J. Dairy Sci. 104:2539–2552
https://doi.org/10.3168/jds.2020-19036

Effect of forage type, season, and ripening time on selected quality properties of sheep milk cheese

E. Renes,[1] D. Fernández,[1] D. Abarquero,[1] V. Ladero,[2] M. A. Álvarez,[2] M. E. Tornadijo,[1] and J. M. Fresno[1]*
[1]Department of Food Hygiene and Technology, Faculty of Veterinary Science, University of León, 24071, León, Spain
[2]Instituto de Productos Lácteos de Asturias (IPLA–CSIC), 33300, Villaviciosa, Asturias, Spain

ABSTRACT

The aim of this research was to study changes in the microbial populations, free AA profile, biogenic amine content, and sensory characteristics of ripened cheeses (100 and 180 d) produced in different seasons (summer, autumn, winter, and spring) from pasteurized sheep milk from 8 commercial flocks fed hay or silage diets. Twenty-one individual AA and 6 biogenic amines were determined by ultra-high performance liquid chromatography. Type of conserved forage for sheep feeding did not affect the variables studied, which is of great interest because hay and silage are low-cost ingredients for sheep feeding. Proteolysis led total free AA concentrations ranging between 35,179.26 and 138,063.71 mg/kg of cheese at 180 d of ripening. γ-Aminobutyric acid, which has been associated with beneficial effects on human health, was the second most abundant AA in all cheese samples, accounting for 15% of total free AA. Spring cheeses showed 2-fold higher concentrations of γ-aminobutyric acid than summer and autumn cheeses at the end of ripening. Overall, spring, winter, and autumn cheeses had lower average concentration of biogenic amines (431.99 mg/kg of cheese) than summer cheeses (825.70 mg/kg of cheese) as well as better sensory characteristics. Therefore, this study could provide the dairy industry with useful information for producing cheeses with valuable nutritional and sensory quality for consumers.

Key words: amino acid, biogenic amine, season, sensory, sheep cheese

INTRODUCTION

Sheep milk has high levels of protein, fat, minerals, and vitamins compared with cow or goat milks, and these compositional characteristics make it an excellent matrix to produce cheese (Balthazar et al., 2017). In fact, most sheep milk production is used to manufacture cheese, which represents a significant percentage of the world agricultural trade (Nudda et al., 2014). Coulon et al. (2004) pointed out that many variables, such as the initial quality of raw milk used and the cheese-making process, can affect the nutritional composition, sensory characteristics, and, consequently, consumer acceptability of cheeses. Sheep management practices, specifically the feeding regimen, have long been identified as important factors affecting the composition and quality of milk (Addis et al., 2005; Morand-Fehr et al., 2007; Renes et al., 2018). In this sense, conserved forages represent an important part of sheep's diet in most common farming systems because the availability and quality of fresh pasture throughout the year can be affected by the phenological stage of the botanical species that are part of the pastures (Cabiddu et al., 2005), and conserved forages constitute a low-cost feed alternative source (Dewhurst et al., 2006). Nevertheless, conservation conditions of forages such as ensiling or wilting can generate physicochemical changes on plants, leading to modifications in milk composition, which can greatly affect cheese characteristics (Kalač and Samková, 2010; Glasser et al., 2013).

Regarding the cheese-making process, sheep milk cheeses are commonly ripened between 3 and 6 mo before sale (Martínez et al., 2011). Proteolysis has been described as the most complex and important biochemical event during cheese ripening (Fox et al., 2017). Hydrolysis of milk casein during primary proteolysis by the residual coagulant activity and by plasmin results in the release of a range of intermediate-sized peptides, which are further broken down into small peptides and AA by the action of the proteinases and peptidases from the starter and nonstarter lactic acid bacteria (**LAB**) as well as from the secondary microbiota (Upadhyay et al., 2004). Therefore, microbial successions that take place during cheese ripening directly affect the course of the proteolysis during cheese ripening, which implies physicochemical changes in the cheese matrix and, consequently, in texture, color, and flavor characteristics of this fermented dairy product (Fox et al., 2017).

Received June 7, 2020.
Accepted October 19, 2020.
*Corresponding author: jmfreb@unileon.es

Although pasteurization of milk for cheese-making reduces the microbial diversity, it has been observed that the final adventitious microbial levels in pasteurized milk cheeses undergo seasonal variations and can highly influence the proteolysis process (Gaya et al., 2005). These authors pointed out that pasteurized sheep milk cheeses produced in winter showed different free AA profile, highlighting the higher content of glutamic acid, from cheeses produced in summer. Despite the large number of studies reporting the great importance of quantity and type of free AA on the sensory characteristics and quality of many cheese varieties (Kilcawley, 2017; Niro et al., 2017), few data are available about the effect of the season of sheep cheese-making on the concentration of AA compounds such as γ-aminobutyric acid (**GABA**) and ornithine. These compounds have attracted the attention of the dairy industry because they have beneficial physiological functions on human health such as hypotensive and fatigue attenuation effects, respectively (Diana et al., 2014; Santiago-López et al., 2018; Diez-Gutiérrez et al., 2020).

An important aspect from a food safety perspective is that some of the free AA generated during cheese ripening can act as precursors of biogenic amines that are mainly synthetized by decarboxylase-positive bacteria (Poveda et al., 2015). Biogenic amines are organic, basic, low-molecular-weight nitrogenous compounds formed mainly by microbial AA decarboxylases, which can cause several health problems in susceptible consumers (Benkerroum, 2016). Under normal conditions, biogenic amines ingested with food are degraded in the organism by the action of monoamine and diamine oxidases. However, when this detoxification process is disturbed because of genetic factors, some medical treatments, or high concentrations of biogenic amines in food, these nitrogenous compounds become toxic metabolites responsible for serious human health problems (Linares et al., 2011). The European Food Safety Authority (EFSA, 2011) pointed out that cheese is the main fermented milk product that can contain potentially harmful levels of biogenic amines and that more control should be exercised. In particular, long-ripened cheeses have been identified as one of the most prevalent foods associated with amine poisoning, mainly related to the presence of high levels of tyramine, resulting in dangerous intoxication characterized by an increase in blood pressure (Ladero et al., 2010).

Currently, there is not a consensus on what should be the maximum permitted concentration of biogenic amines in cheese. It is well known that histamine and tyramine are the biogenic amines most often associated with food poisoning, but it is necessary to highlight that cadaverine and putrescine, which have not been associated with food poisoning, may enhance the toxicity of histamine and tyramine (Linares et al., 2011; Renes et al., 2014). Thus, knowledge of the levels of biogenic amines in cheese is necessary to assess the health hazards arising from consumption of this dairy product. Furthermore, it could be useful as an indicator of the hygienic quality of raw materials and production conditions (Pinho et al., 2001).

Based on the aspects described, the present research could provide relevant information about factors that allow optimizing the hygienic–sanitary, compositional, and sensory quality of long-ripened sheep milk cheeses. Therefore, the objective of this work was to study changes in the microbiological counts, free AA profile (with special emphasis on GABA and ornithine), biogenic amine content, and sensory characteristics of ripened cheeses produced in different seasons with pasteurized sheep milk from commercial flocks fed hay or silage diets.

MATERIALS AND METHODS

Experimental Design

Eight commercial farms of Assaf sheep, located in Castilla y León (Spain), were selected for the present study. Throughout this study, the 8 flocks (1 flock on each farm) received a typical milking ration with a forage:concentrate ratio of 50:50. Four flocks were fed 60% common vetch (*Vicia sativa*) hay, and 4 other flocks were fed the same percentage of common vetch but in the form of silage (Renes et al., 2020). Hay and silage were made from common vetch grown in the same field.

Sheep bulk tank milk (evening and morning milks) was collected from each flock for cheese manufacture 4 times over a 1-yr period: summer (August), autumn (November), winter (February), and spring (May). Cheese-making trials (8 cheese batches × 3 replicates × 4 seasons) were performed in the second week of each collection month at pilot scale (Institute of Food Science and Technology, University of Leon, Spain).

Sheep Cheese Manufacture

The cheese-making procedure was carried out according to Renes et al. (2020). Briefly, 100 L of sheep bulk tank milk was pasteurized at 72°C for 15 s. Calcium chloride (0.2 g/L; Laboratorios Arroyo S.A., Cantabria, Spain) and a starter culture (1% vol/vol; Choozit LYO MA 011 50 DCU; DuPont Ibérica, Madrid, Spain) composed of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* were added. After 30 min, chymosin (Chy-Max Extra, 100% chymosin, 600 international milk-clotting units/mL; Chr. Hansen SL, Madrid,

Spain) was added. After 45 min, the curd was cut and the whey was drained off. The curd was transferred to cylindrical molds (15 cm high, 21 cm in diameter), which were pressed for 2 h. Then, cheeses were salted by immersion (brine density 18°, Baumé 8°C, and pH 5.4) for 17 h. After this time, cheeses were covered with antifungal plastic resin (DelvoCoat, DSM, Heerlen, the Netherlands). Finally, cheeses were taken to a ripening chamber, where they remained at 10°C and 80 to 85% relative humidity for 180 d.

Samples (each sample corresponded to a whole cheese of 3.25 kg) were taken from each cheese batch (4 cheeses) after 100 and 180 d of ripening, dates in which this type of cheese is marketed. Samples were ground, vacuum packed, and stored in a freezer (−30°C) until free AA and biogenic amines analysis. Microbiological, color, texture, and sensory analyses were carried out on fresh samples.

Microbiological Analysis

Fifty grams of cheese samples was homogenized with 200 mL of a 2% (wt/vol) sodium citrate solution (Panreac, Barcelona, Spain). Decimal dilutions were prepared by mixing 10 mL of this homogenate with 90 mL of sterile peptone water (Oxoid, Basingstoke, UK) at 0.1% (wt/vol) according to International Dairy Federation standard 122B (FIL-IDF, 1992).

Microbial counts were determined in triplicate following the method described by Diezhandino et al. (2015). Aerobic mesophilic and psychrotrophic bacteria were enumerated on standard plate count agar (Oxoid) after 48 h of incubation at 30°C and 10 d at 7°C, respectively. The LAB were determined on de Man, Rogosa and Sharpe agar (Oxoid) after incubation at 30°C for 72 h. Lactobacilli were enumerated on Rogosa agar (Oxoid) incubated at 30°C for 5 d. Enterococci were determined on kanamycin esculin azide agar (Oxoid) after incubation at 37°C for 24 h. *Enterobacteriaceae* were enumerated on violet red bile glucose agar (Oxoid) after incubation at 37°C for 18 to 24 h. Molds and yeasts were inoculated on oxytetracycline glucose yeast extract agar (Oxoid) and incubated at 22°C for 5 d.

