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# Polyphasic approach to study physico-chemical, microbiological and sensorial characteristics of artisanal Nicastrese goat's cheese

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ABSTRACT

Nicastrese goat's cheese is produced in the South of Italy under traditional procedures, from raw goat milk without any starter cultures addition. Samples from milk to ripened cheese provided by 4 different farms were subjected to a polyphasic approach to study their physico-chemical, microbiological and sensorial characteristics. In addition, volatile organic compounds formation in the final products was studied. Overall, gross composition and microbiological data revealed a significant variability among samples, which was confirmed by both the volatile organic compounds generated in the final products and by the sensorial data. Conventional technique allowed us to identify 720 isolates, mainly belonging to *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus brevis, Leuconostoc mesenteroides,* and *Enterococcus faecalis.* Culture-independent methods revealed shifts in the microbial community structure, with an increase in biodiversity of metabolically active bacterial species, from milk to cheese samples. Analysis of volatile organic compounds (VOCs) allowed the identification of 36 compounds; free fatty acids and ketones represented the main detected, followed by alcohols and esters. Moreover, statistical analysis was performed in order to correlate VOCs to bacterial species. Data showed that ester compounds as well as alcohol and aldehydes were positively correlated to NSLAB, indicating that the occurrence of *L. casei, L. plantarum* and *L. brevis* species is relevant for the VOCs formation in the final product.



## 1. Introduction

Nicastrese cheese is an artisanal cheese produced in the Calabria region, according to traditional manufacturing practices. The Nicastrese goats are bred in natural grazing land in Catanzaro province, a region characterized by aromatic Mediterranean plants that confer typical sensorial features to the milk. The raw goat's milk is coagulated using lamb rennet (25 g/50–60 l) in 20–30 min at 28–30 °C, without addition of any starter cultures. The curd is cut and manually crumbled into small pieces (rice grain sized) and transferred into moulds (for two days) in order to remove the whey and then brine salted for 24 h. The ripening period ranges from two to six months and takes place in the storage basement of the cheese factories, at 10–15 °C and 70–80% humidity. The unique flavour of the Nicastrese cheese is the result of a complex balance among volatile and non-volatile compounds, originating during the ripening process from milk's fat, protein and carbohydrates (Fox and Wallace, 1997). The main biochemical changes during ripening are determined by autochthonous microbiota, which lead to a unique cheese flavour (Fox et al., 2004). Although several traditional goat's milk cheeses have been widely characterized (Valkaj et al., 2014; Tormo et al., 2015; Picon et al., 2016), up to now no information is available on Nicastrese cheese. For these reasons, in the present study a complete view of physico-chemical, microbiological and sensorial profiles of Nicastrese cheese, provided by four different farms, was carried out. In addition, volatile organic compounds of 60 days ripened cheese were detected.

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## 2. Materials and methods

### 2.1. Cheese manufacture and sampling points

The artisanal Nicastrese cheeses, analysed in the present study, were produced from April to August, on small-scale dairy plant in four different farms (I, II, III and IV), located in the Nicastro area of the Calabria region (Italy). According to the traditional practices, raw goat's milk was processed without the starter cultures addition. The flow-chart of the Nicastrese cheese production is illustrated in Fig. 1. Milk (M), curd (C) and ripened (60 days) cheese (Ch) samples were aseptically collected and subjected to microbiological and physico-chemical analysis within 6 h. The analyses were conducted in triplicate (three independent batches from each farm).

### 2.2. Physico-chemical analyses

Milk (M) samples (250 ml) from each farm were analyzed to measure fat, protein, casein, lactose, non-fat solids, urea, density, citric acid and titration acidity (Soxhlet-Henkel/°SH), using Fourier Transform InfraRed (Milkoscan FT2, Foss Electric, Sweden), calibrated with appropriate goat milk standards. Curd (C) and 60 days ripened cheese (Ch) samples (100 g) were analyzed for moisture, fat, protein and salt content, using Near Infrared Spectroscopy in Transmittance (FoodScanTM Dairy Analyser; FOSS, Italy). In addition, the pH values of M and Ch samples were determined by pHmeter (H19017, Microprocessor, Hanna Instruments). All analyses were carried out in triplicate.

