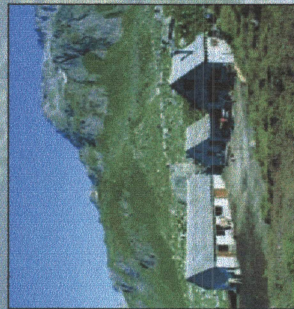
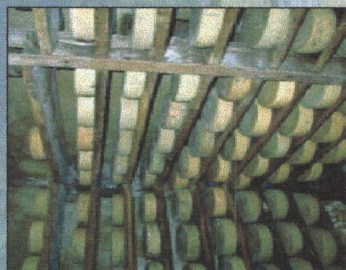


The specific characteristics of taste and aroma of artisanal cheeses may be attributed to the metabolic activities of indigenous lactic acid bacteria. Then increasing information on the natural microbial population present in dairy products can help to prevent the loss of microbial biodiversity in typical foods and consequently the loss of a wide range of cheeses produced by different methods whose typical features depend on local and regional traditions and on the indigenous microbial population present in raw milk and selected by the cheese-making environment. Consequently, there is an increasing interest in the design of specific autochthonous starter cultures for use in large-scale manufacturing of traditional-type cheeses but without changing the fundamental properties of the product.

According to the increasing demand from cheese-makers for new strains able to improve cheese quality, in the present study we isolated, identified and characterised strains from Toma piemontese POD (Protected Origin Denomination) an artisanal cheese, produced in Piedmont (Northwest Italy) to be selected and used as starters in both large-scale and artisanal cheesemaking.

Toma is a semi-cooked cheese produced in Piedmont from raw milk which is warmed to 37-40 °C and rennet is added at a concentration of 0.15-0.20 mL/L. The curd is cut into 5-10 mm particles and collected with muslin, pressed and drained for 24 h. Cheese is ripened at 6-10 °C and 85% relative humidity for 30-60 days. The production and the ripening process depend entirely on the natural microbial population present in milk.

The aim of this work was to study the volatile compounds produced by eight natural lactic bacteria isolated from an artisanal Piedmont cheese (Northern Italy) using SPME-GC-MS.



Materials and Methods

Samples

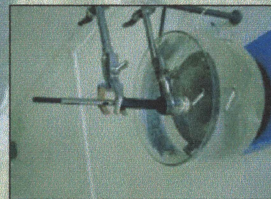
Isolates collected from curd of artisanal mountain cheeses were identified by the combined use of PCR 16S-23S rDNA spacer analysis, species-specific probes and 16S rDNA sequencing.

* Eight isolates of autochthonous lactic bacteria were obtained (six *Lactococcus lactis* subsp. *lactis* (A1.1-A1.4-A1.6-A1.13-A1.18-A1.20), one *Lactococcus lactis* subsp. *cremoris* (A1.8), one *Streptococcus thermophilus* (B1.18), one *Lactobacillus paracasei* (TO.1)).

Aroma analysis

• 3 g of inoculated milk, 10 mL of internal standard (1-heptanol; 10.8 mg/mL) and 28% (w/w) of NaCl in vial with 10 mL of capacity

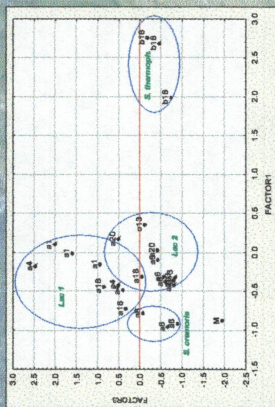
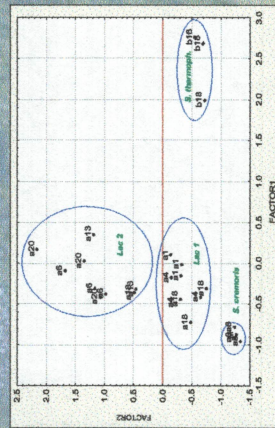
- Stirring at 42 °C for 40 min
- Extraction at 42 °C for 20 min in head space by SPME with DVB/Carboxen/PDMS fiber (2 cm)
- Desorption at 270 °C for 4 min in splitless
- Analysis by DB-WAX capillary column (30 m x 0.25 mm)
- Operating condition: 35 °C for 5 min; 2 °C/min to 183 °C; 1 min at 183 °C; 5 °C/min to 210 °C; 3 min at 210 °C.
- Mass spectra recorded in TIC mode; ionisation voltage of 70 eV; 33–300 amu mass range



SPME extraction for aroma analysis

RESULTS

Analysis highlighted the presence of about 100 organic compounds of which over 40 are not present in the milk. On the basis of Principal Component Analysis performed only for these compounds the presence of four groups of bacteria was underlined. The first and the second, marked as *Lac 1* e *Lac 2*, are both formed by three isolated of *Lactococcus lactis* subsp. *lactis*. The third group is constituted only by *Lactococcus lactis* subsp. *cremoris*, while the fourth, by *Streptococcus thermophilus*. Very interesting is the aroma of *S. thermophilus* for the presence of high quantities of α -dicetones (2,3-butanedione, 2,3-pentanedione), α -chetois (2-hydroxy-3-butanone, 3-hydroxy-2-pentanone) and acids (3-methyl-butanolic; hexanoic; octanoic). For the two groups of *L. lactis* subsp. *lactis* the more important compounds are aldehydes (2-methylbutanal; 2-methyl-propanal) and alcohols (2-methyl-butanol; 3-methyl-butanol; 2-phenyl ethanol). *Lactococcus lactis* subsp. *cremoris* is characterised by low production of reported compounds but a high quantity of α -methyl ester of the 3-OH-4-methyl-pentanoic acid.



