohexanone (25 µg/mL, internal standard) were added into the vial, then immediately sealed with a polypropylene cap with silicon septum. Compounds were released at 50 ºC during 30 min, then the SPME fiber (DVB/CAR/PDMS, 50/30 µm, Supelco Inc.) was exposed for further 30 min the same temperature. After volatiles adsorption into the fiber, 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. The oven temperature was set at 35 ºC during 5 min, increasing to 100 ºC at a rate of 4 ºC/min, followed by an increase of 10 ºC/min until 225 ºC and held for 0.25 min (total of 33.5 min). The transfer line was maintained at 280 ºC and the ion source at 230 °C, with ionization energy of 70 eV. Mass spectra were scanned from 20 to 350 m/z in full scan mode. Identification of the volatile compounds was based on computer matching with the reference mass spectra library (NIST 11), retention times, retention index and with individual standards when available. Volatiles' profile semi-quantitative determination was calculated using cyclohexanone as internal standard equivalents basis, and the results were expressed in µg per 100g of cheese.

2.6 Statistical analysis

All experiments were carried out in triplicate and all analyses were done in triplicate. The different storage conditions were compared using Analysis of Variance (ANOVA), followed by a multiple comparison post hoc test, Tukey's HSD test, at a 5% level of significance. Additionally, principal component analysis

(PCA) was performed in order to identify statistical patterns using the VOC data set obtained for both cheeses.

3. Results And Discussion

3.1 Protein profile

Cow's FC presented a total protein concentration slightly lower compared to goat's FC (Tables 1 and 2), 15.11 ± 0.49 g/100g and 16.99 ± 0.50 g/100g, respectively, similar to what is reported in the literature(Sant'Ana et al., 2013, Van Hekken, Tunick, Farkye, & Tomasula, 2013). Overall, both cow's and goat's FC presented a similar behaviour under the same storage conditions, with no significant changes(p>0.05)observed in all storage conditions, with storage at 75 and 100/RT maintaining 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 and 100 MPa) at variable RT for 60 days (*unpublished data*).

Regarding FAA, cow's FC was initially richer 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 initially a total FAA of 0.9 ± 0.1 µmol/g (Table 2), mainly constituted by glycine, followed by ornithine, glutamine, glutamic acid, valine, and aspartic acid. Similar compositions in initial FAA were also reported for cheeses made with cow's and goat's milk(Atanasova et al., 2021, Teter et al., 2020).

At the 3rd day of storage, no significant variations were observed regarding individual FAA(p>0.05) of cow's FC stored at AP/RF, comparatively to the initial ones, while cheeses at AP/RT exhibited(p< 0.05)a 12-fold increase in alanine and a 3-fold decrease in ornithine, while also several amino acids were now undetected such as glycine, isoleucine, threonine, proline and histidine that were initially present, which could have been used in microbial metabolism(Hoskisson, Sharples, & Hobbs, 2003). Despite these small variations in individual FAA, total FAA content remained similar(p>0.05)to the initial ones for both storage conditions.

As for storage under 50/RT, initially at day 3, changes were only detected in ornithine (decrease of 0.5-fold) without significant variations in all the other individual and total FAA(p>0.05). However, on the following storage periods a remarkable increase in the majority of FAA was detected(p< 0.05), with increments of 100-, 48-, 27- and 21-fold, for alanine, histidine, threonine, and valine, respectively, after 28 days of storage. At this sampling period, FAA were majorly composed of alanine, leucine, serine, glutamic acid, valine, and lysine (altogether representing 66% of total FAA), while being also characterized by the presence of serine, phenylalanine, cystine and threonine that were initially absent, resulting in an overall increase of 7-fold in total FAA. This might be due to 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). Furthermore, 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 log CFU, 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). Nevertheless, this proteolytic effect was lower(p< 0.05)for FC stored at 75 and 100/RT, 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 increases in total FAA of 5.9 and 5.7-fold, under 75 and 100/RT respectively, at day 60 of storage. Interestingly the 100-fold increase in alanine observed after 28 days under 50/RT 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 byEugster, Fuchsmann, Schlichtherle-Cerny, Bütikofer, and Irmler (2019)with the microbial activity of added starter cultures in cheese ripening. New FAA such 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 similar total FAA after 60 days of storage of 6.3 ± 0.8 and 6.1 ± 0.5 µmol/g(p>0.05), respectively. Both storage conditions were able to gradually inactivate the microbial load present in FC samples, in a faster 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 explain partially at least the results of lower FAA increase.