### 2.3. Microbiological analyses

Samples of M (10 ml), C (10 g) and a mixture of both core and surface sections of the 60 days ripened Ch (25 g), collected from each farm, were transferred into a sterile stomacher bag, 10-fold diluted with Ringer's solution (Sigma-Aldrich, Milan, Italy) and homogenised for 2–5 min in a stomacher (Lab-Blender, Seward, London). For each cheese analysed, core and surface sections, from three diametrically opposite points, were sampled using a sterile cutter tool. Decimal dilutions were obtained and inoculated on following media and conditions: Plate Count Agar (PCA) plus 1 g/l of skimmed milk, incubated aerobically at 30 °C for 48–72 h, for total mesophilic counts; de Man Rogosa and Sharpe agar (MRS), adjusted to pH 5.4, incubated at 32 °C for 72 h under microaerophilic condition, for lactobacilli; Violet Red Bile Agar (VRBA), anaerobically incubated for 24–48 h at 37 °C and 45 °C for Enterobacteria and faecal coliforms, respectively; M17 agar, supplemented with 5 g/l of lactose, incubated at 30 °C for 24–48 h, for lactococci; Sabouraud Dextrose Agar (SDA), supplemented with chloramphenicol (0.05 g/l) incubated at 25 °C for 3–5 days, for yeasts and moulds; Mannitol Salt Agar (MSA), incubated at 32 °C for 48 h, for staphylococci; Listeria Selective Agar (LSA) incubated at 35 °C up to 48 h, for *Listeria* spp.; Brilliance *E. coli* (BEC), incubated at 37 °C for 18–24 h for *Escherichia coli*. All media were purchased from Oxoid (Basingstoke, UK). Results were expressed as mean values of three determinations. The average counts and their standard deviations were calculated after log transformation.

### 2.4. LAB reference strains and growth conditions

The lactic acid bacteria (LAB) strains used as reference in the present study are listed in Table 1S. All the strains were cultivated on MRS medium (Oxoid), with the exception of lactococci, enterococci, pediococci and streptococci that were cultivated on M17 medium (Oxoid), supplemented with 5 g/l of lactose. Incubation was performed at the appropriate temperatures, under anaerobic or microaerophilic conditions, depending on the bacteria.

### 2.5. LAB isolation and maintenance

In order to characterize the LAB population of M, C, and Ch samples, seventy colonies from each sample were randomly selected from both M17 and MRS agar plates. After microscopic observation, the colonies were purified by streaking three times then sub-cultured twice on MRS or M17 medium for rods and cocci, respectively. Isolates were frozen at − 20 °C in M17 (for cocci) and in MRS (for rods) broth containing 20% of glycerol (v/v). Overall, 840 isolates were obtained and 720 of them were found gram-positive and catalase-negative. The isolates were subjected to further analyses and identified at the genus and species levels.

### 2.6. Total DNA isolation from LAB isolates and reference strains

DNA extraction from isolates and reference strains was performed by mechanical cell lysis, as previously described by Randazzo et al. (2002) and modified as follows. Cells (2 ml), in the late exponential growth phase, were centrifuged at 8000 rpm for 10 min, washed and re-suspended in 500 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA pH 8.0) with 0.3 g glass beads (diameter 0.106 mm; Sigma, Milan, Italy). Then mechanical lysis was performed twice, each for 3 min, in a bead beater at maximum speed, and cooled on ice. The homogenate was centrifuged at 13,000 rpm for 5 min, and the supernatant was used as template for PCR or stored at −20 °C until use. DNA amount and quality were assessed by using the NanoDrop ND1000 Spectrophotometer (Thermo Fisher).

### 2.7. PCR-RFLP analysis of LAB isolates

All LAB isolates were clustered by PCR-RFLP analysis. For PCR amplification of both isolates and reference strains, the primer pairs 7-f (AGA GTT TGA TC/TA/C TGG CTCAG) and 1510-r (ACG G (C/T) T ACC TTG TTA CGA CTT) were used (Lane, 1991). PCR reaction was performed in a final volume of 50 μl using a Thermal Cycler 2720 (Applied Biosystems, Norwalk, CT, USA). The reaction mixture contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3 mM $MgCl_2$, 50 mM each of the four-deoxynucleoside triphosphates (dNTP), 1.25 U of *Taq* polymerase, 5 pmol of each primer, and 1 μl of appropriately diluted template DNA. The cycling program was: 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 68 °C for 40 s, and 1 cycle at 68 °C for 7 min. Two restriction endonucleases, *Msp*I and *Alu*I (New England, BioLabs), were simultaneous used for the digestion of amplification products, according to the manufacturer's instructions (New England BioLabs). The restriction patterns were analysed using 2% (w/v) agarose gel in 1 × TBE buffer (89 mM Tris–borate, 89 mM boric acid, 2 m MEDTA; pH 8.0) and visualized after staining with Gel Red Nucleic Acid Stain (Biotium, Italy).

### 2.8. Species-specific PCR

The isolates of each PCR-RFLP cluster were subjected to species-specific PCR. PCR reactions were carried out in a total volume of 50 μl, as previously described, using species-specific primers and following the cycle programs suggested by different authors (Table 2S). PCR products were analysed by electrophoresis through 1% agarose gels in a 1 x TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA).