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Short communication: Chemical and sensory characteristics of Canestrato di Moliterno cheese manufactured in spring

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ABSTRACT

Canestrato di Moliterno is an Italian Protected Geographical Indication hard cheese, made in winter and spring from a mixture of ewe and goat milks, that has been poorly investigated. The present study was aimed at characterizing the cheese made in the warm season. Two series of samples, ripened in traditional rooms called *fondaco* as indicated in the official protocol of production, were taken from the main certified producers. The cheeses were analyzed for gross composition; proteolysis and lipolysis; volatile fraction; and organoleptic features. Gross composition was not completely homogeneous among the samples, but primary proteolysis and lipolysis were quite uniform. We observed variations in secondary proteolysis, likely caused by fluctuations in environmental conditions in the *fondaco*. The sensory profiles of the samples were homogeneous: the cheese was soluble, greasy, and adhesive, with a sheepfold and buttery odor. The main taste attributes were fermented, pungent, and bitter. Overall, the results of this study provide an initial contribution to the characterization of Canestrato di Moliterno, and could be used to improve marketing strategies.

Key words: Canestrato di Moliterno, proteolysis, lipolysis, sensory evaluation

Short Communication

Canestrato di Moliterno is an uncooked hard cheese obtained from a mixture of sheep milk (70–90%) and goat milk (10–30%), and it was recently acknowledged by the European Union as a Protected Geographical Indication (PGI) product. It is manufactured under artisanal conditions in the Basilicata region (southern Italy) from December to May. Its shape is cylindrical, and ripening time ranges from 2 mo (fresh type) to 12 mo (ripened type). The matrix is white but evolves to straw yellow with time, and it presents a compact

structure with some small eyes. The flavor becomes more or less piquant as ripening proceeds. The most characterizing points of production are (1) use of unpasteurized milk (raw, without addition of starter, or thermized, with the addition of autochthonous starter); (2) coagulation with paste rennet (lamb or kid); and (3) ripening in traditional rooms called *fondaco* (European Union, 2010). *Fondaco* are basement rooms that have walls at least 40 cm thick and 2 or more windows to allow moderate ventilation. In these rooms, temperature and relative humidity fluctuate in connection with external environmental conditions. Temperature fluctuation during the warm season is more marked than in winter and this, together with the seasonal variations of milk composition, causes differences in cheese quality: the cheese manufactured in spring contains less fat and presents a stronger taste and aroma (Istituto Nazionale di Sociologia Rurale, 1990; Pinarelli, 2006). Even though it is manufactured with a small percentage of goat milk, Canestrato di Moliterno has always been included in the family of Pecorino cheeses. Pecorino is a generic name that indicates Italian cheeses made from raw or thermized sheep's milk in the middle and south of Italy, according to ancient and unique manufacturing techniques (Cevoli et al., 2011; Tofalo et al., 2015). Several dozen varieties of this cheese type have been surveyed in Italy, and 11 are currently acknowledged as European Protected Designation of Origin (PDO) products (the “first-class” European quality label). Adequate information is available in the scientific literature about most important Pecorino cheeses, but information about Canestrato di Moliterno is very scarce, despite its PGI status (Rubino et al., 2009; Pirisi et al., 2011). The present paper is aimed at deepening our understanding of the chemical and biochemical traits, volatile fraction, and sensory characteristics of Canestrato di Moliterno made in the warm season.

Two sets of cheese samples, manufactured between March and May, were taken from 4 certified producers at 30 weeks' ripening. All producers performed vat thermization of the milk (60°C for 15 min; Clerici, Cadorago, Italy), and used the same autochthonous starter (lyophilized lactic acid bacteria starter, developed and copyrighted by the Consorzio per la tutela

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del “Canestrato di Moliterno,” Moliterno, Italy). Milk was coagulated at 38°C by adding lamb rennet paste to cause clotting within 10 to 15 min. After coagulation, the coagulum was left to harden for about 30 min, manually cut to a size of 3 to 4 mm, stirred, and left to compact at the bottom of the vat. Then, the whey was drained off and the curd was cut into small blocks; the blocks were put into plastic molds that resemble ancient rush baskets (baskets are no longer used for hygienic reasons) and slightly pressed by hand. After brine-salting, the cheeses were stored in a primary ripening cellar (12–14°C; 75–80% relative humidity) and, after 3 weeks, transferred to the *fondaco*.

We performed the following chemical analyses: moisture, NaCl, and pH (IDF, 1986, 1988, 1989); fat (Soxhlet method); total and water-soluble nitrogen (Kjeldahl method); and GC determination of total fatty acid composition (Faccia et al., 2015). Primary proteolysis was evaluated using urea PAGE (Andrews, 1983): the gel images were scanned, and the optical density of each casein band was expressed as the percent of total bands. Secondary proteolysis was assessed by determining the free amino acids in water-soluble nitrogen using the EZ:faast LC/MS amino acid analysis kit (Phenomenex, Torrance, CA). The liquid chromatography system employed was a rapid separation UltiMate 3000 coupled with a Velos Pro MS detector (Thermo Scientific Inc., Waltham, MA). The chromatographic conditions were column = 2.1 (i.d.) × 250 mm (length), 5 µm particle size, set at 30°C; solvent A, ammonium formate 10 mM in water, solvent B, ammonium formate 10 mM in acetonitrile; gradient from 10 to 50% of solvent B in 20 min; capillary 320°C, source heater 280°C, sheath gas flow 241.3 kPa, auxiliary gas flow 8 (arbitrary units), full scan mode 100 to 600 Da, positive ionization. For quantitation, we added 3 internal standards (methionine d3, homoarginine, and homophenylalanine) to samples and standard solutions. We assessed lipolysis by GC determination of free fatty acids, extracted according to the method described by Lencioni et al. (1988) and analyzed as indicated for total fatty acids. We extracted the volatile compounds with solid-phase microextraction (SPME) and analyzed them using GC-MS as reported by Felicio et al. (2016), with some modifications. We weighed 1 g of grated cheese in an SPME glass vial and added 3-pentanone as an internal standard. The vial was equilibrated for 10 min at 50°C, and then an SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane 50/30 µm, Supelco, Bellefonte, PA) was exposed for 30 min. For analysis, the fiber was inserted in the split injection port of a Trace 1300 gas chromatograph (Thermo Scientific Inc.) equipped with a TR-Wax-MS capillary column 20 m × 0.1 mm i.d. × 0.1-µm film thickness (Thermo Scientific Inc.). The

thermal gradient used was as follows: 50°C for 0.1 min; to 180°C at 13°C/min; finally, to 220°C at 18°C/min. We used the ISQ Single Quadrupole (Thermo Scientific Inc.) as the MS detector. We identified compounds using the National Institute of Standards and Technology database (NIST MS-Search; <http://chemdata.nist.gov/mass-spc/ms-search/>), comparing with pure standards when possible and considering the Kovats retention time index.

Sensory evaluation was performed by a panel of 9 trained assessors (4 female and 5 male, aged 30–55 yr) who belonged to the staff of the Food Science and Technology section in our department. They were selected following international standards (ISO, 1993) and carried out a quantitative descriptive sensory analysis as reported by Scintu et al. (2010) and Gaze et al. (2015). The panel had 3 open training sessions on 3 commercial samples of different hard ewe cheeses. Panelists then evaluated the Canestrato di Moliterno samples in 2 sessions on different days (a 2-h session for each of the 2 sets of samples) and indicated a series of sensory descriptors. All descriptors were quantified on a 6-point scale and were selected based on weight percentage (frequency of citations × perceived intensity; AFNOR, 1994). Only descriptors with a weight percentage greater than 30% were considered.

All data were processed using SPSS version 19 (IBM, Armonk, NY). Discrete variables were described by their mode value and continuous variables by the mean. We compared producers for each continuous parameter using 1-way ANOVA, and we compared nonparametric variables using the Kruskal Wallis test.

The gross composition of the cheese samples was not fully homogeneous (Table 1). Except for protein and pH, all parameters were significantly different among producers ($P < 0.05$). The fatty acid composition (Table 2) was uniform: the average ratio of saturated to unsaturated was 74:26, and CLA accounted for about 0.5% of total. This latter value was poor compared with those reported for ovine milk and cheese (Addis et al., 2005a; Nudda et al., 2005; Castro et al., 2009). However, it must be noted that the average CLA value was decreased by the very low concentrations detected in cheeses produced in late spring (May): the reduced availability of pasture may have been a cause. Lipolysis was uniform in the cheese samples (Table 2): the mean value of total free fatty acids was 7.21 g/kg of cheese. This was an intermediate level of lipolysis (Collins et al., 2003) and lower than or comparable to those found in Romano and Fiore Sardo (Addis et al., 2005b; Pirisi et al., 2007). The most represented fatty acid was palmitic, followed by stearic and oleic acids. Short- and intermediate-chain fatty acids, which strongly contribute to cheese flavor, accounted for about 26% of the

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