Chemical and biochemical study of industrially produced San Simón da Costa smoked semi-hard cow's milk cheeses: Effects of storage under vacuum and different modified atmospheres

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ABSTRACT

Two batches of smoked, semi-hard (ripened for 45 d) San Simón da Costa cow's milk cheeses with Protected Designation of Origin were used to investigate the chemical, biochemical, and sensorial parameters that may be affected by modified-atmosphere packaging. Cheeses were packaged for 45 d as follows: vacuum packaging, packaging in 100% N₂, packaging in a gas mixture of 20% CO₂/80% N₂, and packaging in a gas mixture of 50% CO₂/50% N₂. The San Simón da Costa cheeses were characterized by high contents of lactic, oxalic, and citric organic acids. The main free amino acids found were isoleucine, phenylalanine, serine, valine, lysine, and glutamic acid, and the most abundant volatile compounds included ethanol, diacetyl, 2-butanol, isopropyl alcohol, furfural, acetaldehyde, 2-butanone, acetone, and 2-methylfuran. Modified atmospheres appeared to alter the ripening processes by affecting lipolysis, as indicated by the lower concentrations of butyric and propionic acids compared with control cheeses. In addition, modified-atmosphere packaging altered the proteolysis processes, yielding higher amounts of branched-chain alcohols. The results revealed that storage under modified atmosphere contributes to the accumulation of several compounds probably derived from smoke, including aldehydes such as 2-furancarboxaldehyde (furfural), alcohols such as 2-methoxyphenol (guaiacol), ketones such as 2-cyclopenten-1-one, and esters such as methyl furancarboxylate, which were negatively correlated with flavor. Vacuum packaging was the most useful technique in terms of preserving the sensory quality of San Simón da Costa Protected Designation of Origin cheeses. Considering the current demands for packaged portions of food at the distribution and retail levels and the potential health risks associated with some smoke-derived compounds usu-

ally present in some smoked foods, the results obtained in this study may be of special interest to the cheese industry.

Key words: smoked cheese, modified-atmosphere packaging, chemical and biochemical compounds, sensory analysis

INTRODUCTION

Modified-atmosphere packaging (MAP) has generated great interest in the manufacture of food products as a means of preserving high-quality fresh products, improving the image of the product, extending the commercial shelf-life, and minimizing the use of additives and preservatives. Moreover, packaging in plastic pouches prevents dehydration and consequent weight loss, as well as absorption of undesirable odors from the environment (for reviews see Robertson, 1993; Hotchkiss et al., 2006). Pouches are usually filled with atmospheres low in oxygen and high in carbon dioxide and nitrogen, which have been shown to slow down respiration rate, reduce microbial growth, and retard enzymatic changes (Hotchkiss et al., 2006). Carbon dioxide has bacteriostatic and fungistatic effects, whereas nitrogen acts principally as filler, preventing package collapse (Robertson, 1993). Modified-atmosphere packaging is also expected to affect beneficial microorganisms involved in sensory attributes of foods during storage. It is obvious that many interactions between microorganisms occur in cheese in relation to flavor formation; however, our knowledge of this area is limited because of the complexity of the cheese microbial ecosystem.

Some studies of the effect of MAP on the shelf-life and quality of hard and semi-hard cheeses as well as fresh cheeses have been carried out (e.g., Robertson, 1993; Eliot et al., 1998; González-Fandos et al., 2000; Kristensen et al., 2000; Juric et al., 2003; Hotchkiss et al., 2006), and MAP is accepted worldwide for use with cheeses. Nevertheless, it has been reported that the use of MAP with high percentages of CO₂ may have a

Received September 18, 2009.

Accepted January 12, 2010.

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detrimental effect on the sensory properties, especially flavor, of semi-hard or hard cheeses such as portioned Parmigiano-Reggiano cheese (Romani et al., 2002), sliced Samsø cheese (Juric et al., 2003), and grated Gravier cheese (Trobetas et al., 2008). Gas mixtures with high percentages of N₂ are considered most suitable for preserving the sensory attributes of these types of cheeses.

Sensory characteristics are very important in cheese analysis, especially with respect to quality control (Bertozzi and Panari, 1993), but become of major importance when dealing with cheeses with Protected Designation of Origin (PDO) status. San Simón da Costa (SSC) is a semi-hard cow's milk cheese produced in Galicia (northwest Spain) and which has PDO status. Milk used for cheese manufacture is curdled with calf rennet at 31 to 33°C for 30 to 40 min. The curd is cut into 5- to 12-mm long pieces and washed with pasteurized water at 33 to 35°C. The cheeses are molded, pressed at approximately 2 to 3 kg/cm² for at least 3 h, and then salted in brine (14 to 17% NaCl) for at least 24 h. The cheeses are then ripened for at least 45 d at 6 ± 2°C, and finally smoked just before delivery to market. Industrial cheeses are made from pasteurized milk, and the smoking operation is carried out, as in traditional raw-milk cheese making, with barkless birch wood (*Betula celtiberica*) for 2 to 7 h. The cheese has a characteristic shape intermediate between pear-shaped and bullet-shaped, terminating at the top in a point, and typically ranges in weight between 0.8 and 1.5 kg. The outer layer of the cheese, 1 to 3 mm deep, is a yellow-ochre color on the surface and somewhat oily. The edible part of the cheese is of fine texture, fatty, semi-hard, semi-elastic, and dense, between white and yellow in color, cuts easily, and has a characteristic smoky aroma and flavor. Eyes should not be present in the mass or must be in low number. Gross composition and pH must be as follows: ≥55% DM; 45 to 60% fat in DM; pH between 5.0 and 5.6 (MAPA, 2005).

Because of its moderate (≤45%) moisture content and the smoking procedures after ripening, SSC cheese is expected to be microbiologically safe and is normally sold unpackaged. Nevertheless, because of ongoing international market expansion, there is now a demand for packaging at distribution and selling points. Sales of SSC cheese have increased by 60% in the last 7 yr, with an annual production of 349,000 kg during 2008 (about €3 million in global value of sales at selling points). There is an increasing demand for portioned SSC cheeses (half portions or wedges), but no studies have been carried out with respect to the sensory features of these portions or of their shelf-life at the distribution and retail levels.

There is little scientific information about SSC cheese, particularly with respect to the microbial flora in raw milk cheeses (García-Fontán et al., 2001; Garabal et al., 2008; Rodríguez-Alonso et al., 2008). Some chemical and biochemical characteristics of industrial SSC cheeses have been reported recently (Nhuch et al., 2008a,b) but no information was provided about the sensorial quality or smoking operations. Moreover, it is unknown if MAP can preserve the typical sensory quality of this traditional PDO cheese. Thus, the aims of the present work were 1) to characterize industrial SSC cheeses from a chemical and biochemical point of view, including analysis of organic acids, free amino acids and volatile compounds, and 2) to evaluate the effects of packaging of portioned SSC cheese under different types of atmosphere, on the chemical and biochemical parameters, and on the sensory quality of the cheeses.

MATERIALS AND METHODS

Collection, Packaging, Storage, and Sampling of the Cheeses

Two batches of 72 SSC industrial (pasteurized milk) PDO certified cheeses (1,000 ± 50 g in weight), each one produced from the same vat of milk, were provided by a local dairy plant known to manufacture premium-grade products. Cheeses were purchased immediately after completion of the 45-d ripening period and subsequent smoking.

Fifty cheeses from each batch were cut into 4 wedges of approximately equal weight (225 to 275 g) to provide a total of 200 wedges. The wedges were packaged in plastic pouches composed of multilayer polyester-polyvinyl chloride (13 μm thick) and polyethylene (80 μm thick) (Polyskin X, Amcor Flexibles Hispania S.L., Barcelona, Spain), with an oxygen permeability of <9 cm³/m² per day at 0% relative humidity (RH) and 23°C, and a water vapor permeability of <4 g/m² per day at 90% RH and 38°C.

The pouches were evacuated, flushed, and then double heat-sealed by using an EV-15-1-CD packaging machine (Technotrip S.A., Terrasa, Spain) with gas injection. The 4 different atmosphere treatments included 1) vacuum 97% (vol/vol) packaged (VP) cheese wedges; 2) cheese wedges packaged in 100% (vol/vol) N₂ (NP); 3) cheese wedges packaged in 20% (vol/vol) CO₂/80% (vol/vol) N₂ (CN1P); and 4) cheese wedges packaged in 50% CO₂/50% N₂ (CN2P). Each treatment was applied to 50 samples. Nitrogen and CO₂/N₂ gas mixtures were supplied by Carbueros Metálicos S.A. (Barcelona, Spain).

Of the remaining 22 whole cheeses from each batch, 3 were sampled for physicochemical, microbiological,

and biochemical analyses before storage (time 0), and 4 were set aside for sensory analysis at time 0. The other 15 cheeses served as controls, and were stored aerobically in a dark cold-storage room for 45 d, together with the 200 packaged cheese wedges. Storage conditions were $85 \pm 3\%$ RH and $6 \pm 2^\circ\text{C}$, simulating retail conditions.

The aerobically stored whole cheeses (control cheeses), and the packaged cheese wedges (VP, NP, CN1P, and CN2P cheeses) were analyzed after 45 d of storage (time 45). Before physicochemical, microbiological, and sensory analyses, cheeses were equilibrated at room temperature ($20 \pm 2^\circ\text{C}$). Physicochemical and microbiological analyses were carried out on refrigerated ($4 \pm 1^\circ\text{C}$) samples within 24 h. Biochemical analyses were carried out on frozen samples ($-30 \pm 1^\circ\text{C}$) wrapped in aluminum foil. The outside layer of 0.5 to 1 cm in depth (including the cheese rind) was removed before sampling for physicochemical, microbiological, and biochemical testing.

The data reported in this paper represent means of values from 2 replicated experiments (2 batches) in which all analyses were performed on triplicate samples, except sensory analyses, which were carried out in duplicate.

Physicochemical and Microbiological Analysis

The SSC cheeses were analyzed for moisture content [International Dairy Federation (IDF) Standard 4A; IDF, 1982], fat content (IDF Standard 152A; IDF 1997a), and protein content (IDF Standard 20A; IDF, 1986) before storage (time 0). After 45 d of storage (time 45), control cheeses and packaged cheeses were analyzed for moisture content.

The pH of the cheese was measured with a digital pH meter (model 507, Crison, Barcelona, Spain) equipped with a probe for solids. Titratable acidity was determined by the method proposed by the AOAC (AOAC, 1990).

Mesophilic microorganisms were enumerated on plate count milk agar (Oxoid, Basingstoke, UK) following the pour-plate method and incubated at $30 \pm 1^\circ\text{C}$ for 3 d. Yeasts and molds were enumerated on rose bengal chloramphenicol agar (Merck, Darmstadt, Germany) at $25 \pm 1^\circ\text{C}$ for 5 d. Sample preparation was carried out in accordance with IDF Standard 122C (IDF, 1996), although the first dilution was 1:5 (wt/vol; 10 g of cheese + 40 mL of 2% sodium citrate).

Gas Composition of Package Headspace

The concentrations of O_2 and CO_2 were measured before physicochemical and microbiological analysis

to verify packaging atmospheres for each treatment, with a Gas Data portable dual analyzer (model PCO2 Plus/100, Gas Data Ltd., Coventry, UK). A rubber septum (Systech Instr. Ltd., Thame, UK) was glued onto the surface of each pouch and pierced with a 23-gauge needle connected to the headspace analyzer. The headspace analyzer gave a direct reading of the percentage of O_2 and CO_2 in the pouch headspace. Gas concentration measurements were taken at the beginning and end of the packaging runs and averaged for each type of gas; packages were then randomized with respect to storage treatments.

Determination of Organic Acids

The organic acids (OA) acetic, butyric, citric, formic, lactic, orotic, oxalic, pyruvic, propionic, succinic, and uric acids, were determined by reversed phase-HPLC following the method proposed by Tormo and Izco (2004). Analysis was carried out on a HPLC Breeze System (Waters, Milford, MA), consisting of a 1525 binary HPLC pump, a 717 plus autosampler, and a 2487 2-channel UV detector set at 210 nm, operated with Breeze software. The separation was performed on an Atlantis dC18 column (Waters, 250 mm $\times$ 4.6 mm, 5- μm particle size). Results were expressed as milligrams of OA per kilogram of cheese.

Determination of Free Amino Acids

Sample extracts obtained previously for OA were also used to study the contents of ammonium and the free amino acids (FAA) Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Tyr, Thr, and Val. Separations were carried out on a AccQ-Tag column (3.9 mm $\times$ 150 mm, particle size 4 μm ; Waters) operated at a flow rate of 1.0 mL/min, and at 37°C . The gradient profile and composition of the mobile phase was adapted from the methodology developed by Van Wandelen and Cohen (1997), and according to the AccQ-Tag method instructions (Waters). Detection was accomplished by fluorescence with excitation at 250 nm and emission at 395 nm. Amino acids were identified by retention time with an amino acid standard. Results were expressed as milligrams of FAA per kilogram of cheese.

Determination of Volatile Compounds

Volatile compounds were determined by GC-MS, according to the method described by Rodríguez-Alonso et al. (2009) with some modifications. The analysis was carried out on an automatic dynamic headspace apparatus (Tekmar Dohrmann 3100 Sample Concentrator, Tekmar, Cincinnati, OH) connected to a GC-MS (Agi-

lent 6890N Network GC System and Agilent 5953 Mass Selective Detector; Agilent Technologies, Santa Rosa, CA). Operating conditions on a DB-Wax column (60 m length $\times$ 0.32 mm i.d.; 0.25- μ m film thickness) were as follows: carrier gas (helium; Carbueros Metálicos) at 1 mL/min, initial temperature 32°C for 7 min, 6°C/min up to 180°C, 10°C/min up to 225°C, and maintained for 2 min. The mass detector was run in scanning mode from 19 to 250 amu, with the ion source at 230°C and ionization voltage at 70 eV. Data were obtained by use of MSD Productivity Chemstation Software (Agilent). Identification of hexane, heptane, dimethyl disulfide, benzaldehyde, butanal, 3-methyl-1-butanol, acetone, 2,3-butanedione, ethanol, 3-methyl-1-butanol, 2-butanol, 1-hexanol, isopropyl alcohol, acetic acid, butyric acid, 3-methyl butyric acid, ethyl esters of linear fatty acids C3-C12, and the reported smoke derived compounds 2-butoxyethyl acetate, 2-furancarboxaldehyde, 2-methylfuran, 2-methoxyphenol, phenol, ethylbenzene, 1,4-dimethylbenzene, and trichloromethane was based on their retention times and on the relative abundances of ions selected for their identification, verified with authentic standard compounds. The other volatile compounds reported in this study were tentatively identified by comparison of their spectra with those contained in the NIST98 (National Institute of Standards and Technology, Gaithersburg, MD) and Wiley 275 (Wiley and Sons Inc., Weinheim, Germany) libraries. Relative abundances of compounds were expressed as percentages of the peak areas in relation to the borneol (internal standard) peak area. Only volatile compounds showing the highest mean relative abundances (>1.0) were considered for analysis. Nonaromatic, nonalicyclic, and nonheterocyclic hydrocarbons were further excluded for MAP analysis because of their relatively high odor threshold values.

Chemicals

The analytical standards used in this study were obtained from Sigma-Aldrich (St. Louis, MO), and gradient HPLC-grade acetonitrile from Scharlab Science (Barcelona, Spain). Milli-Q water (Millipore Co., Bedford, MA) was used to prepare buffers and the stock solutions of each standard compound and samples.

Sensory Evaluation

Sensory evaluation was performed at the sensory room of the Lamas de Abade Catering College (Santiago de Compostela, Spain) by a panel of 10 members responsible for judging the sensory quality of Galician industrial cheeses with PDO status. The sensory judges were formally trained assessors (ISO, 1994; IDF,

1997b), with experience (5 to 10 yr of sensory evaluation practice) in evaluating the product.

Cheeses were transported in portable refrigerators ($4 \pm 1^\circ\text{C}$) and were equilibrated at room temperature ($20 \pm 2^\circ\text{C}$) for 2 h before the sensory analysis sessions. Sensory analyses were carried out at time 0 (cheeses before storage) and time 45 (control and packaged cheeses). Cheeses at time 0 and control cheeses at time 45 were cut into 4 wedges of approximately equal weight and placed in plastic pouches (Polyskin X), which were sealed before being transported. All cheeses were transported in portable refrigerators ($4 \pm 1^\circ\text{C}$) and were equilibrated at room temperature ($20 \pm 2^\circ\text{C}$) for 2 h before the sensory analysis sessions. The pouches containing the packaged (treated) cheeses were opened, and cheeses were immediately placed in sealed plastic pouches just before being given to the panelists for evaluation. All cheese samples (225 to 275 g) were coded with randomly chosen 3-digit numbers and presented in random order to panelists in individual booths with white fluorescent light (IDF, 1997b). The panelists opened the pouches containing each cheese sample, and used unsalted crackers and water to cleanse their palates between samples. All the cheeses were analyzed in duplicate in 2 different sessions.

The judges evaluated the following attributes: appearance (color of the rind and of the cheese mass, cut appearance, and possible presence of eyes), odor (evaluated on the rind and on the cheese mass), texture (evaluated by finger pressing and by chewing the cheese mass in the mouth), acid taste, and flavor (evaluated by chewing and salivating the cheese mass in the mouth). Each attribute was scored on a scale of 1 to 7, where a score of 1 corresponded to those characteristics completely opposing the standards of the industrial PDO cheese, and a score of 7 corresponded to those characteristics that are wholly consistent with the standards of the industrial PDO cheese. The results were expressed as a mean score by the whole panel for each attribute and cheese.

An overall score was obtained by adding the weighted scores for appearance, texture, and flavor attributes (IDF, 1997b), by multiplying the scores by 1, 3, and 6, respectively. Thus, an excellent industrial PDO cheese would receive a total score of 70. It was also arbitrarily considered that cheeses with an overall score below 40 should be rejected. The sensory cards included an "Observations" section, in which the judges were asked to note all the descriptors they considered useful for defining the cheese attributes.

Statistical Analysis

Data corresponding to samples analyzed at time 45 were subjected to ANOVA and where statistical differ-

Table 1. Physicochemical characteristics (pH, acidity, and gross composition), organic acids, and free amino acids in San Simón da Costa cheeses ripened for 45 d (prestorage)

Item	Mean $\pm$ SE
Physicochemical parameters	
pH	5.25 $\pm$ 0.172
Acidity (% lactic acid)	0.021 $\pm$ 0.003
DM (% wt/wt)	56.7 $\pm$ 0.63
Fat/DM (% wt/wt)	55.5 $\pm$ 2.40
Protein/DM (% wt/wt)	38.6 $\pm$ 1.72
Organic acids (mg/kg)	
Lactic	6,858.7 $\pm$ 721.25
Oxalic	1,430.9 $\pm$ 250.84
Citric	703.6 $\pm$ 173.62
Acetic	512.3 $\pm$ 199.76
Formic	429.0 $\pm$ 24.19
Butyric	410.0 $\pm$ 123.32
Succinic	288.8 $\pm$ 57.89
Propionic	341.5 $\pm$ 13.86
Pyruvic	187.6 $\pm$ 45.36
Uric	28.2 $\pm$ 2.69
Orotic	9.6 $\pm$ 1.42
Total organic acids	11,200.1 $\pm$ 830.17
Free AA (mg/kg)	
Ile	1,268.4 $\pm$ 193.57
Ser	653.1 $\pm$ 69.99
Phe	637.0 $\pm$ 93.82
Val	478.1 $\pm$ 59.67
Lis	469.5 $\pm$ 57.97
Glu	452.7 $\pm$ 217.86
His	415.4 $\pm$ 51.98
Pro	399.7 $\pm$ 105.63
Arg	244.3 $\pm$ 59.11
Tyr	211.0 $\pm$ 99.17
Met	201.2 $\pm$ 17.84
Gly	196.8 $\pm$ 26.93
Asp	188.4 $\pm$ 34.72
Leu	173.4 $\pm$ 27.54
Ala	170.6 $\pm$ 28.86
Thr	136.7 $\pm$ 50.73
Ammonium	573.1 $\pm$ 122.68
Total free AA ¹	6,296.4 $\pm$ 846.19

¹Total sum of free AA without ammonium.

ences were noted, differences among the distinct groups (control and packaged cheeses groups) were determined by the Duncan's test. Differences were considered significant at $P < 0.05$. Correlation analysis for flavor and chemical/biochemical variables that differed among the groups was performed by use of a bivariate (2-tailed significance) correlation test. Pearson coefficients were calculated, and differences were considered significant at the $P < 0.05$ and $P < 0.01$ levels. The selected traits that correlated with flavor were subjected to principal components analysis (Varimax rotation, Kaiser normalization; data not shown), and hierarchical cluster analysis (Ward's method, squared Euclidean distance) was then performed on the principal components analysis values to group the samples. The cluster solution was confirmed by calculation of a Wilks' lambda value. All statistical procedures were performed with the SPSS Win 15.0 program (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Physicochemical and Microbiological Analyses and Headspace Gas Composition

Gross composition (DM and fat/DM) of SSC cheeses at time 0 (ripened for 45 d but prestorage) is shown in Table 1. The data fulfilled the requirements of the PDO regulations.

As expected, aerobic storage of control cheeses for 45 d led to loss of weight (estimated mean value of weight loss was 8.1%) and the moisture content decreased to 39.8% (Table 2). The mean moisture content of packaged cheeses was higher than that of control cheeses, although differences were only significant ($P < 0.05$) for VP cheese (Table 2). This is consistent with the findings of other authors who have reported that different gas mixtures did not significantly influence the moisture contents or the weight losses in hard or semi-hard cheeses packaged under modified atmospheres (Romani et al., 2002; Juric et al., 2003; Trobetas et al., 2008).

The mean pH of the SSC cheeses at time 0 was 5.25 and the mean titratable acidity was 0.021% lactic acid (Table 1), although higher pH values were observed for all cheeses groups after storage for 45 d (Table 2). This finding may be attributed to proteolysis and associated formation of amines and ammonium. There was no clear correlation between pH and titratable acidity; the lowest pH ($P < 0.05$) (mean value of 5.32) corresponded to the control cheeses and the highest mean acidity (0.027% lactic acid) corresponded to the CN2P cheeses. The presence of CO₂ in the headspaces of the CO₂-treated cheeses was expected to cause a decrease in pH and an increase in acidity because of its dissolution in cheese and the formation of carbonic acid (González-Fandos et al., 2000). Nevertheless, CO₂ is mainly absorbed on the surface of food, which may lead to acidification of some spots on the surface rather than in the total mass of the sample (Dermiki et al., 2008).

High total counts of mesophilic microorganisms were obtained at time 0 (7.6×10^8 cfu/g) with a slight decreasing trend, particularly in the CN2P atmosphere (1.0×10^8 cfu/g) after 45 d of storage (data not shown). On the other hand, yeasts and molds were only detected in very low numbers (mean counts < 50 cfu/g; data not shown) on some plates of control and NP cheeses. These findings are consistent with the findings of several authors who have reported that MAP with high CO₂ concentrations are very effective for inhibiting bacteria, molds, and yeasts (Eliot et al., 1998; Hotchkiss et al., 2006; Dermiki et al., 2008).

Comparison of the CO₂ observed in the pouches before and after storage showed that the mean percentage

Table 2. Physicochemical and biochemical (organic acids and free AA) characteristics showing significant differences between unpackaged control and packaged San Simón da Costa cheeses after 45 d of storage (means $\pm$ SE)

Item	Unpackaged control cheeses	Packaged cheeses ¹			
		VP	NP	CN1P	CN2P
Physicochemical characteristics					
pH	5.32 ^b ± 0.081	5.47 ^a ± 0.115	5.45 ^a ± 0.080	5.46 ^a ± 0.125	5.40 ^{ab} ± 0.113
Acidity (% lactic acid)	0.025 ^{ab} ± 0.004	0.026 ^{ab} ± 0.005	0.022 ^b ± 0.004	0.023 ^{ab} ± 0.003	0.027 ^a ± 0.001
Moisture (% wt/wt)	39.8 ^b ± 0.96	41.9 ^a ± 1.25	41.6 ^{ab} ± 1.58	41.4 ^{ab} ± 1.55	41.2 ^{ab} ± 1.53
Estimated weight loss (%)	8.1	3.2	4.0	4.5	4.9
CO ₂ in the atmosphere (%)	NT ²	NT	13.8 ^c ± 2.32	28.3 ^b ± 1.34	39.3 ^a ± 2.62
Organic acids (mg/kg)					
Lactic acid	6,469.7 ^a ± 310.99	6,234.1 ^{ab} ± 634.53	5,334.9 ^c ± 671.04	5,532.0 ^{bc} ± 749.30	5,484.9 ^c ± 624.08
Oxalic acid	1,515.4 ^c ± 150.56	2,446.0 ^a ± 225.78	2,022.6 ^b ± 455.13	1,543.0 ^c ± 249.21	2,236.0 ^{ab} ± 112.56
Butyric acid	979.9 ^a ± 220.03	839.7 ^{ab} ± 248.53	653.4 ^b ± 201.64	705.0 ^b ± 336.37	831.5 ^{ab} ± 232.08
Propionic acid	985.1 ^a ± 92.20	538.7 ^{bc} ± 174.49	474.5 ^d ± 150.66	664.0 ^b ± 212.64	529.7 ^{bc} ± 66.09
Citric acid	802.6 ^{ab} ± 78.78	870.7 ^a ± 165.86	631.5 ^c ± 160.57	666.9 ^{bc} ± 108.41	848.2 ^a ± 177.58
Formic acid	435.3 ^a ± 8.20	348.1 ^b ± 32.00	320.4 ^b ± 17.86	348.8 ^b ± 22.15	325.8 ^b ± 40.05
Succinic acid	260.2 ^c ± 22.06	339.9 ^b ± 68.27	312.1 ^{bc} ± 58.31	254.9 ^c ± 112.40	426.3 ^a ± 58.46
Pyruvic acid	76.9 ^c ± 12.73	244.4 ^a ± 47.33	160.2 ^b ± 54.40	133.3 ^b ± 32.20	248.3 ^a ± 43.86
Total organic acids	12,013.4 ^a ± 707.94	12,495.1 ^a ± 1,025.75	10,497.5 ^b ± 1,554.93	10,430.2 ^b ± 1,450.09	11,347.2 ^{ab} ± 831.28
Free AA (mg/kg)					
Phe	1,237.5 ^a ± 44.06	1,123.4 ^{ab} ± 63.55	938.1 ^b ± 92.87	1,039.4 ^{ab} ± 44.10	926.8 ^b ± 118.0
Ser	1,181.5 ^a ± 37.48	1,034.7 ^{ab} ± 117.07	885.8 ^b ± 112.75	1,011.7 ^{ab} ± 71.53	913.2 ^b ± 51.66
Gly	362.0 ^a ± 19.74	328.4 ^{ab} ± 23.23	299.0 ^{bc} ± 16.92	345.1 ^a ± 14.64	273.9 ^c ± 7.75
Thr	47.4 ^a ± 9.33	0.0 ^b ± 0.00	0.0 ^b ± 0.00	0.0 ^b ± 0.00	0.0 ^b ± 0.00
Ammonium	1,064.3 ^a ± 17.14	1,051.2 ^a ± 89.20	873.1 ^b ± 24.70	1,020.0 ^{ab} ± 86.55	929.1 ^{ab} ± 65.70
Total free AA ³	11,013.7 ± 289.13	10,671.8 ± 1,089.53	9,856.6 ± 927.41	10,022.8 ± 964.75	9,482.2 ± 818.11

^{a-c}Means within a row with different superscripts differ significantly ($P < 0.05$).

¹Vacuum packaged cheeses (VP); cheeses packaged under 100% N₂ (NP); cheeses packaged under 20% CO₂/80% N₂ (CN1P); and cheeses packaged under 50% CO₂/50% N₂ (CN2P).

²Not tested.

³Total free AA without ammonium.

of this gas decreased (-10.7%) in the headspaces of the CN2P cheeses, whereas it increased slightly in those of the CN1P and NP cheeses ($+8.3\%$ and $+13.8\%$, respectively; Table 2). The low concentrations of CO₂ detected in the CN2P headspaces may be attributed to dissolution of CO₂ in the cheese matrix, to CO₂ consumption by anaerobic microorganisms, or to a loss of CO₂ through the barrier film (Eliot et al., 1998; Juric et al., 2003; Trobetas et al., 2008). On the contrary, most increases in CO₂ concentrations in the NP and the CN1P headspaces may be caused by Strecker degradation and reduction of sugars, although microbial metabolism plus some oxidative and enzymatic reactions involving oxygen might be involved (Eliot et al., 1998; Trobetas et al., 2008). Increases in CO₂ have also been reported during storage of semi-hard and hard cheeses packed under modified atmospheres (Kristensen et al., 2000; Trobetas et al., 2008). There were no significant differences in O₂ content among the packaged cheeses, and the mean values were between 0.17% and 0.2% (data not shown). These concentrations are similar to those reported for sliced semi-hard Samsø cheese (0.000 to 0.130%; Juric et al., 2003) and sliced Havarti cheese (mean value of 0.023%; Kristensen et al., 2000) stored in 20 to 100% CO₂ atmospheres for 21 d.

Organic Acids

The initial step in cheese ripening is the conversion of lactose to lactate, which is achieved by lactic acid bacteria (**LAB**); however, other OA may appear in cheese as products of enzyme-mediated hydrolysis of milk fat because of growth and metabolism of microorganisms. Before packaging (time 0), the most abundant OA in the cheeses were lactic acid (about 62% of total OA), oxalic acid (about 13% of total OA), and citric acid (about 6% of total OA), with uric acid and orotic acid detected at very low levels (Table 1).

Lactic acid, a major product of the fermentation of lactose by LAB, reaches levels between 1,940 and 17,400 mg/kg in dairy products (Bevilacqua and Califano, 1989). Residual lactose not fermented during the earlier stages of ripening eventually gets used by non-starter lactic acid bacteria (**NSLAB**), leading to the formation of D-lactate as a major end-product (Turner and Thomas, 1980). The unexpected extremely high concentrations of oxalic acid found in the SSC cheeses may be related to the pasture on which the cows were feeding (Franceschi and Nakata, 2005). The low contents of orotic acid may be caused by the intense washing of the curd carried out during cheese making (Larson and

Hegarty, 1979) or utilization by microorganisms as a growth factor during ripening (González de Llano et al., 1996).

Organic acid contents have been considered indicators of microbial metabolism, and the associated profile is useful in terms of identifying the ripening stage (Akalin et al., 2002). Concentrations of OA (means and SD) for which significant differences were found among the different groups of cheeses after 45 d of storage are shown in Table 2. Total contents of OA at 45 d were higher ($P < 0.05$) in control and VP cheeses (Table 2). In general, the main types of OA detected after the different treatments were the same as those detected at the beginning of the study (lactic, oxalic, and citric acids), although butyric and propionic acids were also present in high amounts after storage (Table 2). On the contrary, uric acid (16.9 to 24.4 mg/kg; data not shown) and orotic acid (4.8 to 7.5 mg/kg; data not shown) were detected at the lowest levels and the contents were lower than those detected at time 0.

Butyric acid is probably released from triglycerides through the action of nonspecific esterases and lipases of starter lactococci and secondary microflora (González de Llano et al., 1996). Moreover, butyric acid may be produced from fats or be the result of proteolysis and subsequent deamination of casein (Califano and Bevilacqua, 2000) and may be transformed on demethylation to propionic and formic acids. As in the present study, increases in butyric acid during ripening of SSC pasteurized cheese have previously been reported (Nhuch et al., 2008b). Moreover, increases in butyric and propionic acids have been reported during ripening of other cow's milk cheeses (Bevilacqua and Califano, 1989; Akalin et al., 2002; Lues and Bekker, 2002; Upreti et al., 2006; Nhuch et al., 2008a). Lower concentrations of butyric, propionic, and formic acids in MAP cheeses compared with SSC control cheeses suggest a reduction in the initial formation of butyric acid in the packaged cheeses. This reduction may be related to a decrease in the degree of lipolysis, as noted in other studies on MAP cheeses (Robertson, 1993; Dermiki et al., 2008).

Citric acid was detected in higher amounts ($P < 0.05$) in the VP and CN2P cheeses than in the NP and CN1P cheeses. Moreover, oxalic and pyruvic acids were also detected in significantly higher amounts in the VP cheeses (Table 2). Citric and pyruvic acids are intermediary metabolites and generally show irregular changes during cheese ripening (Akalin et al., 2002; Lues and Bekker, 2002; Upreti et al., 2006). Moreover, citrate can be used as a substrate by the starter lactococci to produce pyruvic and acetic acids (Adda et al., 1982). Pyruvate may also be produced by mesophilic LAB through the glycolytic pathway, and may act as a substrate of several metabolic reactions with the forma-

tion of formic acid, ethanol, and carbonyl compounds such as diacetyl and acetoin (Adda et al., 1982). On the other hand, LAB can fix CO₂ using pyruvate carboxylase and convert pyruvate to oxaloacetate, which may be further converted to citrate through the action of citrate synthase (Upreti et al., 2006).

Additionally, succinic acid, which was detected in higher amounts ($P < 0.05$) in CN2P cheeses (Table 2) could also be generated by lactobacilli, either by citrate metabolism or by degradation of serine (Skeie et al., 2007).

Free Amino Acids

Lactic acid bacteria are nutritionally fastidious microorganisms, which are nevertheless able to hydrolyze milk peptides to FAA. Certain FAA are extremely important in terms of flavor and taste development; for example, Arg is related to bitterness, whereas Pro, Ser, and Asn are related to sweetness (Pappa and Sotirakoglou, 2008). The most abundant FAA in SSC cheeses were Ile (about 20% of total FAA), Ser (about 10% of total FAA), and Phe (about 10% of total FAA); Thr, Ala, and Leu were detected at the lowest levels (Table 1). This FAA profile was different from that reported in a recent study (Nhuch et al., 2008b), in which Glu, Trp, and Leu were the most abundant FAA at 45 and 60 d of ripening. These 3 amino acids together with Asn and Gln in pasteurized milk SSC cheese (Nhuch et al., 2008b) or together with Arg and Phe in raw milk SSC cheese (Nhuch et al., 2008a) accounted for 60% of the total FAA. A considerable variation in composition between manufacturers, probably because of variations in manufacturing parameters, was observed by the latter authors. These studies did not provide any information about the sensorial quality of the cheese under study. Moreover, variations in the use of different commercial starters and in the methods used for FAA detection may result in different FAA profiles.

The branched-chain amino acids Ile and Val and the aromatic amino acid Phe are recognized precursors of key aroma compounds (Yvon and Rijnen, 2001). Isoleucine, Phe, and Ser, together with Glu, Lys, and Val (the next most abundant FAA in SSC), have been reported as the principal FAA in cow's milk Parmigiano-Reggiano (Battistotti and Corradini, 1993) and Ragusano (Fallico et al., 2004) cheeses. Glutamic acid, Val, Phe, and Ile were also the main FAA in Mahón cow's milk cheese ripened for 4 mo (Polo et al., 1985). Unlike in the present study, Leu was also found to be one of main FAA in several hard and semi-hard cow's milk cheeses (Battistotti and Corradini, 1993; Gorostiza et al., 2004; Pappa and Sotirakoglou, 2008). Arginine, which has been reported to be responsible for an unpleasant bit-

tersweet taste in cheese (Lemieux and Simard, 1992), was detected at high levels (244.3 mg/kg) in SSC cheeses.

At the end of the storage time, the total amounts of FAA in all cheese samples were approximately 2 times higher than those observed at the beginning of the study (Table 1 and Table 2), suggesting a high rate of proteolysis during the 45 d of storage. The main FAA detected at the end of the storage period were the same as those detected at the beginning of the study (Ile, Phe, and Ser; Table 2; some data not shown). On the contrary, Thr was only detected in control cheeses and Tyr was determined in mean amounts lower than 200 mg/kg (data not shown).

The amounts of Phe, Ser, and Gly were higher ($P < 0.05$) in control cheeses compared with MAP conditions (Table 2; statistics not shown), and Thr was only detected in the control cheeses (Table 2). Variations in Ser contents may have been the result of the different growth rates of lactobacilli able to degrade Ser to yield OA (Skeie et al., 2007). Moreover, some reversible reactions with Tyr-Phe, together with transamination reactions by NSLAB (Gummalla and Broadbent, 2001), may cause changes in these amino acids. On the other hand, Thr can be converted to acetaldehyde and Gly by means of threonine aldolase (Marshall and Cole, 1983). Thus, microbial degradation involving several different enzymes may be favored by low concentrations of oxygen in the modified atmospheres tested.

Although the highest total amounts of FAA were observed in the control and VP cheeses, no differences were found among groups for this trait (Table 2). Vercelino et al. (1996) reported a lack of any effect of the CO₂ package on proteolysis during storage. In other studies (González-Fandos et al., 2000; Dermiki et al., 2008), a higher degree of proteolysis has been reported in air-packaged and vacuum-packaged cheeses than in CO₂-treated cheeses. González-Fandos et al. (2000) related the higher proteolytic index in vacuum-packaged cheeses to the higher counts of microorganisms in the cheeses.

Volatile Compounds

The conversion of amino acids to aroma compounds is mainly initiated by amino acid transamination, which requires an α -keto acid as an amino group acceptor for the aminotransferases. Keto acids produced by transamination can further be transformed to various aroma compounds. Thus, catabolism of amino acids is a major contributor to flavor formation in ripened cheeses (Yvon and Rijnen, 2001).

Two hundred twenty volatile compounds were identified from the chromatograms of SSC cheeses ripened for

45 d (time 0), but only 67 compounds were present with mean relative abundances higher than 1.0 (Table 3). When the amounts were compared with those observed in control cheeses after 45 d of storage (time 45; Table 4, some data not shown in tables), higher amounts ($P < 0.05$; statistics not shown) were observed at time 0 for furfural, 5-methylfurfural, and 2-methyl-1-propanal among aldehydes; ethanol, 2-methyl-2-propanol, 2-propen-1-ol, 1-pentanol and guaiacol among alcohols; diacetyl, 2-butanone, acetoin, 3-methyl-3-buten-2-one, 2,3-pentanedione, cyclopentanone, 2-methyl-2-cyclopenten-1-one, 1-(2-furanyl)-ethanone, 2-heptanone, 3-methyl-2-butanone, 2-cyclopenten-1-one, and 2-methylcyclopentanone among ketones; toluene, p-xylene, ethylbenzene, β -pinene, ethyl-cyclopentane, and 1,3,5-trimethylbenzene among aromatic and alicyclic hydrocarbons; and 2-methylfuran, furan, benzofuran, 2-(2-propenyl)-furan, and 2-methylbenzofuran among heterocyclic compounds. On the other hand, lower amounts ($P < 0.05$) were found at time 0 for 3-methyl-1-butanal, hexanal, nonanal, and benzaldehyde among aldehydes; 1-propanol, cyclopentanol, 3-methyl-2-buten-1-ol, 2-heptanol, and 3-methyl-2-butanol among alcohols; acetone among ketones; methyl butanoate, and ethyl hexanoate among esters, and pyridine among heterocyclic compounds.

Carbonyl derivatives, cyclopentane derivatives, and benzene derivatives are the most abundant compounds probably derived from smoke (Jónsdóttir et al., 2008). Most compounds probably derived from smoke were found at higher relative abundance in the SSC before storage than at time 45 for control cheeses (Table 3 and Table 4). Some hydrocarbons such as alkyl substituted benzene, naphthalene derivatives, and xylenes may also be derived from the pasture (Barron et al., 2007), and chloroform, toluene, and other benzene derivatives may occur in cheeses as artifacts from adsorbents used in volatile extraction, as a result of packaging material, or as result of environmental contamination during freezing of the samples (Barron et al., 2007; Félix et al., 2008). In this regard, the cheese samples of this study were frozen at -30°C for up to 6 mo. Among the furans, the highest mean abundances corresponded to furfural and 2-methylfuran (Table 3). The furans present in cheese may be result of a Maillard reaction between an amino acid and a sugar, although the effect of pasteurization on the content of furans in cheese is still unclear. Furans such as 2-methylfuran and principally benzofuran have been detected by other authors in Idiazábal cheese (Barron et al., 2007) and Abundance cheese (Bugaud et al., 2001). Phenolic compounds were detected in generally low abundances in SSC cheeses (Table 3), and were mainly represented by guaiacol, 3-methylphenol, and phenol. Phenols show very low

Table 3. Relative abundances of the main volatile compounds in San Simón da Costa cheeses ripened for 45 d

