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Effect of subclinical intramammary infection on milk quality in dairy sheep: II. Matured-pressed cheese (Manchego) produced from milk of uninfected and infected glands and from their blends

Maristela Rovai[a,1], Gerardo Caja[a], Joan Miquel Quevedo[b], Carmen L. Manuelian[a], Jordi Saldo[b], Ahmed A.K. Salama[a,c], Paloma Torre[d], Ines Arana[d], Xavier Such[a], Gabriel Leitner[e,*]

[a] *Grup de Recerca en Remugants (G2R), Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[b] *Centre Especial de Recerca Planta Tecnologia dels Aliments (CERPTA), XaRTA, Universitat Autonòma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[c] *Sheep and Goat Research Department, Animal Production Research Institute, Dokki 12311, Giza, Egypt*
[d] *Department of Environmental Sciences, Universidad Pública de Navarra, Campus Arrosadía, 31006 Pamplona, Navarra, Spain*
[e] *National Mastitis Reference Center, Kimron Veterinary Institute, P.O. Box 12, Bet Dagan 50250, Israel*

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ABSTRACT

Manchego is a high-fat, long ripened hard cheese, which is produced by enzymatic coagulation of sheep milk. Manchego produced in the La Mancha region in Spain and has Protected Designation of Origin (**PDO**) status. The objective of the study was to evaluate the effects of intramammary infection (IMI) in Manchega and Lacaune dairy sheep with various coagulase negative staphylococci bacteria (CNS) on milk coagulation properties and cheese whey syneresis. The effect of the length of milk storage before processing on cheese yield and its quality was also evaluated. The study included 20 Manchega and 16 Lacaune dairy ewes. The half-udder model was applied: each of the selected animals had one gland free of infection, while the contra-lateral gland was infected with various CNS species. Pasteurized milk (10 L) from uninfected glands, and 50:50 and 75:25 milk blends from uninfected:CNS-infected glands was used for cheese production. Overall, 23 batches of cheese were made and ripened for 63 d. Cheeses were analyzed for organic components, physical and mechanical structure. The taste and sensual qualities of the cheese were evaluated by experts and consumers in two separate panels.

Milk yield in the glands infected with CNS was significantly lower than in control glands. The milk of infected glands had higher somatic cell count and poorer coagulation properties in comparison to controls. Cheese made from infected milk had a significant effect on % fat, total fat, fat loss, total protein, protein loss and dry matter in both breeds. However, cheeses made of blends of 25% or 50% milk from infected glands had only minor losses of fat and protein content, which were not associated with noticeable changes in the final weight of the cheese; though, as discussed herein, further research in this line is warrant. The cheeses

* Corresponding author. Tel.: +972 3 9681745; fax: +972 3 9681692.
E-mail address: leitnerg@moag.gov.il (G. Leitner).
[1] Current address: Department of Dairy Science, College of Agriculture and Biological Science, South Dakota State University, Brookings, SD 57007, USA.

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made of milk stored under refrigerated conditions were clearly perceived as different from the other cheeses because it had more intense attributes related with tangy taste (i.e., bitter, sharp, intense taste, acid) and soft texture (i.e., soft, pasty, not tough).

1. Introduction

Queso manchego (hereafter Manchego) is a PDO cheese made in the La Mancha region of Spain from the milk of Manchega sheep. Manchego cheese has a typical pale yellow or dark fawn color; it is not very elastic as other sheep milk cheese and has a strong aroma and flavor as well as a good aftertaste (González Vinas et al., 2001).

Widely accepted milk quality criteria for cheese production can be divided into three major classes: hygienic, technological and sensorial (Raynal-Ljutovac et al., 2005). However, for many years the focus of the dairy industry was on milk post-harvest microbiology, while the bacteria associated with infected udders were not addressed (Åkerstedt et al., 2012). Recently, many studies have dealt with the effects of IMI by different pathogens on milk composition and quality and on cheese yield and cheese quality (Forsbäck et al., 2011; Leitner et al., 2006; Merin et al., 2008; Revilla et al., 2007, 2009a,b; Silanikove et al., 2014a).