Determination of Free AA and Biogenic Amines

Free AA and biogenic amines were determined in triplicate following the method described by Redruello et al. (2013), as follows: 1 g of cheese was homogenized with 10 mL of 0.1 *M* HCl–0.2% 3,3′thiodipropionic acid (Sigma-Aldrich, Madrid, Spain) using an IKA T-18 Ultra-Turrax (IKA-Werke GmbH & Co, Staufen, Germany) for 2 min at 20,000 rpm. This mixture was kept in a Bransonic 221 ultrasonic bath (Branson Ultrasonics, Danbury, CT) for 30 min and then centrifuged at 5,000 × *g* for 20 min. The supernatant was deproteinized by passing through ultrafiltration inserts (Amicon Biomax 5K; Millipore, Billerica, MA) by centrifugation at 3,500 × *g* for 1 h. Then, derivatization of samples was carried out according to the method described by Redruello et al. (2013), as follows. A mixture of 20 μL of sample, 175 μL of 1 *M* borate buffer, 75 μL of methanol, 2 μL of L-2-aminoadipic acid (as internal standard 2 g/L; Sigma-Aldrich), and 3 μL of diethyl ethoxymethylenemalonate (Sigma-Aldrich) was incubated at 30°C in an ultrasonic bath for 45 min. Then, samples were heated at 70°C for 2 h; after this time, they were filtered through 0.22-μm membranes coupled to a syringe needle into chromatography vials (Waters, Milford, MA).

The chromatograph system consisted of an H-class Acquity UPLC system (Waters) coupled to a photodiode array detector. Free AA and biogenic amine separation was carried out using a Waters Acquity UPLC BEH C18 column (1.7-μm particle size, 100 mm × 2.1 mm i.d.) held at 35°C. The mobile phase consisted of 25 m*M* acetate buffer (pH 6.7) plus 0.02% sodium azide (eluent A), methanol (eluent B), and acetonitrile (eluent C). Samples (1 μL) were applied to the column and eluted at a flow rate of 0.45 mL/min according to the linear gradient used by Redruello et al. (2013). The target compounds were identified by their retention times and their spectral characteristics at 280 nm, and were quantified using the internal standard method. Data were acquired and analyzed using the software Empower 2 (Waters).

Color and Texture Instrumental Analyses

Color analysis of sheep milk cheeses was performed, measuring 12 different places on the longitudinal cheese sample surface (1 cm thick), using a reflectance spectrophotometer (CM-700 d; Konica Minolta, Osaka, Japan) provided with a measuring glass head of 8 mm in diameter, an illuminant D65, and a 10° observer. The L*, a*, and b* color measurements were determined according to the CIELab color space, where L* corresponds to light–dark chromaticity (0% = dark to 100% = light), a* to corresponds to green–red chromaticity (−60% = green to 60% = red), and b* corresponds to blue–yellow chromaticity (−60% = blue to 60% = yellow; O'Callaghan et al., 2017).

Texture profile analysis was carried out on 8 cube-shaped (1.9 cm^3) samples obtained from each cheese batch at room temperature (20 ± 2°C). The cheese samples were kept at room temperature for approximately 3 h before analysis, and a 0.5-cm layer was removed from the surface of the cheese.

Texture properties of cheese were determined in 2 successive cycles of 80% compression with a cross-head constant speed of 0.5 mm/s using a TZ-XT2 texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a plate-plate sensor system with a stainless SMS P/75 (75 mm) probe. The textural characteristics were determined from the resultant force–time curve using Texture Expert software (Stable Micro Systems).

Sensory Analysis

Sensory evaluation of sheep milk cheeses manufactured in each season with milk from commercial flocks fed different types of forage at 100 and 180 d of ripening was carried out by 20 panelists aged between 22 and 60 yr. Panel members were recruited from the Food Hygiene and Technology Department of the University of León, Spain, and they were previously trained in 5 sessions of 1 h with commercial sheep milk cheeses. In this training, odor, flavor, and texture attributes were defined and quantified using a descriptive test (ISO, 2005) and according to the methodology previously described by Fresno and Álvarez (2012) for semi-hard and hard cheeses.

Cheese pieces of the same dimensions (4 cm × 1.5 cm × 0.5 cm) were presented to the panel at ambient temperature (20 ± 2°C) and identified with a random 3-digit code in 4 sessions for each cheese batch produced in the same season at the same ripening time. The panel assessed 13 sensory characteristics, divided into 4 main groups: appearance, which included color intensity [ranging from white (1) to off-white (4) to yellowish-brown (7)], number of holes, and hole homogeneity; odor, which included odor intensity, pungent, and moldy; taste, which included taste intensity, saltiness, and bitterness; and texture, which included elasticity, adhesiveness, firmness, and friability. These attributes were recorded on a 7-point intensity scale containing the following descriptors: 1 = nonexistent, 2 = very weak, 3 = weak, 4 = moderate, 5 = strong, 6 = very strong, and 7 = extremely strong.

Statistical Analysis

Statistical analysis of the experimental data was performed using SPSS v. 23 (SPSS Inc., Chicago, IL). Microbiological, free AA, biogenic amine, color, and texture variables were tested for the assumption of normality using the Lilliefors-corrected Kolmogorov-Smirnov test and for homoscedasticity using the Levene test. Subsequently, data were analyzed using a general linear model of ANOVA to investigate the effect of season (summer, autumn, winter, spring), ripening time (100 or 180 d), type of forage feeding (hay, silage), and the interaction between them. Turkey's honestly significant difference post hoc test was applied at 5% significance level to compare sheep cheeses manufactured in different seasons throughout the different ripening times. Student's *t*-test was applied at 5% significance level to compare sheep cheeses manufactured with milk from commercial flocks fed hay or silage in each season at 100 and 180 d of ripening.

Sensory variables were analyzed using the Kruskal-Wallis test by ranks at 5% significance level. The Mann-Whitney test was applied at 5% significance level to compare sheep cheeses at 100 or 180 d of ripening in each season of cheese-making. In addition, Spearman's rank correlation coefficient (ρ) was applied to estimate the relationship between the microbial populations, free AA, and biogenic amine contents as well as between the free AA contents and sensory attribute values of cheese samples.

RESULTS AND DISCUSSION

Microbial Populations of Sheep Milk Cheeses

The values for the microbial counts of sheep milk cheeses at 100 and 180 d of ripening are reported in Figures 1 and 2. Microbial counts in cheese samples were significantly influenced ($P \leq 0.05$) by the type of forage feeding, season of cheese-making, and ripening time as well as by the interaction between season and ripening time. The highest levels for aerobic mesophilic microbiota were observed at 100 d of ripening in cheeses produced in autumn with milk from sheep fed silage. Regarding the effect of the season of cheese-making, it was observed that cheeses produced in autumn showed significantly higher ($P \leq 0.05$) aerobic mesophilic bacteria counts than spring cheeses (Figure 1A). Lactic acid bacteria were the predominant microorganisms during the ripening period studied, which was reflected by the similar counts of aerobic mesophilic bacteria and LAB (Figure 1A and C). In addition, as can be seen in Figure 1D, lactobacilli were the main LAB present in the cheese samples at the end of ripening. The starter culture used for cheese-making only contained lactococci; the reason why viable lactobacilli were the main LAB at 100 and 180 d of ripening could be because the adventitious nonstarter bacteria, mainly composed of lactobacilli, dominate cheese microbiota during long ripening periods due to their good tolerance of the hostile environment (Settanni and Moschetti, 2010; Blaya et al., 2018). Consequently, lactobacilli can strongly influence the biochemistry of curd ripening, playing a crucial role in the development of the final character-

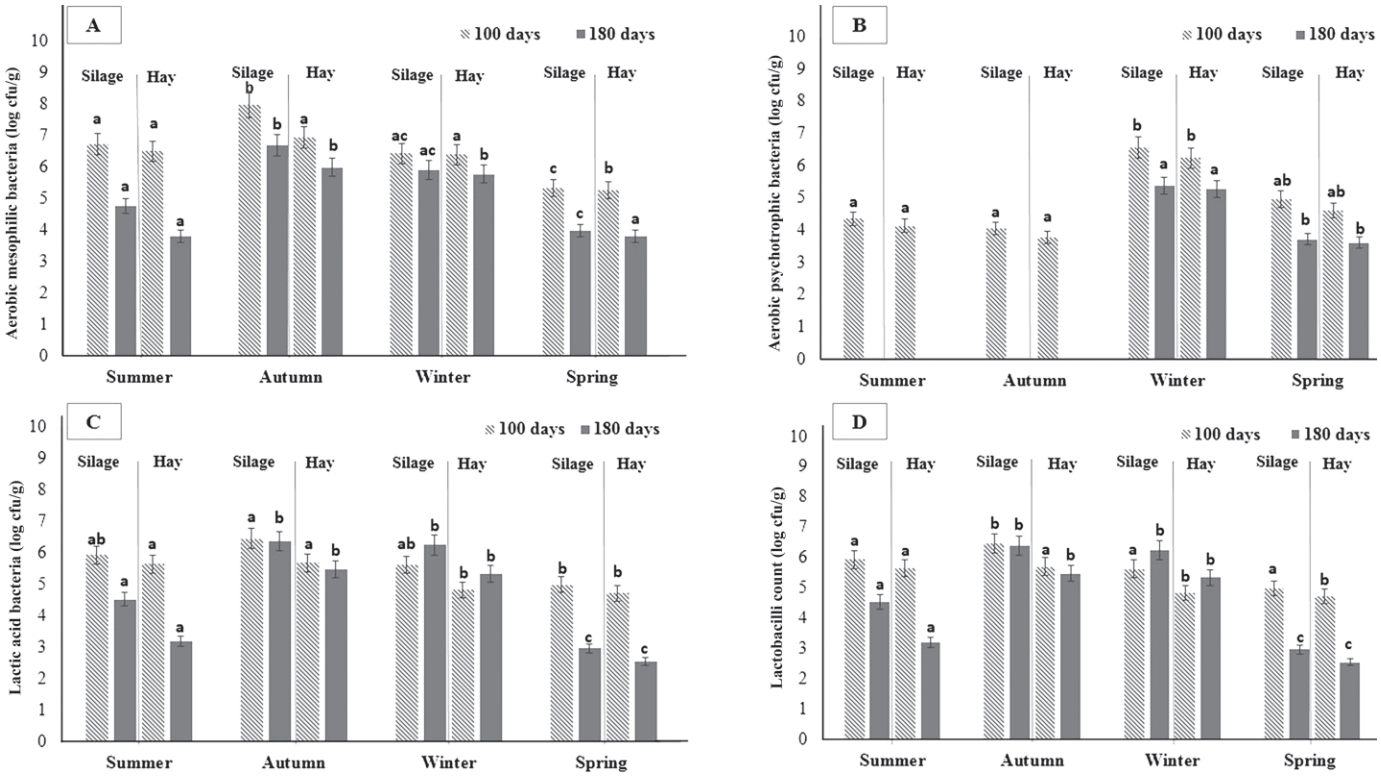

Figure 1. Counts (log cfu/g) of aerobic mesophilic bacteria (A), aerobic psychrotrophic bacteria (B), lactic acid bacteria (C), and lactobacilli (D) at 100 and 180 d of ripening for cheeses made with bulk tank milk from sheep fed silage or hay during summer, autumn, winter, and spring. Data represent mean ± SD; n = 36. Different letters (a–c) in the same bar for each season at the same ripening time point and type of forage feeding denote significant statistical differences ($P \leq 0.05$). Fixed effects (season, type of forage, and ripening) and the interaction between season and ripening were significant ($P \leq 0.05$). Remaining interactions between fixed effects were not significant ($P > 0.05$).

istics of cheese. In the present study, winter cheeses showed the highest counts of lactobacilli at 180 d of ripening, whereas summer, autumn, and spring cheeses reached the highest counts at 100 d.