Compound	Mean $\pm$ SE
Aldehydes	
2-Furancarboxaldehyde (furfural) ^{1,2}	408.2 $\pm$ 223.55
Acetaldehyde	243.1 $\pm$ 33.99
5-Methylfurfural ^{1,2}	33.8 $\pm$ 26.35
3-Methyl-1-butanal ²	30.5 $\pm$ 21.06
Hexanal	24.0 $\pm$ 22.10
Propanal	11.6 $\pm$ 1.76
2-Methyl-1-propanal	9.8 $\pm$ 6.09
Butanal ²	6.9 $\pm$ 1.48
Nonanal	3.9 $\pm$ 3.84
Benzaldehyde ^{1,2}	3.4 $\pm$ 0.53
Alcohols	
Ethanol ²	20,953.5 $\pm$ 2,207.00
2-Butanol ²	778.2 $\pm$ 233.51
Isopropyl alcohol ²	672.6 $\pm$ 133.30
2-Methyl-2-propanol	154.5 $\pm$ 14.42
1-Butanol	139.3 $\pm$ 19.28
1-Propanol	75.4 $\pm$ 12.67
3-Methyl-1-butanol ²	33.9 $\pm$ 9.13
2-Butoxyethanol ¹	32.0 $\pm$ 6.87
2-Propen-1-ol	23.3 $\pm$ 3.92
3-Methyl-2-butanol	18.7 $\pm$ 1.68
1-Pentanol	11.2 $\pm$ 8.94
2-Methoxyphenol (guaiacol) ^{1,2}	10.8 $\pm$ 6.13
2-Methyl-1-propanol	6.8 $\pm$ 6.18
2-Furanmethanol (furfuryl alcohol) ¹	4.5 $\pm$ 2.15
3-Methylphenol	3.6 $\pm$ 3.94
Phenol ^{1,2}	2.0 $\pm$ 1.54
2-Heptanol	1.1 $\pm$ 1.61
Esters	
Ethyl acetate ²	23.9 $\pm$ 5.58
2-Butoxyethyl acetate ^{1,2}	19.5 $\pm$ 13.62
Methyl acetate	18.0 $\pm$ 15.28
Ethyl hexanoate ²	1.6 $\pm$ 1.04
Sulfur compounds	
Dimethyl disulfide ²	30.9 $\pm$ 6.79
Carbon disulfide	12.3 $\pm$ 6.32
Dimethyl sulfide	4.6 $\pm$ 2.77
Ketones	
2,3-Butanedione (diacetyl) ²	1,148.8 $\pm$ 237.49
2-Butanone	207.7 $\pm$ 67.61
Acetone ²	187.6 $\pm$ 63.06
3-Hydroxy-2-butanone (acetoin)	102.8 $\pm$ 23.64
3-Methyl-3-buten-2-one	83.0 $\pm$ 11.71
2,3-Pentanedione	41.2 $\pm$ 23.51
Cyclopentanone ¹	37.1 $\pm$ 4.21
2-Methyl-2-cyclopenten-1-one ¹	31.7 $\pm$ 5.72
1-(2-Furanyl)-ethanone ¹	30.4 $\pm$ 3.24
2-Heptanone	24.6 $\pm$ 7.43
3-Methyl-2-butanone	22.5 $\pm$ 6.20
2-Cyclopenten-1-one ¹	19.4 $\pm$ 5.87
2-Nonanone	8.8 $\pm$ 1.44
2-Methylcyclopentanone ¹	8.07 $\pm$ 6.11
Hydrocarbons ³	
Trichloromethane (chloroform) ^{1,2}	265.8 $\pm$ 103.57
Methylbenzene (toluene) ^{1,2}	149.6 $\pm$ 25.89
Alpha-pinene ¹	15.5 $\pm$ 0.62
1,4-Dimethylbenzene (<i>p</i> -xylene) ^{1,2}	13.4 $\pm$ 8.77
Ethylbenzene ^{1,2}	11.2 $\pm$ 2.98
Beta-pinene ¹	5.6 $\pm$ 1.42
Ethyl-cyclopentane ¹	5.9 $\pm$ 0.88
1,3,5-Trimethylbenzene ¹	5.1 $\pm$ 3.22
1-Ethyl-2-methyl-benzene ¹	3.4 $\pm$ 3.25
1-Ethyl-3-methyl-benzene ¹	2.1 $\pm$ 2.89
1,2-Dimethylbenzene ¹	1.1 $\pm$ 0.97

*Continued***Table 3 (Continued).** Relative abundances of the main volatile compounds in San Simón da Costa cheeses ripened for 45 d

Compound	Mean $\pm$ SE
Heterocycles ⁴	
2-Methylfuran ^{1,2}	107.1 $\pm$ 30.64
Furan ¹	25.5 $\pm$ 22.38
Benzofuran ¹	5.9 $\pm$ 1.15
2-(2-Propenyl)-furan ¹	5.8 $\pm$ 3.60
Pyridine ¹	3.5 $\pm$ 2.51
2-Methylbenzofuran ¹	3.3 $\pm$ 1.78
2-Pentylfuran ¹	1.5 $\pm$ 1.71
2,5-Dihydrofuran ¹	1.1 $\pm$ 1.31

¹Compounds probably originating from smoke.²Volatile compounds verified with authentic standard compounds.³Only aromatic and alicyclic hydrocarbons probably derived from smoke are listed.⁴The group includes nonsubstituted furans and other heterocyclic compounds probably derived from smoke.

sensory threshold values (Rychlik et al., 1998) and are considered the main cause of the smoky flavor (Maga, 1988).

Only 35 volatile compounds showed differences ($P < 0.05$) among the different MAP treatments at time 45 (Table 4). Of these, aldehydes are transitory compounds that may be easily reduced to the corresponding alcohols in the low redox environment of cheese, and therefore, low amounts of aldehydes are expected in ripened cheeses (Rodríguez-Alonso et al., 2009). Among the MAP cheeses, the branched-chain aldehyde 3-methyl-1-butanal was the most abundant aldehyde, reaching the highest abundance in CN2P cheeses (Table 4). This compound has also been reported as the major aldehyde (about 50% of the total aldehydes) in shredded Cheddar cheese packaged in 100% CO₂ (Colchin et al., 2001). 3-Methylbutanal has been suggested to originate from Strecker degradation of Leu and contributes considerably to aroma because of its synergistic effect with alcohols (Yvon and Rijnen, 2001; Rodríguez-Alonso et al., 2009). Relative abundances of the linear-chain aldehydes nonanal, propanal, and butanal were significantly higher in NP cheeses than in at least one of the groups of CO₂-treated cheeses. Other authors have reported a lack of or lower amounts of aldehydes in hard cheeses packed under N₂ than in aerobically stored cheeses, suggesting that most of the aldehydes were derived from lipid oxidation (Colchin et al., 2001; Trobetas et al., 2008).

Alcohols were the most abundant chemical group in SSC cheeses (Table 3). The highest amounts of primary alcohols (1-butanol, 1-pentanol, and 1-hexanol) and branched-chain alcohols (2-methyl-2-propanol, 3-methyl-1-butanol, 3-methyl-2-butanol, and 2-ethyl-1-hexanol) were found in the CO₂-treated cheeses (Table 4). Colchin et al. (2001) also found higher

Table 4. Relative abundances of the main volatile compounds showing major differences among unpackaged control and packaged San Simón da Costa cheeses after 45 d of storage (means $\pm$ SE)

