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# Ripening-induced changes in microbial groups of artisanal Sicilian goats' milk cheese

Alessandro Zumbo<sup>1</sup>, Ambra Rita Di Rosa<sup>1</sup>, Biagina Billone<sup>2</sup>,  
Domenico Carminati<sup>3</sup>, Paolo Girgenti<sup>4</sup>, Vincenzo Di Marco<sup>2</sup>

<sup>1</sup>Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali,  
Università di Messina, Italy

<sup>2</sup>Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Barcellona P.G. (ME), Italy

<sup>3</sup>CRA - Centro di Ricerca per le Produzioni Foraggere e Lattiero Casearie di Lodi, Italy

<sup>4</sup>Assessorato Regionale Agricoltura e Foreste, Sicilia, Italy

*Corresponding author:* Alessandro Zumbo. Dipartimento MOBIFIPA, sezione di Zootecnica e Nutrizione animale, Università di Messina. Polo Universitario Annunziata, 98168 Messina, Italy – Tel. +39 090 3503591 – Fax: +39 090 3503973 – Email: alessandro.zumbo@unime.it

**ABSTRACT** - Changes in the microbial flora of “Caprino dei Nebrodi”, a raw goat’s milk cheese produced in Sicily, were studied during ripening. From 2 batches of cheese, 4 samples were taken at day 0, 2, 15, and 30 of ripening. Also, samples of curd and milk used in the manufacturing process were analyzed. By the end of the ripening process (day 30), high  $\log_{10}$  cfu/g were found for *Lactobacilli* (7.20), *Lattococci* (7.10), and *Enterococci* (7.00), whereas counts of *Enterobacteriaceae* (3.91), *Escherichia coli* (3.30), and *Staphylococcus* (3.89) were found to be lower. The study provides useful information on the microbiological properties of “Caprino dei Nebrodi” cheese, and the results obtained suggest that in order to increase the quality of this artisanal product, it is necessary to improve the sanitary conditions of milking and cheese-making. The study was intended as a preliminary step towards the isolation and identification of bacterial species found in this type of goat’s cheese.

*Key words:* Goat’s cheese, Raw milk, Microbiological changes.

**Introduction** – The Mediterranean region is known for its ancient tradition of goat’s cheese production, and the quality of these cheeses is closely connected with the territory of production and its traditions. Safeguarding these products means safeguarding the uniqueness of their historical and cultural environment (Boyazoglu *et al.*, 2001). In Sicily, a population of dairy goats known as “Capra Messinese” is used to produce goat’s cheese in the Nebrodi mountains. This semi-hard cheese is made from raw milk without the addition of lactic starters. Methods and tools of cheese-making are craftsman-like and traditional, and the entire manufacturing process is manual. The aim of this study was to perform the sanitary assessment and to evaluate the microbiological ripening changes in “Caprino dei Nebrodi” cheese. This is a preliminary step towards the isolation and identification of bacterial species.

**Material and methods** – Two batches of “Caprino dei Nebrodi” cheese were produced according to the following procedure: 1) use of fresh morning milk and milk of the preceding evening, chilled at 3°C; 2) double-filtration of the milk, using disposable filters of cellulose; 3) heating of the milk at about 35°C; 4) coagulation with kid’s rennet; 5) waiting for the milk to coagulate: 30-40 minutes; 6) breaking the curd into grain-size pieces; 7) depositing the curd into plastic moulds by careful pressing; 8) salting of surface approximately 24 hours after the preparation and subsequent reversal of the whole cheese;

9) ripening on wooden boards for 30 days at approximately 18°C. For each of the 2 batches, 4 samples of milk prior to the heating process, 4 samples of curd immediately after the breaking process and 4 samples of “tuma” (cheese at day 0), 2-, 15- and 30-day-old cheese were taken (each sample consisted of one whole cheese of approx. 800g), transported to the laboratory and kept at that temperature for max. 24 hours before analysis. **Sampling and processing of the dilutions was performed in accordance with International Standard Methods.** The pH value was determined by means of a pH-meter WTN 597-S with a penetrating electrode. **Milk, curd and cheese samples were analyzed for the following parameters:** a) *Lactobacilli* mesophilic by double sowing for inclusion in MRS agar (Oxoid) and incubation at 37°C for 72 hours in anaerobiosis; b) *Lactococci* by double sowing for inclusion in M17 agar (Oxoid) and incubation at 22°C for 24 hours; c) *Enterococci* by sowing on surface in Slanez Bartley (Oxoid) and incubation at 37°C for 48 hours; d) *Enterobacteriaceae* by double sowing for inclusion in VRBLA (Violet red bile lactose agar) (Oxoid) and incubation at 30°C for 48 hours; e) *Staphylococcus* coagulase-positive by surface sowing on plates with Baid Parker Agar (Oxoid) and incubation at 37°C for 48 hours; f) *Escherichia coli* by sowing for inclusion in TBX (X-Tryptone Bile Glucose Agar) (Oxoid) and incubation at 44°C for 24 hours. The microflora was quantified as number of colony-forming units (cfu) per gram of cheese, and the experimental data were subjected to logarithmic transformation in order to stabilize variance and normalize the distribution of residuals. Then, analyses of variance (ANOVA) were performed on data obtained at different stages of ripening (SAS, 2001).