Score-plot for the first components of the eight isolates and the milk (M) samples

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄	X ₁₅	X ₁₆	X ₁₇	X ₁₈	X ₁₉	X ₂₀	X ₂₁	X ₂₂	X ₂₃	X ₂₄	X ₂₅	X ₂₆	X ₂₇	X ₂₈	X ₂₉	X ₃₀	X ₃₁	X ₃₂	X ₃₃	X ₃₄	X ₃₅	X ₃₆	X ₃₇	X ₃₈	X ₃₉	X ₄₀	X ₄₁	X ₄₂	X ₄₃	X ₄₄	X ₄₅	X ₄₆	X ₄₇	X ₄₈	X ₄₉	X ₅₀	X ₅₁	X ₅₂	X ₅₃	X ₅₄	X ₅₅	X ₅₆	X ₅₇	X ₅₈	X ₅₉	X ₆₀	X ₆₁	X ₆₂	X ₆₃	X ₆₄	X ₆₅	X ₆₆	X ₆₇	X ₆₈	X ₆₉	X ₇₀	X ₇₁	X ₇₂	X ₇₃	X ₇₄	X ₇₅	X ₇₆	X ₇₇	X ₇₈	X ₇₉	X ₈₀	X ₈₁	X ₈₂	X ₈₃	X ₈₄	X ₈₅	X ₈₆	X ₈₇	X ₈₈	X ₈₉	X ₉₀	X ₉₁	X ₉₂	X ₉₃	X ₉₄	X ₉₅	X ₉₆	X ₉₇	X ₉₈	X ₉₉	X ₁₀₀	X ₁₀₁	X ₁₀₂	X ₁₀₃	X ₁₀₄	X ₁₀₅	X ₁₀₆	X ₁₀₇	X ₁₀₈	X ₁₀₉	X ₁₁₀	X ₁₁₁	X ₁₁₂	X ₁₁₃	X ₁₁₄	X ₁₁₅	X ₁₁₆	X ₁₁₇	X ₁₁₈	X ₁₁₉	X ₁₂₀	X ₁₂₁	X ₁₂₂	X ₁₂₃	X ₁₂₄	X ₁₂₅	X ₁₂₆	X ₁₂₇	X ₁₂₈	X ₁₂₉	X ₁₃₀	X ₁₃₁	X ₁₃₂	X ₁₃₃	X ₁₃₄	X ₁₃₅	X ₁₃₆	X ₁₃₇	X ₁₃₈	X ₁₃₉	X ₁₄₀	X ₁₄₁	X ₁₄₂	X ₁₄₃	X ₁₄₄	X ₁₄₅	X ₁₄₆	X ₁₄₇	X ₁₄₈	X ₁₄₉	X ₁₅₀	X ₁₅₁	X ₁₅₂	X ₁₅₃	X ₁₅₄	X ₁₅₅	X ₁₅₆	X ₁₅₇	X ₁₅₈	X ₁₅₉	X ₁₆₀	X ₁₆₁	X ₁₆₂	X ₁₆₃	X ₁₆₄	X ₁₆₅	X ₁₆₆	X ₁₆₇	X ₁₆₈	X ₁₆₉	X ₁₇₀	X ₁₇₁	X ₁₇₂	X ₁₇₃	X ₁₇₄	X ₁₇₅	X ₁₇₆	X ₁₇₇	X ₁₇₈	X ₁₇₉	X ₁₈₀	X ₁₈₁	X ₁₈₂	X ₁₈₃	X ₁₈₄	X 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CONCLUSIONS

Aroma analysis performed with SPME-GC-MS followed by the PCA of detected compounds have highlighted the differences between the volatile fraction of eight autochthonous lactic bacteria isolates. Most interesting are the differences between the *L. lactis* subsp. *lactis* where the different isolates can produce the same volatile compounds. These differences were confirmed also with technological trials. The use of SPME is very important for this research as it allows very quick, careful analysis without using solvent.

Values for each bacteria group of mean concentrations (X) as ppb and standard deviation (s) of compounds used in the PCA. Results of Analysis of variance are also reported (* $p < 0.01$; * $p < 0.05$; ns = not significant). For the unidentified compounds is reported the base peak ion.

This study was performed within a research project set-up and supported by Ministero delle Politiche Agricole e Forestali (MIPAF, Rome, Italy).