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Characterisation of biochemical changes during ripening in Argentinean sheep cheeses

C.V. Bergamini*, I.V. Wolf, M.C. Perotti, C.A. Zalazar

Instituto de Lactología Industrial, Universidad Nacional del Litoral - Consejo Nacional de Investigaciones Científicas y Técnicas, Santiago del Estero 2829, S3000AOM, Santa Fe, Argentina

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ABSTRACT

In recent years, there has been an increase in the production of sheep milk in our country, which has been used mainly in cheese-making. This production, however, is not well standardised, as there are neither defined protocols nor well-characterised products for this procedure. Previously, different methodologies for making two types of sheep cheeses were developed at our institute. In the present work, the levels of free amino acids, organic acids, free fatty acids and volatile compounds for these sheep cheeses were studied in order to thoroughly characterise them. Therefore, we have produced two types of cheeses: one using a starter of *Streptococcus thermophilus*, in which the curd was cut into 5-mm pieces, washed and then heated to 43 °C (S cheeses); the other one using a mixed starter composed of *S. thermophilus*, *Lactobacillus helveticus* and *Lactobacillus bulgaricus*, in which the curd was cut into smaller pieces, was not washed and was heated to 47 °C (L cheeses). These cheeses were analysed at 2 and 180 days of ripening. Free amino acids (FAAs) and organic acids were studied by HPLC, free fatty acids (FFAs) were quantified by GC and volatile compounds were analysed by SPME-GC-FID/MS. The concentrations of all FAAs were significantly higher in the L cheeses than in the S cheeses, and this was evident from the beginning of the ripening. Both types of cheeses showed similar changes in the concentrations of some FAAs during ripening, but S cheeses were characterised by higher percentages of Phe, Leu and Val, while L cheeses had higher percentages of Pro, Ile, His and Asp. Lactic and citric acid were the most important organic acids present in both types of cheeses. At the end of the ripening, L cheeses presented higher levels of succinic and formic acids, while S cheeses showed a much higher amount of acetic acid. The levels of FFAs increased during ripening, and myristic, palmitic, stearic and oleic acids were the most abundant ones in both types of cheeses. L cheeses showed significantly higher levels of all FFAs at the end of the ripening and presented a greater increase in the percentages of short-chain fatty acids during ripening compared to S cheeses. Regarding volatile compounds, higher levels of aldehydes and ketones characterised the L cheeses, whereas S cheeses had higher proportions of esters and alcohols. Both types of cheeses presented similar areas in the most compounds of acids group, but the levels of butanoic and hexanoic acids were significantly higher in S cheeses than in L cheeses. The results of the present work offer an important contribution to this field: they have provided a better understanding of the changes that occur during the ripening of the two sheep cheeses manufactured with technologies developed in our group. These technologies could also be used by small sheep farm producers located in our region with the aim of increasing the economic yield of their products.

* Corresponding author. Tel.: +54 342 453 0302; fax: +54 342 457 1162.
E-mail address: cvberg@fiq.unl.edu.ar (C.V. Bergamini).

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1. Introduction

The highest production of sheep milk and sheep dairy products is concentrated in the Mediterranean region. Some of the most popular sheep cheeses around the world are Roquefort (France), Feta and Teleme (Greece), Pecorino (Italy), Idiazabal and Manchego (Spain) and Serra da Estrela (Portugal) (Kalantzopoulos, 1993). These cheeses, together with other less popular ones, have previously been characterised in different studies (Raynal-Ljutovac et al., 2008).

However, sheep cheese production in Argentina has not been developed much until now, as sheep have been primarily used for their wool and meat. As a result, sheep cheeses are produced in only a few plants distributed mostly in the provinces of Buenos Aires and Chubut (Mc Cormick and Lynch, 2003). In Santa Fe, the province in which our Instituto de Lactología Industrial is located, the production of sheep cheese is almost nonexistent, but there are some sheep farms that are seeking another way to expand their production possibilities and take advantage of all of their available resources. Therefore, sheep cheese production seems to be a good opportunity. The Escuela de Agricultura, Ganadería y Granja (EAGyG), a secondary school that works in association with our University, has a small sheep herd, which had not been previously exploited for cheese production. Therefore, because we have a lot of experience in cow cheese production, we faced the challenge of developing the technology for sheep cheese production by setting up a small plant at the EAGyG. This small plant would also set an example to other sheep farms of how profitable cheese production could be for them too.