Compound	Unpackaged control cheeses	Packaged cheeses ¹				
		VP	NP	CN1P	CN2P	
Aldehydes						
3-Methyl-1-butanal ²	66.6 ^{ab} ± 4.62	61.1 ^b ± 6.14	65.1 ^{ab} ± 1.36	62.0 ^{ab} ± 14.11	87.5 ^a ± 20.97	
Nonanal	14.0 ^{ab} ± 1.95	11.3 ^{ab} ± 3.80	15.5 ^a ± 1.58	8.9 ^b ± 2.89	9.2 ^b ± 1.50	
Propanal	8.6 ^{ab} ± 2.58	11.6 ^a ± 2.05	11.0 ^a ± 1.77	7.3 ^b ± 2.01	8.1 ^{ab} ± 2.31	
Butanal ²	4.2 ^{ab} ± 0.99	5.1 ^a ± 1.26	5.4 ^a ± 1.60	4.2 ^{ab} ± 0.96	2.0 ^b ± 2.27	
2-Furancarboxaldehyde (furfural) ^{2,3}	3.8 ^{bc} ± 1.03	2.8 ^{bc} ± 2.77	2.1 ^c ± 1.87	7.8 ^{ab} ± 4.20	12.7 ^a ± 1.13	
Alcohols						
1-Butanol	144.2 ^b ± 44.72	176.4 ^{ab} ± 31.49	175.7 ^{ab} ± 11.02	214.2 ^a ± 31.16	210.4 ^a ± 8.21	
2-Methyl-2-propanol	91.7 ^c ± 3.66	105.6 ^c ± 15.45	81.9 ^c ± 12.94	144.6 ^b ± 25.60	180.6 ^a ± 35.67	
3-Methyl-1-butanol ²	30.2 ^b ± 4.59	39.3 ^c ± 1.77	53.8 ^b ± 7.48	71.9 ^a ± 8.18	65.0 ^{ab} ± 8.09	
3-Methyl-2-butanol	26.0 ^b ± 4.68	30.6 ^b ± 2.85	88.7 ^a ± 3.23	128.6 ^a ± 15.71	87.2 ^a ± 52.06	
Cyclopentanol ³	6.9 ^c ± 2.57	9.3 ^c ± 2.72	16.7 ^b ± 2.39	26.3 ^a ± 1.35	21.4 ^{ab} ± 6.41	
Phenol ^{2,3}	2.5 ^{bc} ± 0.46	4.0 ^{bc} ± 2.78	9.5 ^{ab} ± 1.00	11.3 ^a ± 3.24	6.6 ^{abc} ± 5.71	
2-Methoxyphenol (guaiacol) ^{2,3}	1.7 ^c ± 2.12	6.3 ^b ± 3.04	12.7 ^a ± 1.37	7.1 ^b ± 3.54	7.0 ^b ± 1.20	
1-Pentanol	0.0 ^c ± 0.00	17.1 ^{ab} ± 2.86	15.3 ^b ± 2.92	17.5 ^{ab} ± 2.22	21.4 ^a ± 2.37	
1-Hexanol ²	0.0 ^b ± 0.00	6.3 ^a ± 5.75	8.7 ^a ± 1.19	9.9 ^a ± 1.61	9.5 ^a ± 1.86	
2-Ethyl-1-hexanol	0.0 ^b ± 0.00	0.0 ^b ± 0.00	10.8 ^a ± 1.08	9.4 ^a ± 5.00	14.9 ^a ± 8.66	
Ketones						
2-Butanone	47.9 ^c ± 17.71	62.5 ^{bc} ± 34.84	122.4 ^{ab} ± 35.80	104.9 ^{abc} ± 23.49	134.9 ^a ± 37.80	
2-Methyl-2-cyclopenten-1-one ³	15.4 ^b ± 3.69	30.3 ^a ± 5.86	36.8 ^a ± 3.09	36.8 ^a ± 3.10	36.1 ^a ± 5.44	
2-Cyclopenten-1-one ³	0.0 ^c ± 0.00	0.0 ^c ± 0.00	8.9 ^b ± 2.13	10.7 ^{ab} ± 3.38	13.8 ^a ± 4.66	
Esters						
2-Butoxyethyl acetate ^{2,3}	18.1 ^b ± 10.74	21.9 ^b ± 11.02	41.3 ^a ± 6.81	26.2 ^b ± 12.12	30.8 ^{ab} ± 4.30	
Ethyl hexanoate ²	6.3 ^b ± 0.28	8.1 ^{ab} ± 2.05	11.1 ^a ± 3.05	8.9 ^{ab} ± 2.02	10.1 ^{ab} ± 1.56	
Ethyl acetate ²	0.0 ^b ± 0.00	39.5 ^{ab} ± 12.04	155.6 ^a ± 36.92	93.2 ^{ab} ± 14.82	101.1 ^{ab} ± 27.58	
Methyl furancarboxylate ³	0.0 ^b ± 0.00	0.0 ^b ± 0.00	8.1 ^a ± 3.35	6.1 ^a ± 5.28	4.0 ^{ab} ± 4.06	
Sulfur compounds						
Dimethyl disulfide ²	43.8 ^a ± 4.12	24.5 ^b ± 6.77	23.2 ^b ± 0.19	29.4 ^b ± 2.48	27.8 ^b ± 7.48	
Dimethyl sulfide	2.8 ^a ± 0.91	0.0 ^b ± 0.00	0.7 ^b ± 1.29	0.0 ^b ± 0.00	0.0 ^b ± 0.00	
Carbon disulfide	2.1 ^a ± 1.41	0.0 ^b ± 0.00	0.0 ^b ± 0.00	0.0 ^b ± 0.00	0.0 ^b ± 0.00	
Hydrocarbons ⁴						
Trichlorometane (chloroform) ^{2,3}	275.7 ^b ± 73.76	587.1 ^b ± 114.58	2,587.8 ^a ± 196.05	2,351.1 ^a ± 470.08	2,207.8 ^a ± 1,127.28	
1,4-Dimethylbenzene (<i>p</i> -xylene) ^{2,3}	4.7 ^c ± 4.04	11.2 ^{bc} ± 0.70	46.2 ^a ± 14.19	10.6 ^{bc} ± 3.30	19.6 ^b ± 3.39	
Decahydro-,trans-naphthalene ³	2.6 ^b ± 1.76	0.0 ^c ± 0.00	4.4 ^b ± 1.52	9.2 ^a ± 2.29	7.1 ^a ± 0.56	
Ethyl-cyclopentane ³	0.0 ^d ± 0.00	10.8 ^{bc} ± 2.05	17.0 ^a ± 1.01	7.9 ^c ± 4.58	15.2 ^{ab} ± 5.18	
Ethylbenzene ^{2,3}	0.0 ^c ± 0.00	0.0 ^c ± 0.00	24.5 ^a ± 5.91	0.0 ^c ± 0.00	8.5 ^b ± 10.01	
1,3,5-Trimethylbenzene ³	0.0 ^c ± 0.00	0.0 ^c ± 0.00	2.7 ^b ± 1.97	2.4 ^{bc} ± 2.92	5.4 ^a ± 0.91	
Heterocycles ⁵						
Pyridine ³	6.8 ^c ± 1.55	10.5 ^a ± 0.99	7.7 ^{bc} ± 0.83	9.2 ^{ab} ± 1.78	10.2 ^a ± 1.79	
2-Methylfuran ^{2,3}	0.0 ^b ± 0.00	0.0 ^b ± 0.00	20.7 ^a ± 3.20	15.0 ^a ± 5.43	18.8 ^a ± 8.05	
Benzofuran ³	0.0 ^b ± 0.00	0.0 ^b ± 0.00	5.3 ^a ± 2.67	1.1 ^b ± 1.33	3.4 ^a ± 0.72	
Pyrazine ³	0.0 ^b ± 0.00	0.0 ^b ± 0.00	0.0 ^b ± 0.00	2.1 ^b ± 1.82	5.0 ^a ± 0.77	

^{a-d}Means within a row with different superscripts differ significantly ($P < 0.05$).

¹Vacuum-packaged cheeses (VP); cheeses packaged under 100% N₂ (NP); cheeses packaged under 20% CO₂/80% N₂ (CN1P); and cheeses packaged under 50% CO₂/50% N₂ (CN2P).

²Volatile compounds verified with authentic standard compounds.

³Compounds probably derived from smoke.

⁴Only those aromatic and alicyclic hydrocarbons probably derived from smoke are listed.

⁵The group includes nonsubstituted furans and other heterocyclic compounds probably derived from smoke.

concentrations of primary alcohols in Cheddar cheese stored under CO₂ (100%) than cheese stored under N₂. The most intense reducing conditions of CO₂-treated cheeses may have favored reduction of aldehydes to their corresponding alcohols, such as the reduction of the aldehyde 3-methylbutanal to the branched-chain alcohol 3-methylbutanol (Adda et al., 1982; Rodríguez-Alonso et al., 2009). The lower amounts of most of the

alcohols in the control and VP cheeses also support this suggestion.

Among the ketones, the highest abundances of 2-butanone were found in NP and CO₂-treated cheeses. Diacetyl, which is mainly produced from citrate metabolism, may be reduced to acetoin, which in turn may be reduced by NSLAB to 2-butanone (Urbach, 1993). Tentatively identified alicyclic ketones 2-cyclopenten-

Table 5. Sensory scores for unpackaged control and packaged San Simón da Costa cheeses after 45 d of storage (means $\pm$ SE)

Attribute	Unpackaged control cheeses	Packaged cheeses ¹			
		VP	NP	CN1P	CN2P
Appearance	5.2 ^a $\pm$ 0.29	4.7 ^{ab} $\pm$ 0.52	4.3 ^b $\pm$ 0.58	4.3 ^b $\pm$ 0.42	4.7 ^{ab} $\pm$ 0.27
Odor ²	5.5 ^a $\pm$ 0.50	4.9 ^{ab} $\pm$ 0.22	4.2 ^b $\pm$ 0.29	4.6 ^b $\pm$ 0.25	4.1 ^b $\pm$ 0.65
Texture	3.8 ^{bc} $\pm$ 0.58	4.7 ^a $\pm$ 0.52	3.6 ^c $\pm$ 0.58	4.3 ^{ab} $\pm$ 0.26	4.3 ^{ab} $\pm$ 0.29
Acid taste	4.8 ^{ab} $\pm$ 0.29	5.3 ^a $\pm$ 0.65	3.8 ^b $\pm$ 0.35	3.5 ^b $\pm$ 0.71	4.3 ^{ab} $\pm$ 0.58
Flavor	4.6 ^a $\pm$ 0.29	4.9 ^a $\pm$ 0.38	3.9 ^b $\pm$ 0.25	3.4 ^{bc} $\pm$ 0.55	3.1 ^c $\pm$ 0.22
Overall score ³	44.4 ^a $\pm$ 2.13	48.2 ^a $\pm$ 2.08	38.1 ^b $\pm$ 4.00	34.8 ^b $\pm$ 2.49	36.3 ^b $\pm$ 2.25

^{a-c}Means within a row with different superscripts differ significantly ($P < 0.05$).