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

Under HS conditions, generally goat's FC presented signs of proteolysis throughout the storage, however at different rates. Storage at 50/RT resulted in an estimated raise of 641.63 nmol/g FAA per day (*supplementary material Figure S2*), with significant 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. A more prominent abundance (p< 0.05)in FAA was observed for alanine, leucine, valine, glutamic acid, and lysine, 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 (around 6 log CFU, for LAB, *unpublished data*), that increased under 50/RT (reaching almost 8 log CFU/g after the 7th day of storage), which can contribute 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. Under 75 and 100/RT, this increase in total FAA was lower (16- and 8-fold increase, respectively), resulting in a proteolysis rate almost 2-fold slower, with increases of 151.57 and 71.73 nmol/g FAA per day (*supplementary material Figure S2*), respectively, reaching values of 9.5 ± 0.9 and 4.8 ± 0.4 µmol/g for total FAA after 60 days of storage, respectively. Interestingly, TAM and LAB counts were strongly inactivated under those conditions, however the inactivation rate was almost 3-fold faster under 100 MPa (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 factor responsible for the proteolysis observed. Despite the almost half concentration in most FAA between cheeses stored under 75 and 100/RT, both presented a greater abundance(p>0.05)in leucine, valine, aspartic and glutamic acid. Also similarly with storage at 50/RT new amino acids were now present, such as isoleucine, phenylalanine, serine, and tryptophan. Increased proteolysis of FC in

prolonged storage under RF is to be expected, as reported bySant'Ana et al. (2013), who observed an increased proteolysis in FC stored at AP/RF after 21 days, attributed mainly to the action of LAB, extracellular proteases, and to a smaller degree to plasmin.

Globally, for both FCs, storage under HS at 75 and 100/RT resulted over time in an increased concentration in FAA, although even after 60 days, values were significantly lower than the ones reported byAbellán et al. (2012)for goat cheese at day 1 of maturation. Still, the possible impact of these increases should be further investigated in the sensory properties of HS cheeses.

Regarding protein digestibility, cow's (Table 1) and goat's (Table 2) FC presented values prior to storage of 81.2 ± 2.1 and 75.8 ± 1.1%, respectively. Under 100/RT, after 60 days no significant variations were observed for cow's FC (81.4 ± 2.7%), while an increase (p< 0.05)to 81.0 ± 1.8% was detected for goat's FC. As mentioned previously, after 60 days of storage at 100/RT an increase in FAA of 5.7 and 8-fold was observed for cow's and goat's FC, respectively, indicating a higher proteolysis in goat's FC, which could be responsible for the increased protein digestibility.

3.2 Fatty acids profile

Cow's and goat's FC fatty acid profile are presented in *supplementary materialTable S1* and *S2*, respectively. Cow's and goat's FCs had an overall similar fatty acid content, with slight variations, both with a higher composition in saturated fatty acids (SFA), followed by monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), overall similar to the composition described byVan Nieuwenhove, Oliszewski, and González (2009). Initially, 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, while goat's FC had initially total SFA, MUFA and PUFA content of 66.16 ± 0.93%, 27.94 ± 0.83% and 5.09 ± 0.18%, respectively. Regarding SFA, cow's and goat's FC were rich in 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 difference being related with a higher capric acid (C10:0) percentage observed for goat's FC (9.28 ± 0.82%) comparatively with cow's FC (2.83 ± 0.20%), similar to what is reported in the literature(Sant'Ana et al., 2013). As for MUFA cow's and goat's FC 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).

Throughout the different storage conditions, cow's FC fatty acid profile presented some variations when compared to the profile prior storage. In general, longer HS periods tended to present increased(p>0.05)values in SFA content, with storage at 75 and 100/RT reaching values of 65.46 ± 1.04% and 64.96 ± 0.65%, respectively. This tendency was more pronounced especially under 75/RT, presenting a tendency for higher amounts of palmitic acid (p< 0.05), stearic acid and myristic acid (p< 0.05). In accordance, for MUFA and PUFA, HS tended to present lower values, with the major differences (p< 0.05) being related to storage at 100/RT after 7 and 14 days, presenting values similar to the initial ones on the following storage periods(p>0.05). Despite the fluctuations (p< 0.05) detected regardingoleic,linoleic, and α-linolenic acids (C18:3*c*6,*c*9,*c*12), overall, the majority of MUFA and PUFA content was not affected during HS(p>0.05).

As for goat's FC storage, despite some variability in few individual fatty acids during the different storage conditions, under HS no significant changes(p>0.05)were observed after 60 days, comparatively to the initial cheese, although the same tendency was observed similarly to cow's FC storage, with cheeses at 75 and 100/RT presenting higher values(p>0.05)regarding SFA, accompanied by a decrease(p>0.05)for MUFA and PUFA content. Similar results were also found in HS of raw milk for 60 days, with a more pronounced increase SFA content (p< 0.05)being observed especially for storage at 75/RT, while MUFA and PUFA contents decreased throughout the storage (*unpublished data*).

Overall, storage under 75 and 100/RT was able to successfully keep a similar fatty acid profile of both cow's and goat's cheeses, throughout the duration of the study.

3.3 Secondary Lipid oxidation by-products

Regarding lipid oxidation throughout storage under the different storage conditions, it was clear 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, respectively (*supplementary materialTable S3*). Lipid oxidation was more pronounced (p< 0.05)in cows' FC stored under 50/RT after 14 days of storage, reaching 2.78 µg MDA/g. Under 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. As for goats' FC, lipid oxidation was overall stable in most of the storage conditions, while at 75/RT a strong increase (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 significant slower lipid oxidation rate was achieved under 100/RT throughout storage, reaching 1.15 ± 0.05 µg MDA/g (1.7-fold) after 60 days of storage, which was comparable the one observed on the 7th day of storage (0.86 ± 0.11 µg MDA/g). Lipid oxidation can be affected by several factors such as the presence of light, oxygen or enzymes, promoting the formation of several volatile compounds, giving rise to off-flavours, with increasing 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 were reported byZamora, Juan, and Trujillo (2015), observing increases of 2.5-fold after 13 days, whileErcan, Soysal, and Bozkurt (2019)observed increases around 3.4-fold after 21 days, both higher than the ones reported 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. Significantly higher increases in TBARS values under HS/RT were reported for fish (29-fold) and meat products (4.5-fold), but when a lower temperature (10 ºC) was combined with HS a slower decreasing trend in TBARS evolution was achieved, to 6.6 and 3.9-fold, for fish and meat products, respectively(Fidalgo et al., 2019, Fidalgo, Lemos, Delgadillo, & Saraiva, 2018, Santos et al., 2020). However, results equivalent the ones observed for FCs were obtained in HS of raw milk (*unpublished data*), 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

Initially in cow's FC a total of 18volatile organic compounds (VOC) were detected (Table 3) and consisted mainly of free fatty acids (FFA), esters, ketones, and aldehydes, without alcohol compounds, an overall similar composition to what is reported for this kind of dairy product(Tunick, Iandola, & Van Hekken, 2013). The composition in FFA consisted of butanoic, hexanoic, octanoic and decanoic acids, with sorbic acid ((2E,4E)-hexa-2,4-dienoic acid) also being detected, added in the form of potassium sorbate as a preservative by the producer, as stated in the product label. Ethyl butanoate and hexanoate were the main esters present, as for ketones, pentan-2-one and heptan-2-one were the most abundant compounds, and nonanal was the main aldehyde, which was only present in the cheese prior to storage.

After 3 days, cheeses under AP/RF presented a similar VOC profile(p>0.05), regarding to the cheese prior storage, with a slight increase(p>0.05)in most FFA, aldehydes, esters, and a decrease in ketones, with alcohol compounds such as pentan-2-ol, cyclohexanol and hexan-1-ol being now detected. Storage under AP/RT after 3 days, resulted in a clear distinguished VOC profile of cheeses, with increased concentrations (p< 0.05) ofFFA, aldehydes, esters, and alcohols. An increase up to 10-fold was observed in almost all FFA and their respective ethyl esters after 3 days, with acetic and nonanoic acid, ethyl octanoate and dodecanoate being now present. As for aldehydes and alcohols the main increases resulted from 2-methylbut-2-enal and hexan-1-ol, respectively, with no significant changes observed regarding ketones(p>0.05). High microbial or/and enzymatic activity can promote lipolysis and the release of FFA, as well as lactose and amino acids degradation, with ethyl esters formed by esterification of the FFA, and alcohols resulting possibly from reduction of aldehydes formed by amino acids degradation(Muñoz, Ortigosa, Torre, & Izco, 2003, Toso, Procida, & Stefanon, 2002).

After 7 days at 50/RT, cheese VOC profile presented overall an evolution similar to storage at AP/RF regarding esters, alcohols, and FFA, that continuously arose over storage (p< 0.05), while ketones and aldehydes decreased on the 28th day. An overall increase in all FFA was observed especially in hexanoic and octanoic acids, with the now detected acetic and nonanoic acids, contributing to an estimated increase of 52.01 µg/100g of FFA per day (*supplementary material, Figure S3*). In parallel, esters increased around 21.49 µg/100g per day, mainly due to increases observed in ethyl decanoate, butanoate and hexanoate, and from ethyl octanoate and dodecanoate that were initially undetected. Ketones presented an estimated reduction over time of 0.32 µg/100g per day, possibly due to reduction to alcohols, which increased around 0.67 µg/100g per day (*supplementary material Figure S3*), mainly due through the formation of heptan-2-ol and butane-2,3-diol. Despite 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 the significant content reduction after 28 days of storage (p< 0.05). Changes in the VOC profile of cheeses stored at 50/RT can be attributed to the high microbial load under this condition (above 6 and 5 log units for TAM and LAB, respectively, *unpublished data*), resulting in an overall quality loss of cheeses. Interestingly, storage under 75-100 MPa maintained total aldehydes, esters, ketones and alcohols at constants levels(p>0.05)throughout the storage, with exception for FFA at 100/RT that presented an estimated increase of 6.54 µg/100g per day (*supplementary material Figure S3*), which was more pronounced on the 42nd day of storage on forward. And thus, these storage conditions resulted generally in a cheese VOC profile resembling more the initial one prior to storage.

The conducted PCA presented in Figure 1, resulted from multivariate statistical analyses of the VOC detected throughout the storage of cow's FC. Figure 1 shows the score plots of the different variables, with PC 1 and PC 2 accounting for 52.13% and 22.57% of total variability, respectively. As it can be seen, cheeses from storage at AP/RF, 75 and 100/RT at all storage periods are closer to the cheese prior storage (on the positive PC 1), while cheeses stored under 50/RT are more far apart as the storage period increased, with cheeses under AP/RT being more distant (negative PC 1) from the cheese prior to storage. In the loadings of the two principal components (*supplementary materialTable S4*), compounds more associated 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 spoilage, especially higher concentrations of FFA, esters and some alcohols.