At our Institute, some of the well-known technologies and starters were adapted for the cheese-making of two types of sheep cheeses: S cheeses with a starter of *Streptococcus thermophilus* and L cheeses with a starter of a mixture of *S. thermophilus*, *Lactobacillus helveticus* and *Lactobacillus bulgaricus* (Candioti et al., 2010). Gross composition, pH and proteolysis of these cheeses have been described in a previous work (Candioti et al., 2010). S and L cheeses presented differences regarding pH and secondary proteolysis (soluble fraction in trichloroacetic acid 12% and phosphotungstic acid 2.5%); L cheeses had lower pH and showed more proteolysis than S cheeses. Moreover, a great diminution of the lactobacilli in the starter was observed in L cheeses during ripening, and these cheeses were characterised by a more intense flavour than S cheeses. It is important to mention that the milk used for this study was from Pampinta ewes, a native breed from Frisia and Corriedale, which was developed by the Instituto Nacional de Tecnología Agropecuaria (INTA), Anguil, La Pampa, Argentina, an institute that has extensive expertise in the management of sheep herds. By means of this study, we obtained valuable information for an initial characterisation of Argentinean sheep cheeses, as there were no data previously published about them.

Important biochemical events such as proteolysis, lipolysis and glycolysis occur in the cheese matrix during ripening, which are responsible for the final characteristics of the taste, aroma and texture of the product. These enzymatic processes must occur in a coordinated way in order to give a unique and well-appreciated flavour characteristic to each type of cheese (Fox and McSweeney, 2004). The FAAs and FFAs produced during proteolysis and lipolysis may contribute directly to the cheese flavour, but their most important contribution is made indirectly, via the production of substrates for further catabolic reactions in which large amounts of volatile compounds are produced (Marilley and Casey, 2004).

In the present work, we describe in detail the characterisation of two different Argentinean sheep cheeses by studying the changes in free amino acids, organic acids, free fatty acids and volatile compounds during ripening.

2. Materials and methods

2.1. Cheese-making

Raw sheep milk, provided by INTA (Instituto Nacional de Tecnología Agropecuaria) (Anguil, La Pampa, Argentina) was refrigerated and transported at 4 °C to the pilot plant of Instituto de Lactología Industrial (INLAIN) during all of the sheep milking period (October to March). On each cheese-making day, 40 L of sheep milk was batch pasteurised at 65 °C for 20 min, and cooled down to 36 °C. Then calcium chloride (Merck, Darmstadt, Germany) was added to a final concentration of 0.02% (w/v). After that, the milk was divided into two aliquots of 20 L each, one destined for the making of S cheeses and the other for L cheeses. A lyophilised culture of *S. thermophilus* (Chr. Hansen) was added as the primary starter for S cheeses to reach a final concentration of 10^6 CFU mL^{-1} in the cheese milk. Then, 15 min later, 0.014 g L^{-1} of chymosin (Maxiren® 150, France) was added, and when the curd reached the appropriate strength, this batch was cut into 5-mm pieces. The curd was washed with hot water, which replaced 10% of the whey. After that, the mixture was heated to 43 °C, and later, the curd was finally moulded.

For L cheeses, a mix of lyophilised cultures of *S. thermophilus* (60%), *L. helveticus* (20%) and *L. bulgaricus* (20%) (all of Chr. Hansen) was used as the primary starter and was added to reach a final concentration of 10^6 CFU mL^{-1}. This cheese-making process differed from the one used for S cheeses in the following ways: the curd was not washed and was cut into smaller pieces, and, finally, the heat treatment was at 47 °C.

Both S and L cheeses were pressed during 18 h, brined in 20% w/v, pH 5.4 brine for 7 h. Cheeses were ripened at 12 °C and at 80% relative humidity for 6 months. They were sampled at the beginning of the ripening (at 2 days) and at 180 days of ripening. Four cheese replicates were made on different fabrication days, with different milk obtained throughout the whole sheep milking period. In addition, on each fabrication day, four cheeses of approximately 700 g each for each type of cheese were made, using the same milk, that is, four S cheeses and four L cheeses. On each sampling day, a different cheese of each type (S and L cheeses) was sampled, and approximately 100 g of cheese was taken, finely grated and frozen (−20 °C) for the analysis of free amino acids, free fatty acids and organic acids. For the analysis of volatile compounds, the cheese samples were sliced as wedges, wrapped in aluminium foil and stored at −20 °C until analysis.

2.2. Free amino acids

A pre-column derivatization method using 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate (AQC) followed by high-performance liquid chromatography was used for the FAAs analysis in cheese samples. For that, the Chemistry Package of the Waters AccQ·Tag® Amino Analysis Method (Waters Corporation, Mildford, MA, USA) was employed, which comprises the reagent kit for the derivatization reaction, the column, a mixture of amino acid standard, sample tubes and the eluents. The HPLC equipment consisted of a quaternary pump, an on-line degasser and UV/VIS detector, all Series 200 (Perkin Elmer, Norwalk, CT, USA). An interface module connected to a computer was used for acquisition of chromatographic data with the software Turbochrom® (Perkin Elmer, Norwalk, CT, USA). A 3.9 mm × 150 mm Nova-Pak™ C_{18}, 4 μm column (Waters Corporation, Mildford, MA, USA) specifically certified for use with the AccQ·Tag Method and a 15 mm × 3.2 mm, 7 μm guard column (Perkin Elmer, Norwalk, CT, USA) were used. Sample preparation, derivatization reaction and chromatographic separation were performed according to Bergamini et al. (2009).