**Results and conclusions** – The microbial counts found in the raw goat’s milk used for the manufacturing of “Caprino dei Nebrodi” cheese, as well as the counts found in the cheese during ripening, are shown in table 1. The milk used for cheese-making showed high microbial counts: Lactic acid bacteria represented the most prominent microbial group in the milk, being 1-2log units higher than the other groups. The number of microorganisms that indicates the bacteriological quality (*Enterobacteriaceae*, *Micrococcaceae*, *E. coli*, *Staphylococci*) was high in the milk, but lower than the number reported by Zárate *et al.* (1997) in Tenerife goat’s cheese, suggesting less contamination of the cheeses that may occur during milking and/or manufacturing.

Lactic acid bacteria (mainly *Lactobacilli* and *Lactococci*) also represented the predominant microbial group in the cheese (Table 1). It is safe to assume that the marked prevalence of homofermentative (*Lactococci*) both within the milk and the cheese, as well as of heterofermentative (*Lactobacilli*) is of particular importance during the acidification of the curd (Dellaglio *et al.*, 1995). In fact, their

Table 1. Changes in pH and in log<sub>10</sub> cfu/g of main microbial groups of “Caprino dei Nebrodi” cheese.

	Milk	Curd	Ripening time (days)			
	(log <sub>10</sub> cfu/ml)		0	2	15	30
pH	6.65±0.07	6.31±0.47	6.34±0.28 <sup>A</sup>	5.61±0.13 <sup>AB</sup>	5.38±0.17 <sup>AB</sup>	5.32±0.08 <sup>B</sup>
Lactobacilli	5.54±0.10	5.54±0.33	6.49±0.49 <sup>A</sup>	7.20±0.12 <sup>B</sup>	7.12±0.16 <sup>B</sup>	7.20±0.04 <sup>B</sup>
Lactococci	5.32±0.66	4.68±0.81	5.31±0.01 <sup>A</sup>	6.64±0.57 <sup>B</sup>	7.12±0.16 <sup>BC</sup>	7.10±0.14 <sup>BC</sup>
Enterococci	4.61±0.18	5.16±0.23	6.45±0.39	6.81±0.39	6.93±0.38	7.00±0.32
Micrococcaceae	3.48±0.25	4.49±0.69	4.15±0.02 <sup>A</sup>	7.11±0.20 <sup>B</sup>	5.08±0.35 <sup>BC</sup>	5.49±0.58 <sup>BC</sup>
Enterobacteriaceae	3.86±0.9	4.04±0.76	5.26±0.65 <sup>A</sup>	5.04±0.82 <sup>B</sup>	4.15±1.02 <sup>BC</sup>	3.91±0.34 <sup>BCD</sup>
Escherichia coli	2.45±1.03	2.95±0.96	3.52±0.74 <sup>A</sup>	4.81±0.13 <sup>B</sup>	3.35±1.20 <sup>A</sup>	3.30±0.81 <sup>A</sup>
Staphylococci	3.22±0.95	3.67±0.12	4.15±0.78	3.91±0.82	3.82±0.79	3.89±0.56

Data are the average ±standard deviation values of 2 batches.

Values in the same line with different superscript letters were significantly different. <sup>A, B, C, D</sup> P<0.001.

metabolic properties induce a notable decrease in pH, demineralizing the casein, thus triggering the first important step towards cheese-making. This results in an optimal overall acidification process of the cheese, that, at day 30 of ripening, reaches a pH value of 5.32 ( $P < 0.001$ ) (Table 1). Due to the multiplication of bacteria and the retention of microorganisms in the curd after drainage of the whey, most microbial groups show an increase from milk to 2-day-old cheeses (Zárate *et al.*, 1997). A similar increase in the microflora has been observed in goat's cheeses (Caridi *et al.*, 2003), accompanied by a sharp decline in pH (Table 1), due to the production of lactic acid by the high numbers of lactic acid bacteria. Lactic acid bacteria were the predominant microorganisms throughout ripening. Lactobacilli increased significantly ( $P < 0.001$ ) during maturation, probably due to this germs' ability to grow at low pH values (Nunez, 1978) and to resist high salt concentration. Furthermore, *enterococci* (Table 1) were found in abundance in the goat's cheese. Apart from the fact that they typically belong to the microflora of artisanal raw milk cheese products (Mucchetti *et al.*, 2006), they might have a significant function during ripening because of their proteolytic potential. The presence of *enterococcus*, which tends to increase during ripening, even though not significantly, could be due to poor sanitary conditions during milking and the cheese-making procedures, as well as to the resistance of *enterococci* to adverse conditions (high temperatures, high salt concentrations and high acid levels) (Mundt, 1986). *Micrococcaceae* (Table 1) were found at significant numbers in the cheeses at ripening, particularly at day 2, and due to their lipolytic activity, they may contribute to the flavour development in these cheeses (Zárate *et al.*, 1997). Ripening time had a significant ( $P < 0.001$ ) influence on the number of microorganisms indicative of the bacteriological quality (*Enterobacteriaceae*, *E. coli*), since they diminish in quantity during ripening (Table 1). The reduction of these micro-organisms, observed during ripening, suggests that they could be inhibited by highly competitive lactic acid bacteria, able to prevail during ripening. It is well known that ripening acts as a natural selector, during which lactic acid bacteria normally inhibit pathogens (Nunez *et al.*, 1985). Therefore, in order to maintain both the cheese's characteristics and the use of raw milk in the cheese-making procedure, the ripening time might be prolonged, as it is practiced throughout the world (Zárate *et al.*, 1997; Nuñez *et al.*, 1985). In conclusion it can be said therefore that the bacterial species identified in this trial all performed a specific function during the cheese-making and the ripening of our raw goat's milk cheese made without the addition of lactic starters. Therefore, some of the strains might be utilized in terms of an autochthonous starter to be used for a production characterized by sanitary, organoleptic, and merchandizing aspects that are more stable in time.

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