Presence of psychrotrophic bacteria in pasteurized milk cheeses has been widely reported, and the number of these bacteria depends on their level in raw milk, which in turn is subjected to seasonal variations (Pyz-Łukasik et al., 2018). In fact, in the present study, summer and autumn cheeses showed the lowest counts, followed by spring cheeses. In addition, psychrotrophic bacteria counts decreased significantly ($P \leq 0.05$) from 100 d to 180 d of ripening in all cheese samples and were not detected in summer and autumn cheeses at the end of the ripening time, regardless of the type of forage used for sheep feeding.

Enterococci, like lactobacilli, also show high adaptability to cheese environmental conditions during ripening and, due to their proteolytic activity, are responsible for the development of particular sensory characteristics of many cheese varieties (Mrkonjic Fuka et al., 2017). Figure 2A shows that summer cheeses had the lowest values for enterococci counts (1.07 log cfu/g) compared with the cheeses produced in other seasons (2.28–3.01 log cfu/g).

Molds and yeast counts were very low in all cheese samples at 100 and 180 d of ripening (Figure 2C). One of the reasons for the molds and yeast counts observed in cheese samples could be the application of plastic resins with antifungal compounds to the cheese surface (Civelek and Cagri-Mehmetoglu, 2019).

Enterobacteriaceae counts are an indicator of the hygienic conditions applied during the cheese-making process (Metz et al., 2020). The good sanitary quality of the cheese batches was evidenced because *Enterobacteriaceae* counts were detected only at 100 d of ripening in summer and spring cheeses, with values lower than 1.50 log cfu/g (Figure 2B).

Cheese Free AA Content

The concentrations of free AA in ripened sheep milk cheeses produced in different seasons are depicted in Figure 3. In all cheese samples, no significant differences ($P > 0.05$) were observed for the total free AA (**TFAA**) or for the 21 individual AA contents ana-

lyzed depending on the type of conserved forage used for sheep feeding. Similarly, no effect of different grass and TMR diets on TFAA was observed in other cheese varieties such as Cheddar (O'Callaghan et al., 2017) or Maasdam (Panthi et al., 2019).

However, as expected, TFAA concentrations of the cheese samples increased from an average mean of 55,074.18 mg/kg of cheese at 100 d to 83,168.39 mg/kg of cheese by 180 d, as a possible result of an increase in the secondary proteolysis due to the intracellular enzymes released into the cheese matrix mainly by starter and nonstarter LAB (Bergamini et al., 2010). The trend of increasing TFAA in sheep milk cheeses during ripening has been widely reported. For example, Bustamante et al. (2003) and Hernández et al. (2009) described a linear response between the content of TFAA in Idiazábal cheese and a ripening period of 180 d, and Tofalo et al. (2019) reported an increase in the TFAA content of raw sheep milk cheeses during ripening regardless of the type of rennet used. In the present study, TFAA average concentrations in cheese samples at the end of ripening were higher than those reported before in Argentinean sheep cheeses produced with raw milk (6,270 mg/kg of cheese) and ripened for 180 d (Bergamini et al., 2010) or those observed by Poveda et al. (2004) and Renes et al. (2019) in sheep cheeses produced with pasteurized milk using different starter cultures (23,939 and 79,662 mg/kg of cheese, respectively) at 150 and 180 d of ripening, respectively.

In addition, concentrations of TFAA in cheese samples were significantly influenced ($P \leq 0.001$) by the season of manufacture. It is noteworthy that spring cheeses presented the highest concentrations of TFAA at 100 and 180 d of ripening, which could be correlated with the course of ripening as well as with the evolution of lactic microbiota in these cheeses. In this sense, after 100 d of ripening, spring cheeses showed TFAA concentration similar to that shown by winter cheeses at the end of ripening and 2-fold high TFAA contents than summer and autumn cheeses. Therefore, the production of sheep milk cheeses in spring could reduce the ripening time, thus providing technological

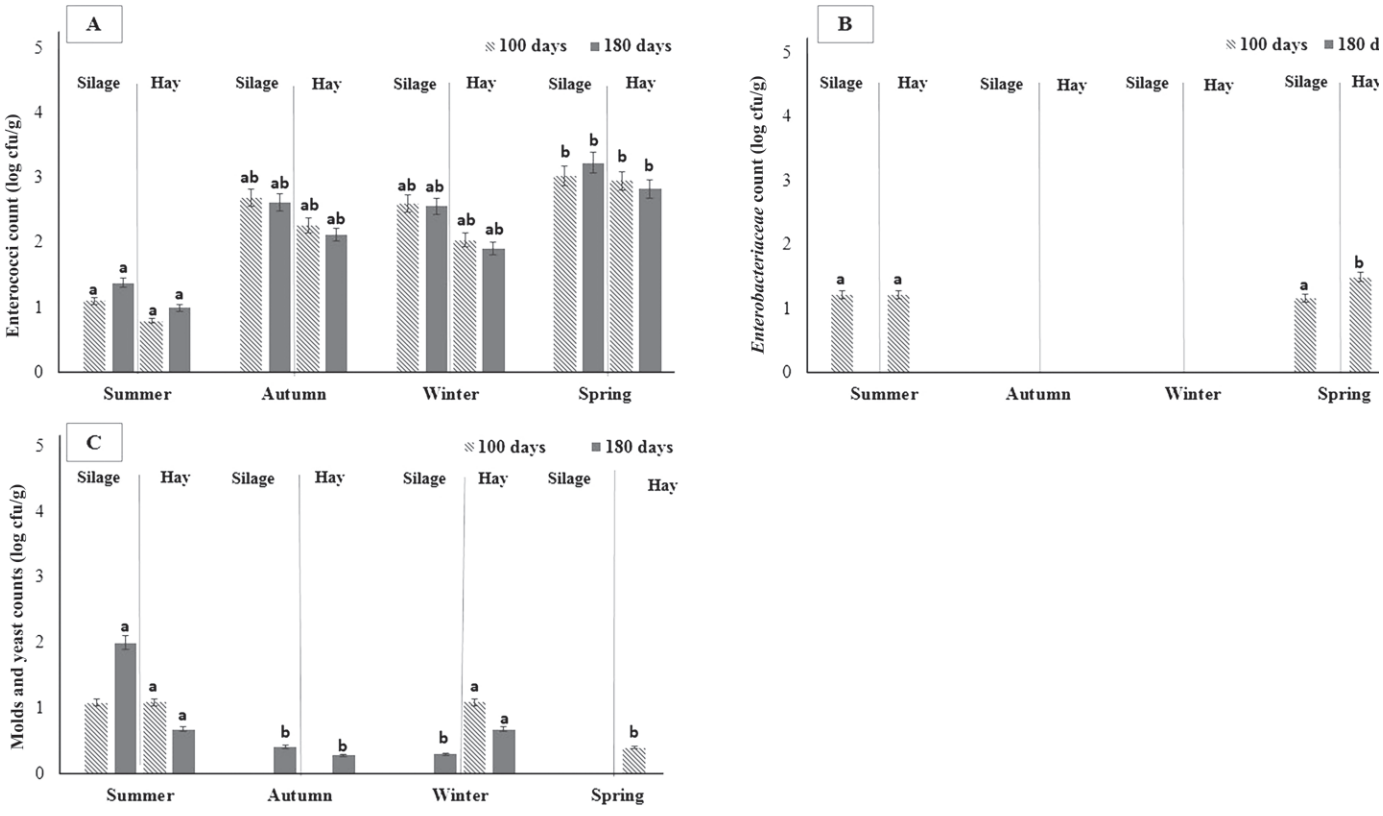

Figure 2. Enterococci (A), *Enterobacteriaceae* (B), and molds and yeast (C) counts (log cfu/g) at 100 and 180 d of ripening for cheeses made with bulk tank milk from sheep fed silage or hay during summer, autumn, winter, and spring. Data represent mean ± SD; n = 36. Different letters (a, b) in the same bar for each season at the same ripening time point and type of forage feeding denote significant statistical differences ($P \leq 0.05$). Fixed effects (season, type of forage, and ripening) and the interaction between season and ripening were significant ($P \leq 0.05$). Remaining interactions between fixed effects were not significant ($P > 0.05$).