¹Vacuum-packaged cheeses (VP); cheeses packaged under 100% N₂ (NP); cheeses packaged under 20% CO₂/80% N₂ (CN1P); and cheeses packaged under 50% CO₂/50% N₂ (CN2P).

²Odor of packaged cheeses evaluated by smelling immediately after taking the samples out of the bags.

³Result of multiplying appearance by 1, texture by 3, and flavor by 6.

1-one and 2-methyl-2-cyclopenten-1-one compounds, probably derived from smoke, were also detected in higher amounts in the CO₂-treated cheeses and NP cheeses than in the control and VP cheeses.

The highest amounts ($P < 0.05$) of the esters ethyl acetate and ethyl hexanoate were found in the NP cheeses compared with the control cheeses (Table 4) but not relative to VP or CO₂-treated cheeses. Colchin et al. (2001) also found higher concentrations of esters in cheeses stored under N₂ relative to cheeses stored under CO₂ (100%). The inhibitory effect of CO₂ toward the formation of esters and other flavor compounds in cheeses has been reported by others (Colchin et al., 2001; Trobetas et al., 2008).

Sulfur compounds (dimethyl disulfide, dimethyl sulfide, and carbon disulfide) were detected at lower relative abundances in SSC, but the abundances were higher ($P < 0.05$) in control cheeses (Table 4), suggesting that MAP treatments impose an inhibitory effect on microbial proteolysis (Adda et al., 1982; Yvon and Rijnen, 2001) during aging.

In general, aromatic and alicyclic hydrocarbons were found at lower amounts in SSC (excluding chloroform), although they were higher in NP cheeses and CO₂-treated cheeses (Table 4). The heterocyclic compounds 2-methylfuran, benzofuran, and pyrazine, together with ethylbenzene and 1,3,5-trimethylbenzene, were not detected in control cheeses or in VP cheeses. Pyrazine, which was only detected in CO₂-treated cheeses, may be formed via the Maillard reaction during aging of the cheeses, but has also been found as a result of microbial action contributing positively to flavor when present at low concentrations (Curioni and Bosset, 2002).

Sensory Analysis

The SSC cheeses generally had a buttery, hazelnut, and smoky flavor that ranged from mild to piquant (data not shown), consistent with the standards of the

PDO council (MAPA, 2005). Cheeses before storage (time 0) were awarded a mean overall score of 64.0, with mean scores of 6.4 for appearance, 5.8 for texture, and 6.7 for flavor (data not shown). However, after 45 d of storage, the sensorial scores of all treatments decreased (Table 5). In this study, it was arbitrarily considered that cheeses with an overall score below 40 should be rejected, and NP, CN1P, and CN2P treatments (3 out of 4 treatments) resulted in scores below 40. The VP and control cheeses, which achieved higher scores for flavor and overall scores ($P < 0.05$) than the other cheeses (Table 5), were frequently (6 out of 10 panelists; data not shown) reported to have an intense buttery flavor (a positive characteristic in accordance with the PDO regulations). Nevertheless, although the mean abundance of diacetyl was higher in VP cheeses (467.2 relative abundance; data not shown), no differences ($P > 0.05$) were found for amounts of this compound among the groups of cheeses. The CN2P cheeses were sometimes (4 out of 10 panelists; data not shown) defined as “sour” and “piquant” in taste, descriptors also used for other hard cheeses packaged under high concentrations of CO₂ (Trobetas et al., 2008). The texture of NP and CO₂-treated cheeses was frequently (7 out of 10 panelists; data not shown) defined as “granulous” or “friable,” a feature that has also been observed in hard cheeses packaged under high concentrations of CO₂ (Romani et al., 2002; Juric et al., 2003). On the contrary, the texture of VP cheeses was generally (8 out of 10 judges; data not shown) defined as “softer” and “more elastic,” than that of control cheeses, perhaps closer to the “semi-elastic” descriptor proposed by the PDO regulations. Fat migration phenomena in VP cheese may be involved in the evolution toward a less friable and more elastic structure (Romani et al., 2002).

A relationship between flavor and the different biochemical traits analyzed throughout this study is shown by the main correlation coefficients in Table 6.

Table 6. Main correlations between flavor and chemical and biochemical parameters in San Simón da Costa industrial cheeses

Trait correlated with flavor	Pearson correlation coefficient (r)
Free AA	
Aspartic acid	0.654*
Lysine	0.675*
Phenylalanine	0.651*
Total free AA	0.625*
Alcohols	
3-Methyl 1-butanol	-0.896**
3-Methyl 2-butanol	-0.784**
2-Ethyl-1-hexanol	-0.773**
Ketones and esters	
2-Butanone	-0.610*
Ethyl acetate	-0.604*
Compounds of smoke origin	
Furfural	-0.626*
Cyclopentanol	-0.922**
1,3,5-Trimethylbenzene	-0.664*
2-Methylfuran	-0.744**
Pyrazine	-0.698**
2-Methylfuran	-0.744**

* $P < 0.05$; ** $P < 0.01$.

Moreover, the hierarchical cluster analysis graphically assigned the VP cheeses as very close to control cheeses (Figure 1). Strong negative correlations for flavor with high statistical significance ($P < 0.01$) were found with

branched-chain alcohols (3-methyl-1-butanol, 3-methyl-2-butanol, and 2-ethyl-1-hexanol) and compounds probably derived from smoke (cyclopentanol, 2-methylfuran, and pyrazine) (Table 6). When interpreting all of these correlations, it must be taken into account that the members of sensory panel who participated in this study were specifically trained to recognize the standards of industrial PDO cheeses; thus, flavors caused by compounds such as branched-chain aldehydes and alcohols would perhaps not be considered desirable or typical. Moreover, intense smoke odor because of the presence of higher quantities of compounds such as phenol, cyclopentanol, or 2-methyl-2-cyclopenten-1-one may be considered a negative feature.

CONCLUSIONS

Modified-atmosphere packaging appears to alter the ripening processes as regards lipolysis and proteolysis. The lower concentrations of butyric and propionic acids observed under MAP conditions may be attributed to a reduction in the degree of lipolysis. On the other hand, proteolysis yielded greater amounts of branched-chain alcohols, which were negatively correlated with flavor. Storage under modified atmospheres for a period simulating distribution and retail conditions appears to

Figure 1. Hierarchical cluster analysis generated from the chemical and biochemical traits that were correlated with flavor after 45 d of storage: vacuum-packaged cheese (VP cheeses); cheese packaged under 100% N₂ (NP cheeses); cheese packaged under 20% CO₂/80% N₂ (CN1P cheeses), and cheese packaged under 50% CO₂/50% N₂ (CN2P cheeses). The dashed line indicates the level of statistical significance of discrimination between unpackaged/VP cheeses and NP/CN1P/CN2P cheeses.

contribute to the accumulation of smoke-derived compounds within the cheese, which were also negatively correlated with flavor. For a storage period of up to 45 d, vacuum packaging appears to be the most useful technique for preserving the sensory quality of SSC cheeses.

ACKNOWLEDGMENTS

We are grateful to Raquel Lage (CIAM, Abegondo, Spain) for skilful technical assistance. The authors thank the members of the Sensory Panel of the Regulatory Consortium of Galician cheeses with Protected Designation of Origin, in particular to Angel Ramil Castelo from the SSC PDO Council, for their assistance in carrying out the expert sensorial analysis. This study was financially supported by the Xunta de Galicia, Spain, and FEDER-MAC funds (project PGIDIT-02TAL50301PR and grants PGIDIT03PXIC50301PN-PGIDIT06PXIC503085PN). P. Rodríguez-Alonso acknowledges the receipt of a research fellowship from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain (INIA) during these studies.

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