In goat's FC initially a total of 24 compounds were detected (Table 4), most of the VOC belonged to FFA (n=6), followed by esters (n=5), alcohols (n=5), ketones (n=4), and aldehydes (n=1), resembling the ones reported byQuintanilla, Hettinga, Beltrán, Escriche, and Molina (2020).

Storage at AP/RT resulted in a higher VOC content in most major classes, with the exception for ketones and aldehydes that can be easily converted into acids or alcohols. This raise (p< 0.05)was almost up to 10-fold in alcohols, FFA and ethyl esters, resulting in a considerable increase in 3-methylbutan-1-ol, butane-2,3-diol, acetic, butanoic and octanoic acids, and in ethyl butanoate and hexanoate. Under AP/RF this evolution in cheese VOC profile was not so pronounced, despite the significant increases (p< 0.05)observed in acetic and nonanoic acids, ethyl esters remained within the values initially reported(p>0.05), however with a higher alcohol abundance, mainly from 3-methylbutan-1-ol (p< 0.05), while ketones (p< 0.05)and aldehydes concentration were reduced after 3 days.

Under HS conditions, 50/RT promoted significant changes in cheese VOC profile, with an accentuated formation(p< 0.05)of FFA, ethyl esters and alcohols, while ketones and aldehydes were undetected just after 7 days of storage. Prolonged storage at 50/RT resulted in a rise of all FFA, esters and alcohols, contributing to a distinguished VOC profile comparatively to cheeses prior to storage (p< 0.05). Contrarily, storage under 75 and 100/RT promoted a more stable VOC profile over storage, with a reduction in ketones content slower under these storage conditions, while aldehydes increased slightly only after 60 days under 100/RT (p< 0.05). A greater alcohol formation was observed in the first 14th days (p< 0.05), reaching values similar to the initial ones on the following storage periods, whereas FFA remained constant from the 7th day on forward, without considerable changes(p>0.05) beingdetected for esters over storage.

Changes in the VOC profile under the different storage conditions allowed the elaboration of a PCA considering the individual VOC, which could explain 71.02% of total variance (Figure 2), with 55.42% and 15.60% corresponding from PC 1 and PC 2, respectively, with ketone and aldehyde compounds scoring on the positive PC 1 (*supplementary material Table S5*) associated with unspoiled 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 materialTable S5*).

For both cheeses, storage under HS at 75-100/RT, allowed a more stable VOC profile throughout the storage, and resembling more the VOC profile of cheeses prior to storage, even after 60 days at RT, with a better maintenance of FFA, esters and alcohols over storage, when compared to the other storage conditions. It is worthy to note, that even only after 3 days at low temperature (AP/RF), cheeses stored under HS (75-100/RT) after 60 days presented overall a more resembling VOC profile comparatively to cheeses prior to storage.

4. Conclusions

Overall, HS under 75 and 100 MPa at RT allowed a much better preservation of both cow's and goat's FC, during a considerably longer storage period, when compared with RF. The results at these two pressures are very interesting, as throughout storage especially under 100/RT, a slower lipid oxidation rate and a more stable fatty acid profile and total protein was achieved after 60 days. Additionally, it was verified also an increased FAA abundance in HS cheeses, mainly for the longest storage periods, which should be further assessed to fully understand the real impact in the sensorial properties and consumers acceptability of these cheeses. These two storage conditions retained cheeses volatile organic profile similar to cheeses prior to storage, without noticeable formation of undesirable compounds associated with cheese spoilage, even after 60 days at uncontrolled variable RT, which was corroborated by the PCAs conducted for both cheeses.

In conclusion, a much longer shelf-life can be achieved under HS/RT (up to at least 60 days, the maximum period studied in this work), opening new business opportunities, as FC has only few weeks of shelf-life under RF and with additionally enhanced microbial safety due to considerable microbial inactivation (*unpublished data*). Although further technological development and research regarding HS implementation in the food sector is needed, HS could additionally allow significant energy savings throughout storage, being *quasi* energetically costless, due to needless constant energy supply to maintain temperature, thus being environmentally friendlier and more sustainable and suitable for example for longer transportation.

Declarations

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Authors' Contributions: Ricardo V. Duarte: conceptualization, investigation and writing- original draft preparation. Susana Casal: methodology and resources. Ana M. Gomes: supervision and resources. Ivonne Delgadillo: supervision and resources. Jorge A. Saraiva: supervision, resources, writing-reviewing and Editing.

Data Availability: The data presented in this study are available by request from the corresponding author.

Conflict of Interest: The authors have no conflict of interest to declare.

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Tables

Table 1 - Total protein (g/100g), 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 for each parameter.