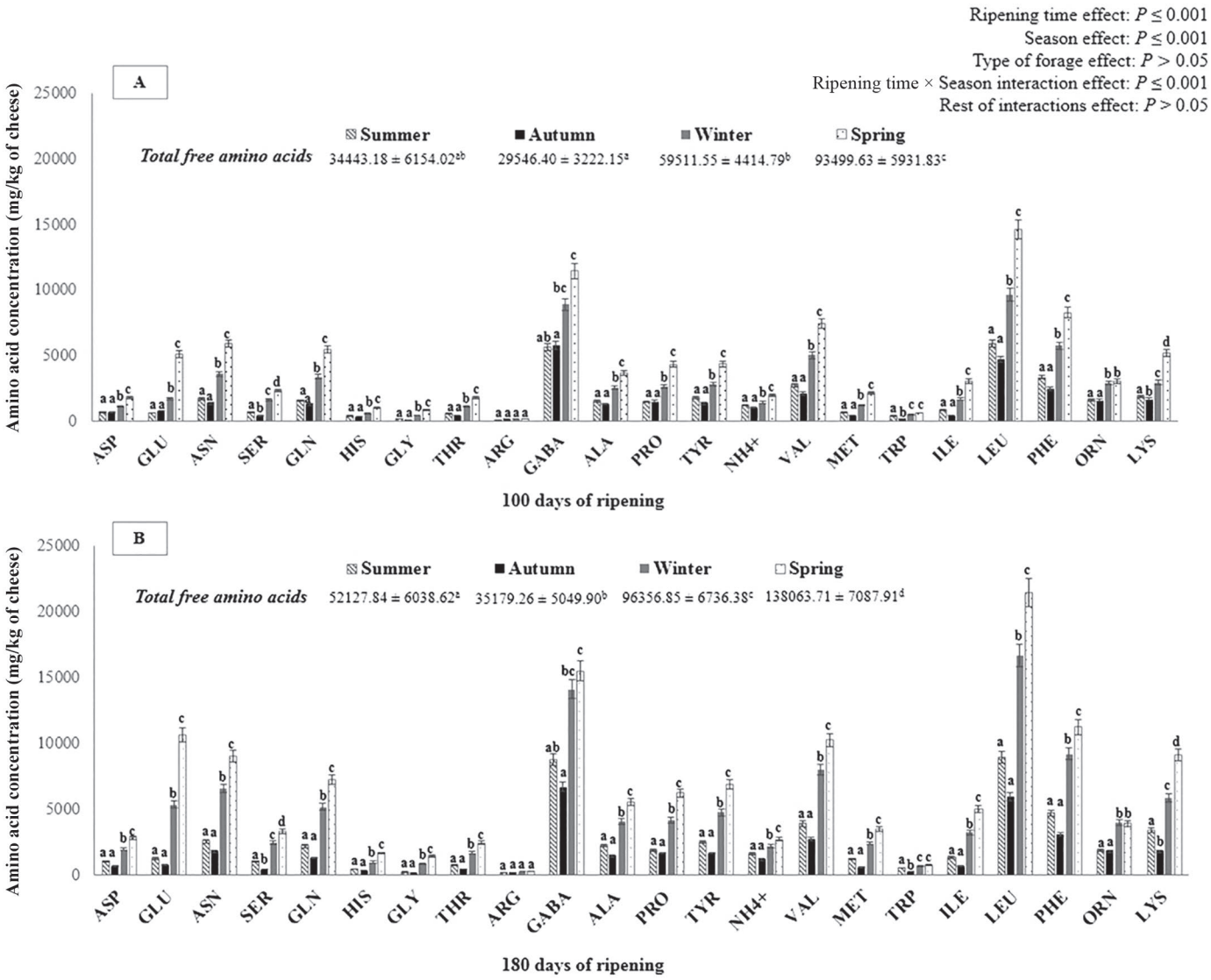

Figure 3. Concentration (mg/kg of cheese) of individual AA at 100 (A) and 180 (B) days of ripening in cheeses made with sheep bulk tank milk during summer, autumn, winter, and spring. Data represent mean ± SD; n = 72. Different letters (a–d) in the same bar for each free AA indicate significant statistical differences ($P \leq 0.05$) between cheese batches. ASP: aspartic acid; GLU: glutamic acid; ASN: asparagine; SER: serine; GLN: glutamine; HIS: histidine; GLY: glycine; ALA: alanine; THR: threonine; ARG: arginine; GABA: γ-aminobutyric acid; PRO: proline; TYR: tyrosine; VAL: valine; MET: methionine; TRP: tryptophan; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; ORN: ornithine; LYS: lysine.

benefits because it has been pointed out that ripening is a relatively expensive process for the cheese industry (Azarnia et al., 2006).

In fact, the content of free AA is related to the cheese-making technology, including ambient conditions, and to the ripening process. Additionally, it has been pointed out that microorganisms in the cheese matrix, mainly LAB, can present complex proteolytic systems formed by different proteinases and peptidases depending on the specie and therefore can lead to different proportions of individual free AA (Poveda et al., 2016; Fox et al., 2017). The major AA in all the cheese samples during the ripening time studied were leucine, GABA, phenylalanine, valine, lysine, and asparagine, accounting for more than 60% of the TFAA (Figure 3). The fact that leucine, phenylalanine, and valine were among the major AA in the cheese samples shows that proteolysis of α_{S1}-casein (rich in these AA) predominated over β-casein (Pappa et al., 2006).

Of importance in this study is the high concentrations of GABA found in all cheese samples. In fact, GABA was the second most abundant AA in all the cheese samples, accounting for 15% of the TFAA. For many years, the presence of GABA in cheese has

been considered an indicator of anomalous fermentations produced by the adventitious microbiota during ripening (Freitas et al., 1998; Gorostiza et al., 2004). However, over the last decade, attention has been paid to the presence of GABA in cheese and dairy products as a result of its health-related benefits (Diana et al., 2014; Manca et al., 2015). Although GABA is not present in milk casein, this nonprotein AA can be synthesized during cheese-ripening from L-glutamate by the glutamate decarboxylase enzymatic complex present in some LAB (Lacroix et al., 2013). As can be seen in Figure 3, the concentration of GABA in the cheese samples increased significantly ($P \leq 0.001$) from 100 d to 180 d of ripening. The highest concentrations of GABA were observed at 180 d of ripening in spring cheeses (15,475.49 mg/kg of cheese) and winter cheeses (14,009.31 mg/kg of cheese) followed by summer and autumn cheeses (8,714.36 and 6,676.22 mg/kg of cheese, respectively). Highlighting the dose of GABA that is in the health-promoting range, a daily intake of only 2 g of spring cheeses ripened at 100 or 180 d would provide the amount of GABA (26.4 mg) that is required to be effective in treating neurological disorders (Okada et al., 2000). Likewise, Pouliot-Mathieu et al. (2013) pointed out that a daily intake of 50 g of an experimental cheese containing 16 mg of GABA decreased blood pressure in humans. In the present study, 50 g of spring cheeses would supply 672.63 mg of GABA. This fact implies that small portions of the spring cheeses would be necessary to achieve the physiological effects indicated.

Ornithine, like GABA, is not present in milk casein, but it can nonetheless be synthesized by the enzymatic activity of LAB metabolism through the precursors arginine and citrulline during cheese ripening (Diana et al., 2014). It could be corroborated by the low concentrations of arginine detected in the cheese samples, which is also a positive aspect from a sensory point of view because arginine has been related to bitter off-flavors in cheese (Poveda et al., 2004). Ornithine is gaining attention because some studies have demonstrated its bioactive functions, such as sedative and hypnotic effects and fatigue attenuation (Sugino et al., 2008; Kurata et al., 2012). Despite this, in the literature no information is available about the effective dose of ornithine to achieve beneficial effects on human health. In the present study, the ripening period studied had no significant effect ($P > 0.05$) on ornithine values (Figure 3). However, cheese samples showed different concentrations of this bioactive compound depending on the season of manufacture. It was observed that spring and winter cheeses showed approximately 2-fold higher concentrations of ornithine than summer and autumn cheeses. Concentration of ornithine in the cheese samples analyzed ranged between 1,559.49 and 3,969.76 mg/kg of cheese, which was comparable with the values reported for other sheep milk cheeses (Renes et al., 2019). However, Diana et al. (2014) found lower levels of ornithine in artisanal sheep and goat cheeses.

Cheese Biogenic Amine Content

Cheese ripening is important for the development of flavor and texture characteristics of each cheese variety (Pinho et al., 2001). However, as has been described in the present study, the casein proteolysis that takes place during cheese ripening led to the accumulation of free AA, some of them being precursors of biogenic amines. It should be highlighted that, as in the case of the TFAA, the type of forage (hay or silage) used for sheep feeding had no significant effect ($P > 0.05$) on the biogenic amine concentrations of the cheeses studied. The ripening times considered were 100 and 180 d because they are the dates in which this type of cheese is marketed. The presence and concentration of individual biogenic amines in the cheese samples were highly influenced by the season of cheese-making (Table 1). Linares et al. (2016) described that histamine and tyramine are the most abundant biogenic amines in cheese. Nevertheless, the information available about the major biogenic amines in sheep milk cheeses is variable because the formation and accumulation of these compounds in cheese depend on multiple factors such as substrate availability, pH, water activity, and bacterial density, among others (Schirone et al., 2013; Renes et al., 2019). In the present study, the most abundant biogenic amines found in cheese samples accounting for more than 90% of the total biogenic amine content were histamine and tyramine, with the exception of summer cheeses at 100 d of ripening, in which cadaverine (25.87%) was the major biogenic amine followed by tyramine (25.00%) and histamine (18.20%). These 3 biogenic amines were present in all cheese samples; however, tryptamine was detected only in summer cheeses ripened 100 d and spring cheeses at 180 d. Tryptamine concentration in summer cheeses was 64-fold higher than that in spring cheeses. In addition, putrescine and phenylethylamine were present in summer cheeses, with average concentrations of 20.94 and 44.66 mg/kg of cheese, respectively. As result, summer cheeses showed the highest concentrations of total biogenic amines compared with the cheeses produced in other seasons, between which there were no significant differences ($P > 0.05$). Therefore, there was not a relationship between the free AA content and biogenic amine concentration detected in cheese samples. This was in agreement with Poveda et al. (2016), who pointed out that environmental factors during ripening that can af-

fect the activity of decarboxylase enzymes may be more important than the availability of precursors (free AA).

It has been recommended that the sum of all the biogenic amines should not exceed the range between 750 and 900 mg/kg of cheese (Manca et al., 2015). In this regard, none of the samples exceeded the indicated limit, except summer cheeses ripened for 180 d, which showed a total biogenic amine concentration of 910.74 mg/kg (i.e., approximately 2-fold higher total biogenic amine concentration than the other cheeses at the same ripening time). The high content of these compounds measured in summer cheeses could be attributed to 2 causes. First, it has been described that adventitious microorganisms derived from the dairy environment and surfaces of equipment used can be responsible for biogenic amine formation (Schirone et al., 2013). Second, despite the fact that all cheeses in the present study were produced from pasteurized milk, it has been pointed out that in pasteurized milk cheeses the presence of biogenic amines is related to the nonstarter LAB in raw milk because some of these microbiota are active producers of biogenic amines and are resistant to thermal treatment (Ladero et al., 2011). In this sense, these microorganisms in summer milks could have been higher than those in milks collected in other seasons. Nevertheless, it cannot be corroborated because it is difficult to find a correlation between the concentrations of biogenic amines in cheese and an increment of a specific group of microorganisms because the ability to produce biogenic amines is mostly related to strain rather than to specie (Novella-Rodríguez et al., 2002).