So far, an evaluation of hygienity status of a given herd was done by measuring bulk milk somatic cells count (BMSCC), which gives a general idea on the association between an increased BMSCC and the number of infected animals in the herd, as well as the decrease in milk yield and quality. However, milk yield and composition may vary greatly in association with the infecting agent (Leitner et al., 2008). *Streptococci*, *Escherichia coli* and *Staphylococcus aureus* are the most common bacteria that cause clinical mastitis in cows and sheep (Le Maréchal et al., 2011). In sheep, many of the bacteria causing clinical infection do not influence the BMSCC milk because in most cases the gland is not milked or even when treated, the gland does not produce milk. Coagulase negative staphylococci (CNS) are the major causes of IMI, which result in subclinical infection in all ruminant species (Bergonier and Berthelot, 2003; Silanikove et al., 2014a). These bacteria decrease milk yield and cause changes in milk composition, with a decrease of the level of lactose and increase of whey proteins and proteolytic enzymes, which have negative effects on the suitability of milk for cheese making (Gonzalo et al., 2000; Leitner et al., 2004, 2008; Le Maréchal et al., 2011; Marti-De Olives et al., 2011; Giadinis et al., 2012). Studies related to the effect of subclinical IMI with CNS in sheep and goats on cheese yield and quality show that CNS influence milk coagulation properties and other functional properties as free fatty acids, moisture content, flavor (Jaeggi et al., 2003; Albenzio et al., 2004; Leitner et al., 2011; Revilla et al., 2007, 2009a,b; Rovai et al., 2014) and decrease its nutritional quality as reflected in reduction the content of vitamin c and total antioxidant capacity (Silanikove et al., 2014b).

Organoleptic properties are considered as basic traits in product characterization and qualification. The evaluation of cheese attributes through a sensorial panel may be useful to the industry to evaluate product consumer acceptance. There are two methods for evaluating the product characteristics which are the classical sensory panel of experts and another, under a more "holistic" approach (e.g., napping), where panelists are not trained and the evaluation follows their own criteria in collecting their own spontaneous product perceptions (Nestrud and Lawless, 2010). The objectives of the present study were: (i) to examine the effects of IMI by various CNS bacteria in Manchega and Lacaune dairy sheep on milk composition, coagulation properties, syneresis and on Manchego cheese yield and its quality, and (ii) to examine the effect of IMI and the length of milk storage before processing.

2. Materials and methods

2.1. Animals

The study was conducted at the Experimental Farm of the SGCE (Servei de Granges i Camps Experimentals) of the Universitat Autònoma de Barcelona (UAB, Bellaterra, Spain). The Ethical Committee on Animal and Human Experimentation (CEEAH) of the UAB approved all the experimental and animal care procedures.

A total of 36 lactating dairy sheep of two breeds (Manchega, MN; $n = 20$; Lacaune; LC, $n = 16$) at mid to late lactation (80–150 d in milk) were studied. Animals were selected for the study according to bacterial culturing and presence of udder infection as previously described (Rovai et al., 2014). The study used the half-udder model where one half was free of infection and the other was naturally intramammary infected with CNS, in order to eliminate animal and experimental effects (Leitner et al., 2004). Sheep housing, management and milking were as previously described (Rovai et al., 2015).

2.2. Milk sampling and analysis

Individual milk samples for physicochemical analyses, somatic cell count (SCC) and level of bacterial infection were collected during the morning milking from each gland as previously described (Rovai et al., 2014, 2015). Individual glands were milked and the entire gland's milk was collected separately from uninfected glands (MUIG) or milk from CNS infected glands (MING). In order to accumulate the required milk volume for the study, milk was collected for 2 d, morning and evening milking, mixed and stored at 4 °C. At the day of cheese production, milk was warmed and sampled for California mastitis test (CMT), rennet clotting time (RCT, min) and curd firmness (CF, V) using the Optigraph (Ysebaert, Frepillon, France).

Milk samples (80 mL) were analyzed with a near-infrared spectrometer Foss NIRSystems 5000 (Foss Electric A/S, Hillerød, Denmark) to determine contents of total solids (TS), fat, total protein ($N \times 6.38$), true protein, and casein (CN) using specific calibrations for sheep milk (Albanell et al., 1999) at the laboratories of the UAB. A second milk sample, preserved with Bronopol antimicrobial tablets (Broad Spectrum Micro-tabs II, D&F Control Systems Inc., San Ramon, CA), was tested for SCC by an automatic cell counter (Fossomatic 360) and for lactose by a Milkoscan 5000 (Foss Electric, Hillerød, Denmark) at the Dairy Herd Improvement Laboratory of Catalonia (ALLIC, Cabrils, Barcelona, Spain).