Condition	**Initial**	**AP/RT**	**AP/RF**	**50MPa/RT**			**75MPa/RT**				**100MPa**
Days	0	3	3	3	14	28	3	14	28	60	3
Total Protein (g/100g)	15.11a	13.09a	1.46a	14.08a	14.47a	12.92a	14.57a	13.49a	15.39a	13.78a	15.32a
Digestibility (%)	81.2a	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*
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
Glycine	69.6a	*ND*	83.0a	116.9abc	154.4bcd	247.9e	106.7abc	156.9bcd	188.1de	172.1cd	89.0ab
Valine*	23.3a	43.0a	11.1a	35.2a	176.5b	486.6e	25.1a	111.6ab	310.5cd	784.0g	26.5a
3-Aminoisobutyric acid	18.8 ab	*ND*	*ND*	12.6 ab	39.0 abc	69.4 abcde	15.0 ab	56.4 abcd	116.6 def	142.7 f	12.0 a
Leucine*	85.6ab	303.8b	50.6a	66.4a	431.7bc	1333.1e	86.1ab	378.7abc	969.4d	1511.1e	145.8ab
Isoleucine*	4.8a	*ND*	*ND*	*ND*	33.7ab	96.2c	*ND*	31.7ab	104.0c	278.9e	*ND*
Threonine	5.0a	*ND*	*ND*	*ND*	30.2ab	136.6c	*ND*	*ND*	46.0abc	63.3abc	*ND*
Serine	*ND*	20.2 a	*ND*	*ND*	166.4 bcd	605.9 e	*ND*	39.1 ab	137.9 abc	182.1 bcd	*ND*
Proline	19.8a	69.7abc	*ND*	*ND*	41.8abc	76.5abc	*ND*	104.2bcde	166.3de	185.9e	*ND*
Asparagine	13.7ab	36.4abcd	11.7ab	21.6abc	26.4abcd	29.7abcd	10.2a	24.9abcd	48.1cd	57.3d	15.1ab
Aspartic acid	181.8 abc	56.8 ab	37.4 a	94.6 ab	350.9 abcde	415.7 cde	128.0 ab	363.0 bcde	568.0 e	471.9 cde	113.7 ab
Methionine*	10.9a	38.0a	14.1a	*ND*	42.7a	108.1b	19.6a	43.1a	72.7ab	51.1ab	19.9a
Hydroxyproline	12.3ab	24.1ab	9.0a	9.6ab	30.3abc	41.3abcd	11.2ab	80.4e	65.8cde	74.1de	14.6ab
Glutamic acid	292.3bcd	224.4ab	209.8abc	58.4a	173.6ab	496.6de	167.5ab	207.3ab	439.8cd	502.4de	255.8abc
Phenylalanine*	*ND*	19.7a	*ND*	*ND*	34.8a	495.7e	*ND*	60.0ab	219.8bc	269.9cd	*ND*
Glutamine	174.6 abcd	69.4 ab	58.3 a	74.5 ab	294.8 bde	286.6 bcde	117.7 abc	320.0 de	607.2 f	392.2 ef	101.6 ab
Ornithine	167.5b	54.3a	201.2b	84.9a	40.5a	75.6a	54.5a	53.2a	42.8a	72.8a	45.1a
Lysine*	14.1a	14.2a	15.1a	32.4ab	166.0bc	493.9e	30.7ab	155.8b	298.6cd	348.0de	33.6ab
Histidine*	5.7a	*ND*	*ND*	*ND*	94.8bc	272.2d	*ND*	57.1ab	144.7c	307.6d	10.1a
Tyrosine*	2.3a	2.0a	3.3a	4.4ab	6.6ab	4.7ab	2.9a	12.2ab	15.4ab	32.5cd	10.1ab
Tryptophan*	*ND*	33.4ab	4.0a	*ND*	65.6ab	309.3e	*ND*	43.4ab	109.2bc	240.2de	*ND*
Cystine	*ND*	*ND*	*ND*	*ND*	39.7a	168.4c	*ND*	11.3a	101.1ab	73.3a	*ND*
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

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

Table 2 - Total protein (g/100g), protein digestibility (%), free amino acids (nmol/g) of goat'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–i) indicate significant differences ($p < 0.05$) between the different storage conditions for each parameter.

Condition	Initial	AP/RT	AP/RF	50MPa/RT			75MPa/RT				100MPa/RT	
Days	0	3	3	3	14	28	3	14	28	60	3	14
Total Protein (g/100g)	16.99 abc	15.36 a	17.02 abc	18.84 c	18.33 bc	15.35 a	18.30 bc	16.43 ab	16.83 abc	15.96 a	18.19 bc	16.75 abc
Digestibility (%)	75.8a	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*	*NP*
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
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
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
3-Aminoisobutyric acid	5.6a	89.2b	*ND*	16.8a	*ND*	*ND*	9.3a	*ND*	82.0b	195.1c	21.4a	*ND*
Leucine*	12.6a	1572.3e	14.5a	332.1ab	3175.8f	4799.8g	50.6a	776.9bc	1308.7de	2839.9f	84.1a	455.4ab
Isoleucine*	*ND*	149.4bc	*ND*	28.4a	263.7d	398.0f	*ND*	86.2abc	118.8bc	355.4ef	*ND*	58.7ab
Threonine	*ND*	95.4b	*ND*	*ND*	10.7a	*ND*	*ND*	12.5a	5.6a	*ND*	*ND*	5.5a
Serine	*ND*	1644.7d	*ND*	*ND*	566.9c	462.6bc	*ND*	31.4a	93.4a	219.7ab	*ND*	16.1a
Proline	*ND*	1488.9c	*ND*	179.0a	547.9b	724.5b	*ND*	14.8a	29.7a	85.5a	*ND*	8.2a
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
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
Methionine*	6.0a	384.7c	*ND*	28.5ab	341.0c	389.6c	*ND*	35.6ab	52.0ab	84.5b	*ND*	24.1b
Glutamic acid	65.7a	1823.3b	*ND*	171.2a	2329.1bc	2889.7c	*ND*	86.2a	222.6a	489.7a	42.4a	109.5a
Phenylalanine*	*ND*	500.4d	*ND*	52.6a	831.4e	1108.0f	*ND*	60.5a	133.6ab	274.1c	*ND*	26.8a
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
Ornithine	83.6 bcd	178.3 e	101.3 cd	39.8 ab	58.5 abc	110.0 d	34.9 a	43.3 ab	36.7 ab	72.1 bcd	41.6 ab	50.5 ab
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
Histidine*	2.6a	292.7b	*ND*	8.9a	576.9c	529.8c	*ND*	75.8a	57.3a	93.1a	*ND*	89.5a
Tyrosine*	1.3a	324.4c	*ND*	11.0a	157.9b	71.3a	*ND*	22.9a	4.0a	3.0a	*ND*	5.3a
Tryptophan*	*ND*	367.0cd	*ND*	81.4ab	467.2d	340.9cd	*ND*	124.2ab	112.5ab	454.8d	*ND*	55.8a
Cystine	*ND*	*ND*	*ND*	*ND*	*ND*	127.3b	*ND*	27.5a	32.8a	73.4ab	*ND*	*ND*
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

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

Table 3 - Volatile organic compounds (µg internal standard equivalents /100g) of cow's FC prior storage (Initial) and stored 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.

Condition	Initial	AP/RT	AP/RF	50MPa/RT		75MPa/RT				100MPa/RT	
Days	0	3	3	7	28	7	14	28	60	7	14
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
acetic acid	*nd*	284.66d	4.69a	28.67b	137.33c	1.32a	0.69a	0.50a	1.19a	*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
hexanoic acid	28.90a	370.52c	56.60a	153.49b	494.08d	31.04a	86.10ab	56.84a	52.48a	62.86a	92.15ab
(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
octanoic acid	47.88ab	315.17e	65.27ab	132.93cd	357.54e	49.36ab	86.07abc	59.90ab	26.93a	60.71ab	82.21abc
nonanoic acid	*nd*	13.64c	*nd*	6.56ab	9.57bc	2.70ab	4.11ab	4.87ab	3.17ab	4.14ab	2.64a
decanoic acid	20.53a	96.63d	39.49abc	65.95bcd	102.98d	26.84ab	35.06abc	40.42abc	19.61a	36.65abc	51.14abc
dodecanoic acid	*nd*	6.54bcd	4.31ab	7.82cd	10.07d	3.33ab	4.71abc	3.56ab	2.40a	4.09ab	4.28ab
Esters	26.60a	339.46c	63.34a	137.31b	617.04d	23.85a	34.64a	32.07a	22.51a	34.84a	45.17a
ethyl acetate	4.84ab	58.49d	8.77b	21.45c	19.47c	5.78ab	3.91a	3.45a	3.78a	6.33ab	4.72ab
ethyl butanoate	9.97a	121.69c	17.56ab	32.71b	138.47c	7.21a	6.61a	6.18a	3.20a	11.32a	9.20a
ethyl hexanoate	11.68a	70.85b	16.62a	21.55a	119.34c	8.16a	8.46a	10.95a	5.86a	10.61a	15.08a
ethyl octanoate	*nd*	51.88c	14.17ab	37.44ab	128.71d	*nd*	8.58a	8.45a	9.80a	*nd*	10.03a
ethyl nonanoate	*nd*	*nd*	*nd*	*nd*	3.13	*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
ethyl dodecanoate	*nd*	1.90a	0.81a	1.52a	7.04b	*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*
pentan-2-ol	*nd*	*nd*	2.63	*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*
hexan-1-ol	*nd*	12.84d	1.42ab	3.93c	ND	1.04ab	ND	1.16ab	2.30b	ND	ND
cyclohexanol	*nd*	*nd*	4.19	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
heptan-2-ol	*nd*	*nd*	*nd*	*nd*	14.00	*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-methylbutanal	*nd*	12.16b	2.42a	8.95b	*nd*	*nd*	*0.83a*	*nd*	1.06a	*nd*	*nd*
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
3-methylbut-2-enal	3.01a	10.14b	3.13a	7.28ab	*nd*	*nd*	*nd*	*nd*	*nd*	2.84a	*nd*
hexanal	2.07a	*nd*	2.05a	*nd*	*nd*	1.91a	1.21a	1.39a	2.14a	2.24a	1.70a
heptanal	0.83a	14.20b	2.80a	*nd*	*nd*	*nd*	*nd*	*nd*	0.67a	*nd*	*nd*
nonanal	8.97	*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
pentan-2-one	4.11a	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	4.11a	6.45a
heptan-2-one	8.46a	9.51a	4.86a	7.07a	*nd*	7.46a	12.01ab	11.39ab	9.23a	11.57ab	18.40bc
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
Others	*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
toluene	3.88b	11.86c	2.60ab	2.75ab	*nd*	2.16ab	1.11a	1.84a	2.23ab	2.60ab	1.75a

nd – stands for not detected

Table 4 - Volatile organic compounds (µg internal standard equivalents /100g) of goat's FC prior storage (Initial) and stored 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.