Texture, Color, and Sensory Analysis

Texture, color, and sensory data revealed that the use of hay or silage diets for sheep feeding had no significant effect ($P > 0.05$) on cheese sample quality. However, other studies have shown that feeding systems based on hay and silage sorghum, fresh pastures, or TMR can highly influence these characteristics in cow milk cheeses (O'Callaghan et al., 2017; Panthi et al., 2019; Serrapica et al., 2020).

Table 2 presents the values for the variables analyzed in the texture profile analysis and in the color study of sheep milk cheeses produced in different seasons and ripened for 100 and 180 d. Texture characteristics analyzed in the cheese samples were fracturability, hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness. The most significant differences ($P \leq 0.05$) observed between the cheese samples for texture characteristics were mainly determined by the season of cheese-making. Summer and autumn cheeses at 100 and 180 d of ripening showed higher values for adhesiveness, gumminess, and chewiness than winter and spring cheeses, which was related to the degree of proteolysis that took place during ripening. In this sense, the depth of proteolysis was higher in spring and winter cheeses, as can be verified by the TFAA contents

Table 1. Concentration (mg/kg of cheese) of biogenic amines at 100 and 180 d of ripening in sheep milk cheeses manufactured during the 4 seasons of the year[1]

Biogenic amine	Ripening time (d)	Season				*P*-value[2]		
		Summer	Autumn	Winter	Spring	S	R	S × R
Histamine	100	134.77 ± 12.85[a]	243.14 ± 10.09[b]	237.59 ± 14.47[b]	202.85 ± 16.67[b]	*	NS	**
	180	233.42 ± 11.88[a]	211.19 ± 15.71[a]	204.24 ± 16.74[a]	202.85 ± 14.24[a]			
Tyramine	100	185.19 ± 10.74[a]	152.61 ± 8.41[a]	183.48 ± 13.11[a]	209.20 ± 15.65[a]	**	***	***
	180	501.65 ± 7.28[a]	210.90 ± 17.26[b]	234.92 ± 16.35[b]	338.24 ± 14.15[c]			
Putrescine	100	35.26 ± 3.52	ND[3]	ND	ND	**	NS	NS
	180	6.61 ± 0.22	ND	ND	ND			
Tryptamine	100	124.17 ± 17.77	ND	ND	ND	**	NS	*
	180	ND	ND	ND	2.00 ± 0.66			
Cadaverine	100	191.59 ± 15.57[a]	6.39 ± 1.38[b]	6.39 ± 1.60[b]	1.28 ± 0.68[b]	***	NS	NS
	180	149.44 ± 13.86[a]	1.28 ± 0.61[b]	7.66 ± 1.58[b]	1.28 ± 0.65[b]			
Phenylethylamine	100	69.68 ± 13.58	ND	ND	ND	*	NS	NS
	180	19.63 ± 1.02	ND	ND	ND			
Total	100	740.65 ± 88.38[a]	402.13 ± 52.52[b]	427.44 ± 30.69[b]	413.32 ± 44.49[b]	***	NS	*
	180	910.74 ± 97.00[a]	423.38 ± 55.81[b]	446.82 ± 55.30[b]	478.84 ± 40.52[b]			

[a–c]Different superscripts within a row denote significant statistical differences ($P \leq 0.05$) between cheese batches.

[1]Results are expressed as mean ± SD; n = 72.

[2]S = season fixed effect; R = ripening time fixed effect; S × R = interaction between season and ripening fixed effects.

[3]ND = not detected. Limit of detection for putrescine, tryptamine, and phenylethylamine was 0.41, 0.26, and 1.53 mg/kg, respectively.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. NS: $P > 0.05$. Type of forage feeding fixed effect and remaining interactions between fixed effects were not statistically significant ($P > 0.05$).

Table 2. Texture profile analysis and color measurement at 100 and 180 d of ripening for the cheese samples made during the 4 seasons of the year with milk from sheep feed hay or silage

		Season										
		Summer		Autumn		Winter		Spring		*P*-value[2]		
Item[1]	Ripening time (d)	Hay	Silage	Hay	Silage	Hay	Silage	Hay	Silage	S	R	F
Fracturability (N)	100	83.08 ± 32.74^{a,A}	93.12 ± 28.90^{a,A}	78.32 ± 20.38^{a,A}	74.99 ± 29.03^{a,A}	70.30 ± 22.15^{a,A}	68.27 ± 13.82^{a,A}	64.52 ± 22.71^{a,A}	78.62 ± 23.85^{a,A}	**	*	NS
	180	101.86 ± 62.00^{a,B}	107.78 ± 71.64^{a,B}	81.66 ± 27.44^{ab,A}	76.24 ± 20.07^{ab,A}	58.46 ± 16.88^{b,B}	54.91 ± 13.71^{b,B}	69.75 ± 22.65^{ab,A}	81.98 ± 32.38^{ab,A}			
Hardness (N)	100	238.59 ± 71.80^{a,A}	260.72 ± 55.35^{a,A}	233.66 ± 72.84^{a,A}	222.47 ± 51.97^{ab,A}	194.10 ± 56.76^{a,A}	189.81 ± 54.78^{b,A}	208.79 ± 86.87^{a,A}	229.47 ± 82.69^{ab,A}	***	NS	NS
	180	284.46 ± 92.85^{a,A}	282.92 ± 90.43^{a,A}	231.93 ± 68.54^{ab,A}	224.28 ± 69.36^{ab,A}	204.69 ± 53.92^{b,A}	192.52 ± 51.93^{b,A}	227.90 ± 85.26^{ab,A}	230.80 ± 83.05^{ab,A}			
Adhesiveness (N·m)	100	−13.30 ± 1.39^{a,A}	−14.82 ± 1.05^{a,A}	−15.03 ± 1.85^{a,A}	−15.60 ± 1.79^{a,A}	−12.95 ± 1.57^{a,A}	−12.63 ± 0.62^{a,A}	−9.38 ± 0.46^{b,A}	−9.32 ± 0.56^{b,A}	*	*	NS
	180	−13.16 ± 1.57^{a,A}	−12.88 ± 1.38^{a,A}	−12.45 ± 1.17^{a,A}	−12.50 ± 1.58^{a,A}	−8.35 ± 1.24^{b,B}	−7.57 ± 0.46^{b,B}	−8.14 ± 0.43^{b,A}	−8.79 ± 0.55^{b,A}			
Cohesiveness	100	0.13 ± 0.01^{aA}	0.13 ± 0.01^{a,A}	0.16 ± 0.04^{b,A}	0.15 ± 0.00^{b,A}	0.12 ± 0.02^{a,A}	0.11 ± 0.02^{a,A}	0.13 ± 0.01^{a,A}	0.13 ± 0.01^{a,A}	**	NS	NS
	180	0.13 ± 0.00^{a,A}	0.13 ± 0.01^{a,A}	0.15 ± 0.01^{b,A}	0.14 ± 0.01^{b,A}	0.12 ± 0.01^{a,A}	0.12 ± 0.01^{a,A}	0.13 ± 0.02^{a,A}	0.13 ± 0.01^{a,A}			
Springiness	100	0.39 ± 0.07^{a,A}	0.40 ± 0.09^{a,A}	0.41 ± 0.07^{a,A}	0.42 ± 0.08^{a,A}	0.33 ± 0.05^{a,A}	0.36 ± 0.08^{a,A}	0.35 ± 0.02^{a,A}	0.35 ± 0.07^{a,A}	NS	NS	NS
	180	0.37 ± 0.07^{a,A}	0.35 ± 0.05^{a,A}	0.45 ± 0.03^{a,A}	0.40 ± 0.04^{a,A}	0.38 ± 0.09^{a,A}	0.47 ± 0.12^{a,A}	0.39 ± 0.14^{a,A}	0.40 ± 0.10^{a,A}			
Gumminess (N)	100	31.52 ± 6.78^{ab,A}	35.44 ± 9.18^{a,A}	36.36 ± 9.50^{a,A}	33.61 ± 8.92^{a,A}	22.10 ± 4.02^{b,A}	21.40 ± 6.17^{b,A}	25.88 ± 4.56^{b,A}	30.63 ± 3.52^{a,A}	***	NS	NS
	180	37.77 ± 11.18^{a,A}	37.38 ± 10.48^{a,A}	34.67 ± 7.64^{a,A}	32.33 ± 9.07^{ab,A}	23.22 ± 5.64^{a,A}	23.54 ± 4.97^{b,A}	30.30 ± 5.98^{ab,A}	29.06 ± 6.90^{ab,A}			
Chewiness (N)	100	12.32 ± 2.92^{ab,A}	13.97 ± 3.08^{a,A}	14.75 ± 6.05^{a,A}	14.01 ± 6.99^{a,A}	7.49 ± 2.53^{b,A}	8.03 ± 2.43^{a,A}	9.22 ± 3.87^{b,A}	10.95 ± 2.62^{a,A}	***	NS	NS
	180	14.32 ± 3.00^{a,A}	12.91 ± 5.81^{a,A}	15.42 ± 5.56^{a,A}	12.93 ± 6.60^{a,A}	8.72 ± 3.23^{b,A}	10.81 ± 2.00^{a,A}	11.39 ± 3.54^{ab,A}	11.91 ± 2.11^{a,A}			
L*	100	83.26 ± 7.81^{bc,A}	82.80 ± 7.92^{ab,A}	86.41 ± 11.95^{a,A}	86.81 ± 11.58^{b,A}	89.67 ± 8.42^{c,A}	88.90 ± 8.34^{a,A}	82.79 ± 11.59^{ab,A}	81.64 ± 7.03^{ab,A}	***	***	NS
	180	79.91 ± 7.43^{a,B}	79.26 ± 7.57^{a,B}	83.37 ± 9.89^{b,B}	81.81 ± 4.11^{ab,B}	81.39 ± 10.69^{b,B}	79.29 ± 9.99^{b,B}	79.90 ± 9.44^{b,B}	78.98 ± 10.34^{c,B}			
a*	100	−2.23 ± 0.51^{a,A}	−2.25 ± 0.44^{a,A}	−1.99 ± 0.59^{a,A}	−2.16 ± 0.38^{a,A}	−2.24 ± 0.86^{a,A}	−2.28 ± 0.79^{a,A}	−2.15 ± 0.76^{a,A}	−2.13 ± 0.82^{a,A}	NS	NS	NS
	180	−2.18 ± 0.36^{a,A}	−2.01 ± 0.44^{a,A}	−1.90 ± 0.48^{a,A}	−2.08 ± 0.52^{a,A}	−2.04 ± 0.86^{a,A}	−2.62 ± 0.89^{a,A}	−2.35 ± 0.59^{a,A}	−2.39 ± 0.72^{a,A}			
b*	100	17.50 ± 1.52^{a,A}	17.55 ± 2.13^{a,A}	16.76 ± 8.75^{a,A}	18.76 ± 10.62^{a,A}	16.46 ± 4.13^{a,A}	16.29 ± 4.26^{a,A}	14.76 ± 9.66^{a,A}	16.09 ± 7.18^{a,A}	NS	NS	NS
	180	17.94 ± 2.44^{a,A}	18.59 ± 4.02^{a,A}	16.76 ± 7.73^{a,A}	17.59 ± 6.11^{a,A}	16.45 ± 6.80^{a,A}	14.79 ± 8.03^{a,A}	15.72 ± 6.38^{a,A}	15.97 ± 6.93^{a,A}			

$^{a–c}$Means ± SD (n = 96 for texture and n = 144 for color) within a row with different superscripts (differences between the cheese batches in each season for the same ripening time point and type of forage feeding) are significantly different ($P \leq 0.05$).

^{A,B}Means ± SD (n = 96 for texture and n = 144 for color) within a column with different superscripts (differences between the cheese batches in each ripening time point for the same season and type of forage feeding) are significantly different ($P \leq 0.05$).

[1]The L*, a*, and b* color measurements were determined according to the CIELab color space, where L* corresponds to light–dark chromaticity (0% = dark to 100% = light), a* corresponds to green–red chromaticity (−60% = green to 60% = red), and b* corresponds to blue–yellow chromaticity (−60% = blue to 60% = yellow; O'Callaghan et al., 2017).

[2]S = season fixed effect; R = ripening time fixed effect; F = type of forage feeding fixed effect.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. NS: $P > 0.05$. Interactions between fixed effects were not significant ($P > 0.05$).

observed in these cheeses. Primary and secondary proteolysis during cheese ripening involves the breakdown of the caseins' elastic network and their transformation into more soluble compounds, reducing the adhesiveness as well as the gumminess, and therefore, chewiness values are reduced (Pinho et al., 2004). In addition, spring and winter cheeses showed lower values for fracturability, hardness, cohesiveness, and springiness than summer and autumn cheeses. It could be because low-molecular-weight compounds released during proteolysis contribute to an increase in pH values of cheese. This increase in pH leads to ionized carboxyl groups, which results in higher repulsion between proteins as well as in enhanced solubilization, with the consequent weakening of the protein matrix, thus decreasing the values of the aforementioned textural properties (Pastorino et al., 2003).

However, in the case of the color characteristics analyzed, the season of cheese-making as well as the ripening time had no significant effect ($P > 0.05$) on a* and b* values. In contrast, lightness (L*) decreased from 100 d to 180 d of ripening in all cheese samples, in concordance with what was described by Serrapica et al. (2020). Summer and spring cheeses had lower ($P \leq 0.001$) L* values than winter and autumn cheeses.

Multivariate statistical analysis of the sensory attribute scores was indicative of significant differences ($P > 0.05$) in some of the attributes considered for the cheese samples produced in different seasons (Table 3). In general, sensory characteristics of the cheese samples at 180 d of ripening were scored with significantly higher values ($P \leq 0.05$) than those ripened for 100 d. Summer and autumn cheeses ripened for 180 d showed the lowest scores for odor intensity (3.21),

Table 3. Sensory analysis of the cheese samples produced during the 4 seasons of the year after 100 and 180 d of ripening[1]

Sensory attribute	Ripening time (d)	Season				*P*-value[2]	
		Summer	Autumn	Winter	Spring	S	R
Appearance							
Color intensity	100	$3.78 \pm 0.32^{a,A}$	$3.21 \pm 0.63^{ab,A}$	$2.91 \pm 0.50^{b,A}$	$3.42 \pm 0.38^{ab,A}$	***	***
	180	$3.97 \pm 0.29^{a,A}$	$3.56 \pm 0.41^{b,A}$	$3.78 \pm 0.42^{ab,B}$	$4.00 \pm 0.48^{a,B}$		
Number of holes	100	$3.05 \pm 0.46^{a,A}$	$2.81 \pm 0.38^{a,A}$	$3.16 \pm 0.59^{a,A}$	$3.05 \pm 0.39^{a,A}$	NS	**
	180	$2.96 \pm 0.30^{a,A}$	$3.34 \pm 0.63^{a,A}$	$3.45 \pm 0.42^{a,A}$	$3.56 \pm 0.33^{a,B}$		
Hole homogeneity	100	$3.38 \pm 0.63^{a,A}$	$3.14 \pm 0.28^{a,A}$	$3.62 \pm 0.43^{a,A}$	$3.19 \pm 0.41^{a,A}$	NS	NS
	180	$2.97 \pm 0.19^{a,A}$	$3.34 \pm 0.48^{a,A}$	$3.50 \pm 0.31^{a,A}$	$3.58 \pm 0.34^{a,A}$		
Odor							
Odor intensity	100	$2.93 \pm 0.19^{a,A}$	$3.06 \pm 0.41^{b,A}$	$3.07 \pm 0.44^{b,A}$	$3.08 \pm 0.27^{b,A}$	*	*
	180	$3.21 \pm 0.33^{a,A}$	$3.21 \pm 0.27^{a,A}$	$3.30 \pm 0.26^{b,A}$	$3.56 \pm 0.28^{c,B}$		
Pungent	100	$1.28 \pm 0.11^{a,A}$	$1.25 \pm 0.06^{a,A}$	$1.18 \pm 0.14^{a,A}$	$1.06 \pm 0.03^{b,A}$	***	NS
	180	$1.42 \pm 0.18^{a,A}$	$1.18 \pm 0.10^{b,A}$	$1.18 \pm 0.08^{b,A}$	$1.19 \pm 0.08^{b,A}$		
Moldy	100	$1.09 \pm 0.04^{a,A}$	$1.07 \pm 0.06^{a,A}$	$1.06 \pm 0.03^{a,A}$	$1.03 \pm 0.02^{a,A}$	*	***
	180	$1.21 \pm 0.09^{a,B}$	$1.17 \pm 0.11^{ab,B}$	$1.11 \pm 0.05^{bc,B}$	$1.04 \pm 0.03^{c,A}$		
Taste							
Taste intensity	100	$3.84 \pm 0.19^{a,A}$	$3.61 \pm 0.17^{a,A}$	$3.75 \pm 0.44^{a,A}$	$3.87 \pm 0.33^{a,A}$	**	***
	180	$4.50 \pm 0.29^{a,B}$	$4.02 \pm 0.44^{b,B}$	$4.10 \pm 0.16^{ab,B}$	$4.32 \pm 0.28^{ab,B}$		
Saltiness	100	$3.49 \pm 0.25^{a,A}$	$3.26 \pm 0.19^{ab,A}$	$3.00 \pm 0.26^{b,A}$	$3.01 \pm 0.25^{b,A}$	***	***
	180	$4.03 \pm 0.29^{a,B}$	$3.45 \pm 0.56^{b,A}$	$3.69 \pm 0.17^{ab,B}$	$3.33 \pm 0.27^{b,B}$		
Bitterness	100	$2.79 \pm 0.17^{a,A}$	$2.13 \pm 0.18^{b,A}$	$1.97 \pm 0.28^{bc,A}$	$1.75 \pm 0.21^{c,A}$	***	**
	180	$2.90 \pm 0.33^{a,A}$	$1.95 \pm 0.21^{b,A}$	$1.57 \pm 0.21^{c,B}$	$1.54 \pm 0.13^{c,B}$		
Texture							
Firmness	100	$4.60 \pm 0.30^{a,A}$	$4.42 \pm 0.38^{ab,A}$	$4.04 \pm 0.30^{b,A}$	$4.47 \pm 0.44^{ab,A}$	***	***
	180	$4.93 \pm 0.34^{a,A}$	$4.54 \pm 0.40^{ab,A}$	$4.48 \pm 0.15^{b,B}$	$4.86 \pm 0.26^{ab,B}$		
Elasticity	100	$3.60 \pm 0.14^{a,A}$	$4.32 \pm 0.44^{b,A}$	$4.21 \pm 0.24^{b,A}$	$3.97 \pm 0.26^{b,A}$	***	NS
	180	$3.53 \pm 0.22^{a,A}$	$4.26 \pm 0.27^{b,A}$	$4.19 \pm 0.16^{b,A}$	$3.91 \pm 0.09^{c,A}$		
Friability	100	$3.55 \pm 0.16^{a,A}$	$3.26 \pm 0.35^{a,A}$	$3.54 \pm 0.28^{a,A}$	$3.25 \pm 0.29^{a,A}$	*	***
	180	$4.01 \pm 0.24^{a,B}$	$3.79 \pm 0.19^{ab,B}$	$3.48 \pm 0.42^{b,A}$	$3.92 \pm 0.21^{a,B}$		
Adhesiveness	100	$3.61 \pm 0.08^{a,A}$	$3.48 \pm 0.28^{a,A}$	$3.04 \pm 0.14^{b,A}$	$3.17 \pm 0.16^{b,A}$	***	***
	180	$3.28 \pm 0.18^{a,B}$	$3.87 \pm 0.34^{b,B}$	$3.24 \pm 0.20^{a,B}$	$3.68 \pm 0.21^{b,B}$		

[a–c]Different superscripts within a row denote significant statistical differences ($P \leq 0.05$) between cheese batches (differences between cheese batches in each season for the same ripening time point).

[A,B]Different superscripts within a column denote significant statistical differences ($P \leq 0.05$) between cheese batches (differences between cheese batches in each ripening time point for the same season).

[1]Results are expressed as mean ± SD; n = 480.

[2]S = season fixed effect; R = ripening time fixed effect.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. NS: $P > 0.05$. Type of forage feeding fixed effect and remaining interactions between fixed effects were not significant ($P > 0.05$).

whereas spring cheeses scored 0.35 point more for this sensory characteristic. This could be explained by the positive correlation ($\rho = 0.87$; $P \leq 0.001$) observed between the TFAA content and odor intensity scores in the cheese samples because it has been described that AA catabolism is a major process for aroma formation in cheese (Yvon and Rijnen, 2001). Furthermore, in all cheese samples, off-aromas such as pungent and moldy scored 1 point (i.e., nonexistent) on the perception scale. Regarding the taste attribute, summer cheeses showed the highest values for taste intensity, saltiness, and bitterness (4.17, 3.78, and 2.97, respectively). The presence of bitterness in cheeses is very frequent as a consequence of the release of hydrophobic peptides of medium or small size. As ripening progresses, they are hydrolyzed to AA, decreasing the amount of these compounds related to bitterness in cheese (McSweeney, 2007). Additionally, differences observed between the cheese samples for elasticity, adhesiveness, friability, and firmness were less than 1 point.

