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Effect of brine concentration on the ripening of an Argentinean sheep's milk cheese

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ABSTRACT

In the present work, it was studied the influence of brine concentration on the ripening of an Argentinean sheep cheese. The aim was to establish the conditions of the salting process for it to lead to a product with good quality and acceptability by consumers. Sheep cheeses were manufactured using a direct-vat-set culture of *Streptococcus thermophilus* as starter and chymosin as coagulant. The salting was carried out using brine with the following concentrations: 20, 15, 10 and 5% (w/v). The cheeses were vacuum packed on the fourth day and ripened for 60 days. In order to evaluate the influence of salt content in brine on cheese quality, there were analyzed gross composition, pH, microbial counts, proteolysis, sodium and calcium concentration, melting capacity and sensory characteristics. The increase of salt concentration in brine corresponded with a decrease in moisture as proteolysis advanced. This suggests some inhibition of proteolytic and peptidolytic enzymes involved in ripening due to these adverse environmental conditions. In the descriptive sensory analysis, differences were only found for bitter taste, which was significantly higher for the less salted cheeses. This was attributable and correlated with an increase in concentration of hybrophobic peptides due to an imbalance in proteolysis/peptidolysis during ripening. The melting capacity of the cheese was not affected by the level of salt used. Therefore, it is proposed that the salting process for an Argentinean sheep cheese should be with a 15% brine.

1. Introduction

Even though there are many world-famous sheep milk cheeses, such as Roquefort (France), Feta (Greece), Romano (Italy) or Manchego (Spain), in recent years, sheep milk and its derived products have gained great importance, as it is reflected in their growing presence in the market (Mc Cormick and Lynch, 2003). This can be attributed to organoleptic properties of these products, whose typical and delicate flavors are derived mainly from its high fat content and the composition of fatty acids (Ramos and Juarez, 2011). However, the biochemical changes that occur during the ripening period also have an important impact on sensory characteristics of cheeses and their acceptability by consumers. In connection with this, it is important to notice that the salt content in cheese has a direct influence on these processes, mainly due to its effect on water activity, microbial growth, protein hydration and enzyme activity in general (Møller et al., 2013; Rulikowska et al., 2013). Therefore, the salting process constitutes a major step in the development of a cheese, since the amount of salt (especially their level in the moisture) plays a critical role not only in the quality but also in conservation and safety (Guinee and Sutherland, 2011; Rulikowska et al., 2013). Thus, the salt level for each type of cheese must be regulated within a certain optimum range, which is defined according to the conditions under which this operation is performed. Salt levels below this range may lead to defects such as the development of undesirable microorganisms, uncontrolled enzyme activities or changes in the cortex (Melilli et al., 2005). Conversely, high salt concentrations may cause defects associated to an inhibition of primary starter or biochemical changes during ripening process, or to a direct effect on the gross composition (Guinee and Sutherland, 2011). In general, the range for salt content in the different varieties of cheeses ranges from 0.7 to 4%, while the salt-in-moisture is from 2 to 10% (Boylston, 2012).

Nowadays, there is a global trend towards the reduction of sodium in processed foods, because of its direct relationship with various diseases, especially hypertension (Appel et al., 2011). Several studies have been conducted about cheese salt reduction, the most common being the partial replacement of sodium chloride by potassium chloride (Sihufe et al., 2006; Grummer et al., 2013). However, based on the foregoing, reducing salt content of a cheese

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requires, in the first place, knowing the levels that are compatible with a product of an acceptable organoleptic quality (Ganesan et al., 2014).

In our country, despite the growing popularity of sheep cheeses in the last years, there is still no standardized technology that ensures uniformity and consistency in their quality (Candioti et al., 2009). In the present work, it has been studied the effect of brine concentration on the ripening process of a sheep cheese. The aim was to establish the salting conditions which result in a safe product with good organoleptic quality in accordance to the needs of small producers.

2. Materials and methods

2.1. Cheese-making

Raw sheep milk, provided by the School of Agriculture, Farm and Livestock from Universidad Nacional del Litoral (EAGyG-UNL), was refrigerated and transported at 4 °C to the pilot plant of our Institute (Instituto de Lactología Industrial, UNL), where it was kept frozen at −20 °C until its use. Each cheese making day, 40 L of raw milk were unfrozen and pasteurized at 65 °C for 20 min, and then cooled to 39 °C (temperature of coagulation). A lyophilized commercial culture of *Streptococcus thermophilus* (ST-M5, Chr. Hansen, Inc., Denmark), previously resuspended in 100 mL of sterile milk, was added at a concentration of 10^6 CFU mL^{-1} of milk. After 10 min, it was added the chymosin produced by fermentation of *Aspergillus niger* var. awamori (Chy-Max, Inc. Chr Hansen, Denmark. 183 IMCU/mL) The amount of rennet was enough to obtain the proper firmness. For cutting the curd in 15–20 min. At this time, the curd was cut in the adequate grain size (approximately 25 mm). After 15 min, the mixture was stirred gently during 15 min to achieve proper moisture when left standing for about 10 min. Then, the whey was removed and the curd was placed into molds, and kept in a warm chamber (40 °C–3 h) until reaching pH 5.10 ± 0.05. Afterwards, the cheeses were placed in a conditioning chamber at 4 °C and 92% relative humidity (with air circulation at reduced speed to avoid excessive surface evaporation), where they were kept for 24 h. This stage has the purpose of regulating the cooling rate and allows the development of fermentation to compensate the rise of pH derived from the salts balance, which occurs during brining (Cuffia et al., 2011). Cheeses obtained, of approximately 4 kg, were divided into eight portions of 500 g each, labeled Q1, Q2, Q3 and Q4, which were salted by immersion in concentrated brines 20%, 15%, 10% and 5%, respectively, for a time equivalent to 1 h per kg cheese. After salting, the cheeses were placed in the same conditioning chamber (4 °C and 92% relative humidity), and on the fourth day they were packed under vacuum in shrink plastic bags, until completing their maturation. Four replicates of cheeses were made on successive cheese-making days.

2.2. Gross composition, pH, sodium and calcium analysis

At two months of ripening, cheeses were analyzed in order to determine: moisture (FIL-IDF, 1982), protein (FIL-IDF, 1993), pH (Bradley et al., 1993), and fat matter (Fil-IDF, 1997). At the same time, the concentration of calcium and sodium by atomic absorption flame (FAAS), and flame atomic emission (FAES) respectively, was determined by standard methods (AOAC, 1995).

For all experiences two replicates were made. Each one was analyzed in duplicate.

2.3. Microbiological analysis

Enumeration of total lactic acid bacteria was performed in cheeses at 3, 30 and 60 days of ripening by plating sample dilutions on skim milk agar (SMA) and counting plate colonies after 48 h of incubation at 37 °C according to American Public Health Association (APHA) standards (Frank et al., 1993). Coliforms were enumerated on Bile Red Violet Agar (BRVA) incubating the plates for 24 h at 37 °C, as stated in APHA (Christen et al., 1993).

The results were obtained from two replicates.

2.4. Proteolysis assessment

Proteolysis was assessed by the techniques described below:

2.4.1. Soluble nitrogen (SN)

Cheese samples were treated to obtain crude citrate extract and soluble fractions at pH 4.6, in TCA 12% and PTA 2.5%, according to Hynes et al. (2003). The crude cheese extract was obtained by adding 20 mL of sodium citrate 0.5 M to 10 g of cheese and grounding to homogeneity using a pestle. Deionized water was added to ~90 mL, and the pH was adjusted to 4.6. After centrifugation (3000 × *g*/15 min), the soluble fraction volume was adjusted to 100 mL. The TCA 12% and PTA 2.5% soluble fractions were obtained from 4.6 soluble fraction according to Gripon et al. (1975). The N content was determined in duplicate by the macro-Kjeldahl method according to the IDF method (FIL-IDF, 1993).

2.4.2. Electrophoresis

The insoluble residue at pH 4.6 was purified. In order to do that, samples were re-dissolved by adding 200 mL of distilled water and bringing the pH to 7 with stirring. After being kept about 10 min in these conditions, the insoluble residue was re-precipitated at pH 4.6, proceeding in the same manner as in the extraction. This operation was repeated twice. Finally, the insoluble residue was washed with distilled water twice (by suspension and centrifugation). Samples thus obtained were preserved in a freezer at −18 °C for subsequent electrophoretic analysis.

Electrophoresis assessment was carried out by Urea-PAGE in a Mini-Protean II cube (BioRad Laboratories, California, USA) by Andrews (1983) method, with a concentration of acrylamide of 7.5%. Proteins were stained by Coomasie blue G-250.

2.4.3. RP-HPLC

The HPLC equipment consisted of a quaternary pump, an online degasser and UV–vis detector, all Series 200, purchased from PerkinElmer (PerkinElmer, Norwalk, CT, USA). An interface module connected to a computer was used for acquisition of chromatographic data with the software Turbochrom® (PerkinElmer). A 220 × 4.6 mm Aquapore OD-300C18, 5 μm – 300 A° analytical column was used (PerkinElmer). Water-soluble extracts of the cheeses were obtained by blending 5 g of cheese and 15 mL of distilled water with mortar and pestle, then warmed up to 40 °C and maintained for 1 h. The suspension was centrifuged (3000 × *g*/30 min), and filtered through fast flow filter paper. The filtered solution was adjusted to a final volume of 25 mL. Samples were filtered through 0.45 μm membranes (Millex, Millipore, São Paulo, Brazil), and 60 μL was injected into the HPLC chromatograph. Detection was performed at 214 nm, and column temperature was 40 °C. The gradient starting from 100% of solvent A (H_2O:trifluoroacetic acid (TFA) 1000:1.1, v/v) and 0% of solvent B (acetonitrile:H_2O:TFA 600:400:1, v/v), was generated 10 min after injection. The proportion of solvent B was increased by 1% min-1 (80 min), 20% min-1 (1 min), 0% min-1 (4 min), and then returned to starting conditions, which took 1 min. These last setting conditions were maintained for 10 min (Hynes et al., 2003). The different profiles were visually compared. In addition, total peak areas were calculated from each chromatogram and divided into 3 groups: peaks which elute between 0 and 30 min are mainly free amino acids and small hydrophilic peptides, peaks which elute between 30 and 70 min