Condition	Initial	AP/RT	AP/RF	50MPa/RT		75MPa/RT					1
Days	0	3	3	7	28	7	14	28	60	7	1
Free fatty acids	166.38a	3170.90c	1017.31ab	3282.07c	4239.54c	1007.95ab	1264.32b	1121.78ab	1286.54b	799.95ab	1
acetic acid	21.64a	422.79d	179.00bc	173.23c	319.21d	74.99abc	71.31abc	46.02abc	48.22ab	56.39abc	5
butanoic acid	14.34a	571.82e	107.32abc	344.72d	535.75e	94.99abc	124.43bc	96.47abc	181.96c	68.04ab	8
hexanoic acid	27.06a	1013.36c	223.33ab	1029.30c	1294.91c	254.32ab	380.46b	301.62ab	454.01b	193.04ab	2
octanoic acid	61.41a	736.95cd	254.26ab	1062.65de	1198.72e	340.61ab	389.41abc	307.57ab	372.90abc	284.01ab	4
nonanoic acid	8.81ab	10.24abc	26.39d	21.55cd	17.44bcd	8.39ab	6.69a	8.71ab	5.25a	3.91a	7
decanoic acid	44.19a	396.63b	168.37ab	710.73c	690.81c	233.13ab	298.83ab	359.02b	122.28ab	164.69ab	3
dodecanoic acid	2.71a	27.18c	7.07a	13.34ab	18.94bc	9.90ab	11.09ab	11.08ab	3.35a	7.77a	1
Esters	55.03a	156.58bc	46.71a	225.24c	563.92d	29.50a	119.02abc	110.92abc	101.84ab	24.83a	1
ethyl acetate	10.16ab	26.43c	15.21b	9.56ab	10.00ab	8.91ab	7.83ab	5.00a	3.40a	10.14ab	1
ethyl butanoate	5.52a	52.20d	12.02ab	27.71bc	75.04e	11.45ab	18.60abc	20.04abc	28.80c	11.50ab	1
ethyl hexanoate	8.98a	30.22a	7.12a	40.56a	138.36b	8.82a	22.03a	17.46a	20.94a	7.02a	1
ethyl octanoate	24.94a	41.05a	*nd*	40.56a	138.36b	*nd*	18.85a	13.83a	14.80a	*nd*	1
ethyl decanoate	3.55a	10.02abc	8.43ab	95.92d	172.44e	*nd*	51.71c	54.58cd	33.89abc	*nd*	5
ethyl dodecanoate	*nd*	*nd*	*nd*	*nd*	4.43	*nd*	*nd*	*nd*	*nd*	*nd*	*n*
Alcohols	93.97a	834.88f	364.48d	649.46e	768.53ef	350.06d	329.98cd	175.64ab	166.17a	343.01bcd	3
2-methylpropan-1-ol	11.05ab	39.41e	18.99bcd	29.16de	24.53cd	12.78ab	11.48ab	8.77a	8.95a	16.05abc	1
3-methylbutan-1-ol	44.09a	482.63c	197.42b	424.03c	555.42c	239.00b	245.46b	103.44ab	105.37ab	212.59b	2
2-methylbutan-1-ol	5.76a	40.86de	14.80ab	30.89cd	54.08e	23.28bc	24.69bc	17.54abc	17.84abc	23.09bc	2
butane-2,3-diol	29.87a	253.64b	79.79a	81.56a	80.82a	75.00a	48.35a	45.89a	34.01a	104.07a	7
cyclohexanol	1.73a	15.24b	14.01b	*nd*	18.69b	*nd*	*nd*	*nd*	*nd*	*nd*	*n*
heptan-2-ol	*nd*	4.66ab	3.66a	7.63ab	8.53b	*nd*	*nd*	*nd*	*nd*	*nd*	*n*
Ketones	98.39b	16.12a	27.54a	*nd*	*nd*	37.49a	15.67a	11.77a	19.17a	28.80a	2
butane-2,3-dione	42.55	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*n*
3-hydroxybutan-2-one	59.97c	16.12ab	23.71ab	*nd*	*nd*	37.49bc	4.90a	3.92a	7.25ab	28.80ab	1
heptan-2-one	7.99a	*nd*	*nd*	*nd*	*nd*	*nd*	10.92ab	7.85a	11.20ab	*nd*	1
nonan-2-one	5.00a	*nd*	*nd*	*nd*	*nd*	8.25abc	12.05bc	4.45a	5.47a	4.38a	7
Aldehydes											
3-methylbutanal	4.75a	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	3.87a	5.28ab	4.05a	3
Others	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*n*
heptane	20.51abc	38.16bcd	24.55abc	51.21de	5.40a	44.97cde	4.63a	17.93abc	14.70abc	62.12e	1
toluene	1.04a	5.85c	*nd*	5.20bc	5.26bc	2.83abc	1.91ab	3.82abc	1.42ab	3.05abc	4

nd – stands for not detected

Figures

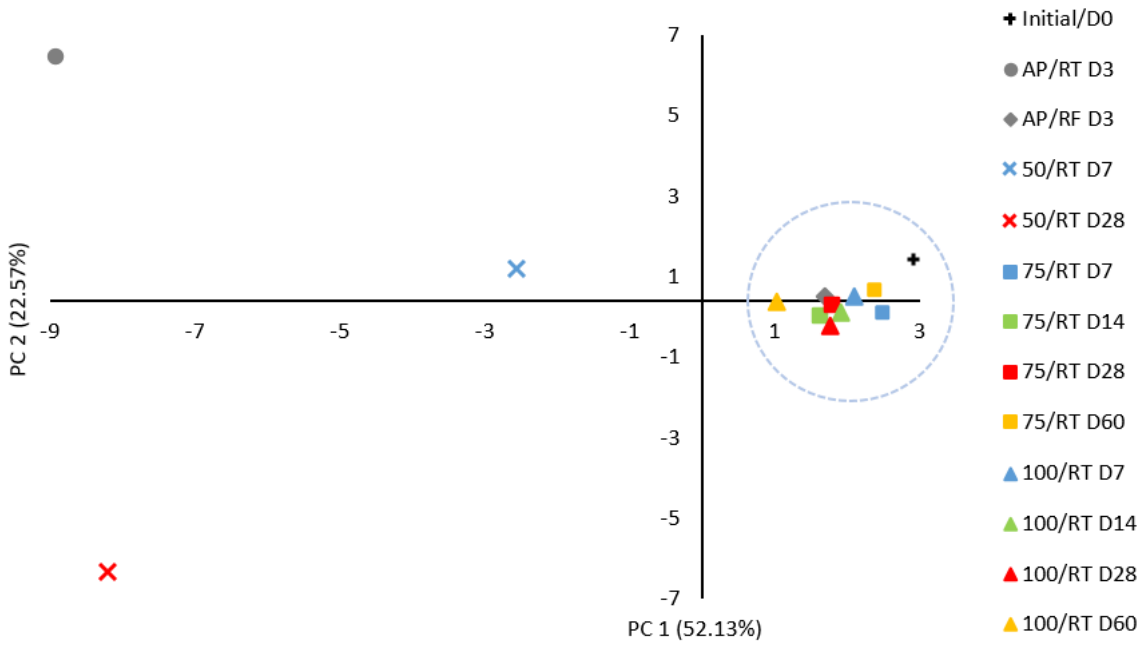

Figure 1

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

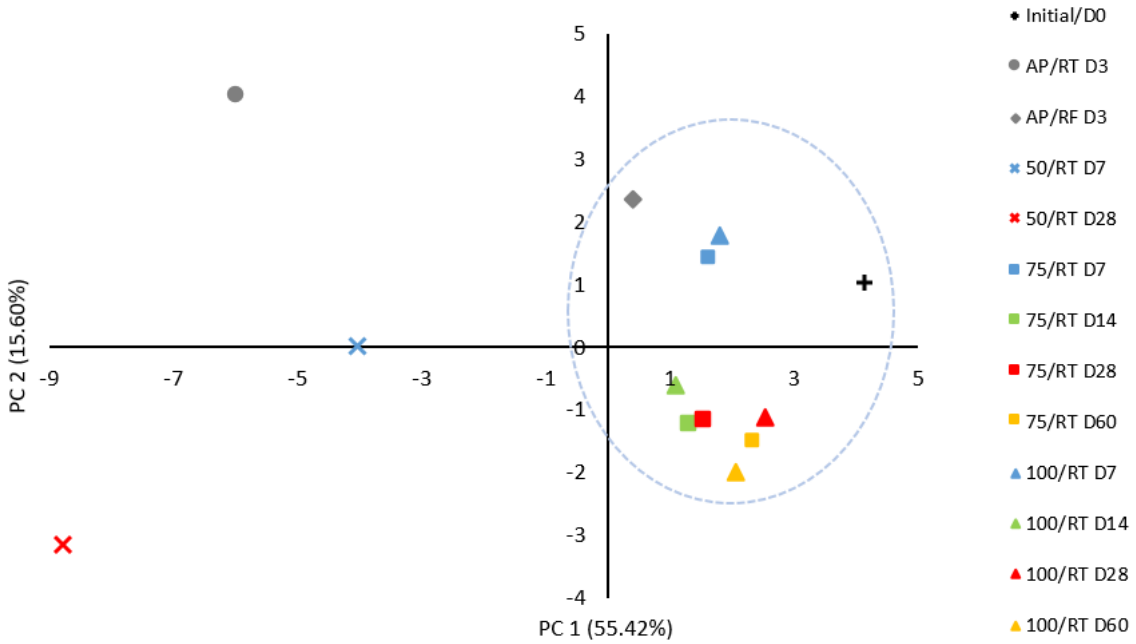

Figure 2

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

Supplementary Files

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- SupplementarymaterialRDetalHSFreshCheese.docx