CONCLUSIONS

This study showed that 2 common methods of forage conservation, haymaking and ensiling, did not affect the free AA profile, biogenic amine content, or sensory characteristics of cheeses produced with milk from commercial sheep flocks fed these types of forages. This is of great interest because conserved forages constitute a low-cost feed alternative source. In contrast, season of cheese-making as well as ripening time highly influenced cheese microbial populations and quality. Lactobacilli were the main LAB present in cheese samples, playing a relevant role in the development of cheese characteristics. It should be highlighted that cheeses produced in spring and ripened for 100 and 180 d showed greater proteolysis than cheeses manufactured in other seasons, leading to high contents of GABA and ornithine, which have been associated with beneficial effects on human health. These cheeses also showed low biogenic amine contents as well as lower texture values for texture and L* attributes. Nevertheless, spring cheeses were scored with good sensory characteristics by panelists.

ACKNOWLEDGMENTS

This work was supported by the Ministerio de Economia y Competitividad (project AGL2016-75159-C2-2-R; Madrid, Spain) and the Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (project TA2014-00069-CO2-02; Madrid, Spain). The authors also acknowledge Entrepinares S.A.U. Company (Valladolid, Spain) for their assistance in the cheese-making trials. The authors have not stated any conflicts of interest.

REFERENCES

Addis, M., A. Cabiddu, G. Pinna, M. Decandia, G. Piredda, A. Pirisi, and G. Molle. 2005. Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9,*trans*-11. J. Dairy Sci. 88:3443–3454. https://doi.org/10.3168/jds.S0022-0302(05)73028-9.

Azarnia, S., N. Robert, and B. Lee. 2006. Biotechnological methods to accelerate cheddar cheese ripening. Crit. Rev. Biotechnol. 26:121–143. https://doi.org/10.1080/07388550600840525.

Balthazar, C. F., T. C. Pimentel, L. L. Ferrão, C. N. Almada, A. Santillo, M. Albenzio, N. Mollakhalili, A. M. Mortazavian, J. S. Nascimento, M. C. Silva, M. Q. Freitas, A. S. Sant'Ana, D. Granato, and A. G. Cruz. 2017. Sheep milk: Physicochemical characteristics and relevance for functional food development. Compr. Rev. Food Sci. Food Saf. 16:247–262. https://doi.org/10.1111/1541-4337.12250.

Benkerroum, N. 2016. Biogenic amines in dairy products: Origin, incidence, and control means. Compr. Rev. Food Sci. Food Saf. 15:801–826. https://doi.org/10.1111/1541-4337.12212.

Bergamini, C. V., I. V. Wolf, M. C. Perotti, and C. A. Zalazar. 2010. Characterisation of biochemical changes during ripening in Argentinean sheep cheeses. Small Rumin. Res. 94:79–89. https://doi.org/101016/j.smallrumres.2010.07.004.

Blaya, J., Z. Barzideh, and G. LaPointe. 2018. Symposium review: Interaction of starter cultures and nonstarter lactic acid bacteria in the cheese environment. J. Dairy Sci. 101:3611–3629. https://doi.org/10.3168/jds.2017-13345.

Bustamante, M. A., M. Virto, M. Aramburu, L. J. R. Barron, M. Pérez-Elortondo, F. J. Albisu, and M. de Renobales. 2003. Lamb rennet paste in ovine cheese (Idiazábal) manufacture. Proteolysis and relationship between analytical and sensory parameters. Int. Dairy J. 13:547–557. https://doi.org/10.1016/S0958-6946(03)00077-3.

Cabiddu, A., M. Decandia, M. Addis, G. Piredda, A. Pirisi, and G. Molle. 2005. Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk. Small Rumin. Res. 59:169–180. https://doi.org/10.1016/j.smallrumres.2005.05.005.

Civelek, I., and A. Cagri-Mehmetoglu. 2019. Determination of antifungal effect of edible coatings containing *Williopsis saturnus* var. saturnus against yeast and mold growth on Kashar cheese. J. Food Sci. 84:311–318. https://doi.org/10.1111/1750-3841.14431.

Coulon, J. B., A. Delacroix-Buchet, B. Martin, and A. Pirisi. 2004. Relationships between ruminant management and sensory characteristics of cheeses: A review. Lait 84:221–241. https://doi.org/10.1051/lait:2004008.

Dewhurst, R. J., K. J. Shingfield, M. R. F. Lee, and N. D. Scollan. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim. Feed Sci. Technol. 131:168–206. https://doi.org/10.1016/j.anifeedsci.2006.04.016.

Diana, M., M. Rafecas, C. Arco, and J. Quílez. 2014. Free amino acid profile of Spanish artisanal cheeses: Importance of gamma-aminobutyric acid (GABA) and ornithine content. J. Food Compos. Anal. 35:94–100. https://doi.org/10.1016/j.jfca.2014.06.007.

Diez-Gutiérrez, L., L. San Vicente, L. J. R. Barrón, M. C. Villarán, and M. Chávarri. 2020. Gamma-aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. J. Funct. Foods 64:103669. https://doi.org/10.1016/j.jff.2019.103669.

Diezhandino, I., D. Fernández, L. González, P. L. H. McSweeney, and J. M. Fresno. 2015. Microbiological, physico-chemical and proteolytic changes in a Spanish blue cheese during ripening (Valdeón cheese). Food Chem. 168:134–141. https://doi.org/10.1016/j.foodchem.2014.07.039.

EFSA. 2011. Scientific opinion on risk based control of biogenic amine formation in fermented foods. EFSA J. 9993:1–93.

FIL-IDF (International Dairy Federation). 1992. Milk and Milk Products. Preparation of Test Samples and Dilutions for Microbiological Examination. FIL-IDF, Brussels, Belgium.

Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017. Biochemistry of Cheese Ripening. 2nd ed. Springer US, Boston, MA.

Freitas, A. C., J. M. Fresno, B. Prieto, I. Franco, F. X. Malcata, and J. Carballo. 1998. Influence of milk source and ripening time on free amino acid profile of Picante cheese. Food Control 9:187–194. https://doi.org/10.1016/S0956-7135(97)00073-X.

Fresno, M., and S. Álvarez. 2012. Chemical, textural and sensorial changes during the ripening of Majorero goat cheese. Int. J. Dairy Technol. 65:393–400. https://doi.org/10.1111/j.1471-0307.2012.00842.x.

Glasser, F., M. Doreau, G. Maxin, and R. Baumont. 2013. Fat and fatty acid content and composition of forages: A meta-analysis. Anim. Feed Sci. Technol. 185:19–34. https://doi.org/10.1016/j.anifeedsci.2013.06.010.

Gorostiza, A., A. J. Cichoscki, A. T. Valduga, E. Valduga, A. Bernardo, and J. M. Fresno. 2004. Changes in soluble nitrogenous compounds, caseins and free amino acids during ripening of artisanal Prato cheese: A Brazilian semi-hard cows variety. Food Chem. 85:407–414. https://doi.org/10.1016/j.foodchem.2003.07.018.

Gaya, P., C. Sánchez, M. Nunez, and E. Fernández-García. 2005. Proteolysis during ripening of Manchego cheese made from raw or pasteurized ewes' milk. Seasonal variation. J. Dairy Res. 72:287–295. https://doi.org/10.1017/S0022029905001019.

Hernández, I., L. J. R. Barrón, M. Virto, C. Pérez-Elortondo, C. Flanagan, U. Rozas, A. I. Nájera, M. Albisu, M. S. Vicente, and M. de Renobales. 2009. Lipolysis, proteolysis and sensory properties of ewe's raw milk cheese (Idiazábal) made with lipase addition. Food Chem. 116:158–166. https://doi.org/10.1016/j.foodchem.2009.02.026.

ISO (International Organization for Standardization). 2005. Sensory analysis—Methodology—General guidance. Standard no. 6658. ISO, Geneva, Switzerland.

Kalač, P., and E. Samková. 2010. The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. Czech J. Anim. Sci. 55:521–537. https://doi.org/10.17221/2485-CJAS.

Kilcawley, K. N. 2017. Cheese flavour. Pages 443–474 in Fundamentals of Cheese Science. 2nd ed. P. F. Fox, T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney, ed. Springer US, Boston, MA.

Kurata, K., M. Nagasawa, S. Tomonaga, M. Aoki, S. Akiduki, K. Morishita, D. M. Denbow, and M. Furuse. 2012. Orally administered L-ornithine reduces restraint stress-induced activation of the hypothalamic-pituitary-adrenal axis in mice. Neurosci. Lett. 506:287–291. https://doi.org/10.1016/j.neulet.2011.11.024.

Lacroix, N., D. St-Gelais, C. P. Champagne, and J. C. Vuillemard. 2013. Gamma-aminobutyric acid-producing abilities of lactococcal strains isolated from old-style cheese starters. Dairy Sci. Technol. 93:315–327. https://doi.org/10.1007/s13594-013-0127-4.

Ladero, V., M. Calles-Enríquez, M. Fernández, and M. A. Álvarez. 2010. Toxicological effects of dietary biogenic amines. Curr. Nutr. Food Sci. 6:145–156. https://doi.org/10.2174/157340110791233256.

Ladero, V., E. Sánchez-Llana, M. Fernández, and M. A. Alvarez. 2011. Survival of biogenic amine-producing dairy LAB strains at pasteurisation conditions. Int. J. Food Sci. Technol. 46:516–521. https://doi.org/10.1111/j.1365-2621.2010.02508.x.

Linares, D. M., B. del Rio, B. Redruello, V. Ladero, M. C. Martin, M. Fernandez, P. Ruas-Madiedo, and M. A. Alvarez. 2016. Comparative analysis of the in vitro cytotoxicity of the dietary biogenic amines tyramine and histamine. Food Chem. 197:658–663. https://doi.org/10.1016/j.foodchem.2015.11.013.

Linares, D. M., M. Martín, V. Ladero, M. A. Alvarez, and M. Fernández. 2011. Biogenic amines in dairy products. Crit. Rev. Food Sci. Nutr. 51:691–703. https://doi.org/10.1080/10408398.2011.582813.

Manca, G., A. Porcu, A. Ru, M. Salaris, M. A. Franco, and E. P. L. De Santis. 2015. Comparison of γ-aminobutyric acid and biogenic amine content of different types of ewe's milk cheese produced in Sardinia, Italy. Ital. J. Food Saf. 4:4700. https://doi.org/10.4081/ijfs.2015.4700.

Martínez, S., I. Franco, and J. Carballo. 2011. Spanish goat and sheep milk cheeses. Small Rumin. Res. 101:41–54. https://doi.org/10.1016/j.smallrumres.2011.09.024.

McSweeney, P. L. H. 2007. Cheese Manufacture and Ripening and Their Influence on Cheese Flavour. B. C. Weimer, ed. Academic Press, Amsterdam, the Netherlands.

Metz, M., J. Sheehan, and P. C. H. Feng. 2020. Use of indicator bacteria for monitoring sanitary quality of raw milk cheeses—A literature review. Food Microbiol. 85:103283. https://doi.org/10.1016/j.fm.2019.103283.

Morand-Fehr, P., V. Fedele, M. Decandia, and Y. Le Frileux. 2007. Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Rumin. Res. 68:20–34. https://doi.org/10.1016/j.smallrumres.2006.09.019.

Mrkonjic Fuka, M., I. Zgomba Maksimovic, A. Tanuwidjaja, N. Hulak, and M. Schloter. 2017. Characterization of enterococcal community isolated from an artisan Istrian raw milk cheese: Biotechnological and safety aspects. Food Technol. Biotechnol. 55:368–380. https://doi.org/10.17113/ftb.55.03.17.5118.

Niro, S., M. Succi, P. Tremonte, E. Sorrentino, R. Coppola, G. Panfili, and A. Fratianni. 2017. Evolution of free amino acids during ripening of Caciocavallo cheeses made with different milks. J. Dairy Sci. 100:9521–9531. https://doi.org/10.3168/jds.2017-13308.

Novella-Rodríguez, S., M. Veciana-Nogués, A. Roig-Sagués, A. Trujillo-Mesa, and M. C. Vidal-Carou. 2002. Influence of starter and non starter on the formation of biogenic amine in goat cheese during ripening. J. Dairy Sci. 85:2471–2478. https://doi.org/10.3168/jds.S0022-0302(02)74329-4.

Nudda, A., G. Battacone, O. Boaventura Neto, A. Cannas, A. H. D. Francesconi, A. S. Atzori, and G. Pulina. 2014. Feeding strategies to design the fatty acid profile of sheep milk and cheese. Rev. Bras. Zootec. 43:445–456. https://doi.org/10.1590/S1516-35982014000800008.

O'Callaghan, T. F., D. T. Mannion, D. Hennessy, S. McAuliffe, M. G. O'Sullivan, N. Leeuwendaal, T. P. Beresford, P. Dillon, K. N. Kilcawley, J. J. Sheehan, R. P. Ross, and C. Stanton. 2017. Effect of pasture versus indoor feeding systems on quality characteristics, nutritional composition, and sensory and volatile properties of full-fat Cheddar cheese. J. Dairy Sci. 100:6053–6073. https://doi.org/10.3168/jds.2016-12508.

Okada, T., T. Sugishita, T. Murakami, H. Murai, T. Saikusa, T. Horino, A. Onoda, O. Kajimoto, R. Takahashi, and T. Takahashi. 2000. Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. Nippon Shokuhin Kagaku Kogaku Kaishi 47:596–603. https://doi.org/10.3136/nskkk.47.596.

Panthi, R. R., A. L. Kelly, D. Hennessy, M. G. O'Sullivan, K. N. Kilcawley, D. T. Mannion, M. A. Fenelon, and J. J. Sheehan. 2019. Effect of pasture versus indoor feeding regimes on the yield, composition, ripening and sensory characteristics of Maasdam cheese. Int. J. Dairy Technol. 72:435–446. https://doi.org/10.1111/1471-0307.12590.

Pappa, E. C., I. Kandarakis, E. M. Anifantakis, and G. K. Zerfiridis. 2006. Influence of types of milk and culture on the manufacturing practices, composition and sensory characteristics of Teleme cheese during ripening. Food Control 17:570–581. https://doi.org/10.1016/j.foodcont.2005.03.004.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003. Effect of pH on the chemical composition and structure-function relationships of Cheddar cheese. J. Dairy Sci. 86:2751–2760. https://doi.org/10.3168/jds.S0022-0302(03)73871-5.

Pinho, O., I. M. Ferreira, E. Mendes, B. M. Oliveira, and M. Ferreira. 2001. Effect of temperature on evolution of free amino acid and biogenic amine contents during storage of Azeitão cheese. Food Chem. 75:287–291. https://doi.org/10.1016/S0308-8146(01)00109-1.

Pinho, O., E. Mendes, M. M. Alves, and I. M. P. L. V. O. Ferreira. 2004. Chemical, physical, and sensorial characteristics of "Terrincho" ewe cheese: Changes during ripening and intravarietal comparison. J. Dairy Sci. 87:249–257. https://doi.org/10.3168/jds.S0022-0302(04)73163-X.

Pouliot-Mathieu, K., C. Gardner-Fortier, S. Lemieux, D. St-Gelais, C. P. Champagne, and J. C. Vuillemard. 2013. Effect of cheese containing gamma-aminobutyric acid-producing lactic acid bacteria on blood pressure in men. PharmaNutrition 1:141–148. https://doi.org/10.1016/j.phanu.2013.06.003.

Poveda, J. M., L. Cabezas, and P. L. H. McSweeney. 2004. Free amino acid content of Manchego cheese manufactured with different starter cultures and changes throughout ripening. Food Chem. 84:213–218. https://doi.org/10.1016/S0308-8146(03)00204-8.

Poveda, J. M., R. Chicón, and L. Cabezas. 2015. Biogenic amine content and proteolysis in Manchego cheese manufactured with *Lactobacillus paracasei* ssp. *paracasei* as adjunct and other autochthonous strains as starters. Int. Dairy J. 47:94–101. https://doi.org/10.1016/j.idairyj.2015.03.004.

Poveda, J. M., G. M. Molina, and S. Gómez-Alonso. 2016. Variability of biogenic amine and free amino acid concentrations in regionally produced goat milk cheeses. J. Food Compos. Anal. 51:85–92. https://doi.org/10.1016/j.jfca.2016.06.012.

Pyz-Łukasik, R., P. Knysz, and M. Gondek. 2018. Hygiene quality and consumer safety of traditional short- and long-ripened cheeses from Poland. J. Food Qual. 2018:1–7. https://doi.org/10.1155/2018/8732412.

Redruello, B., V. Ladero, I. Cuesta, J. R. Álvarez-Buylla, M. C. Martín, M. Fernández, and M. A. Alvarez. 2013. A fast, reliable, ultra high performance liquid chromatography method for the simultaneous determination of amino acids, biogenic amines and ammonium ions in cheese, using diethyl ethoxymethylenemalonate as a derivatising agent. Food Chem. 139:1029–1035. https://doi.org/10.1016/j.foodchem.2013.01.071.

Renes, E., F. De la Fuente, D. Fernández, M. E. Tornadijo, and J. M. Fresno. 2018. Effect of feeding regimen on the fatty acid profile of sheep bulk tank milk. Int. J. Dairy Technol. 71:857–867. https://doi.org/10.1111/1471-0307.12553.

Renes, E., I. Diezhandino, D. Fernández, R. E. Ferrazza, M. E. Tornadijo, and J. M. Fresno. 2014. Effect of autochthonous starter cultures on the biogenic amine content of ewe's milk cheese throughout ripening. Food Microbiol. 44:271–277. https://doi.org/10.1016/j.fm.2014.06.001.

Renes, E., P. Gómez-Cortés, M. A. de la Fuente, D. Fernández, M. E. Tornadijo, and J. M. Fresno. 2020. Effect of forage type in the ovine diet on the nutritional profile of sheep milk cheese fat. J. Dairy Sci. 103:63–71. https://doi.org/10.3168/jds.2019-17062.

Renes, E., V. Ladero, M. E. Tornadijo, and J. M. Fresno. 2019. Production of sheep milk cheese with high γ-aminobutyric acid and ornithine concentration and with reduced biogenic amines level using autochthonous lactic acid bacteria strains. Food Microbiol. 78:1–10. https://doi.org/10.1016/j.fm.2018.09.003.

Santiago-López, L., J. E. Aguilar-Toalá, B. Hernández-Mendoza, A. Vallejo-Cordoba, A. M. Liceaga, and A. F. González-Córdova. 2018. Bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption. J. Dairy Sci. 101:3742–3757. https://doi.org/10.3168/jds.2017-13465.

Schirone, M., R. Tofalo, G. Fasoli, G. Perpetuini, A. Corsetti, A. C. Manetta, A. Ciarrocchi, and G. Suzzi. 2013. High content of biogenic amines in Pecorino cheeses. Food Microbiol. 34:137–144. https://doi.org/10.1016/j.fm.2012.11.022.

Serrapica, F., P. Uzun, F. Masucci, F. Napolitano, A. Braghieri, A. Genovese, R. Sacchi, R. Romano, C. M. A. Barone, and A. Di Francia. 2020. Hay or silage? How the forage preservation method changes the volatile compounds and sensory properties of Caciocavallo cheese. J. Dairy Sci. 103:1391–1403. https://doi.org/10.3168/jds.2019-17155.

Settanni, L., and G. Moschetti. 2010. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiol. 27:691–697. https://doi.org/10.1016/j.fm.2010.05.023.

Sugino, T., T. Shirai, Y. Kajimoto, and O. Kajimoto. 2008. L-Ornithine supplementation attenuates physical fatigue in healthy volunteers by modulating lipid and amino acid metabolism. Nutr. Res. 28:738–743. https://doi.org/10.1016/j.nutres.2008.08.008.

Tofalo, R., G. Perpetuini, N. Battistelli, A. Pepe, A. Ianni, G. Martino, and G. Suzzi. 2019. Accumulation γ-aminobutyric acid and biogenic amines in a traditional raw milk ewe's cheese. Foods 8:401–414. https://doi.org/10.3390/foods8090401.

Upadhyay, V. K., P. L. H. McSweeney, A. A. A. Magboul, and P. F. Fox. 2004. Proteolysis in cheese during ripening. Pages 391–433 in Cheese: Chemistry, Physics and Microbiology. 3rd ed. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Academic Press, Amsterdam, the Netherlands.

Yvon, M., and L. Rijnen. 2001. Cheese flavour formation by amino acid catabolism. Int. Dairy J. 11:185–201. https://doi.org/10.1016/S0958-6946(01)00049-8.