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# PROTEOLYSIS IN MINIATURE TOMA PIEMONTESE CHEESE MADE USING SINGLE STRAINS OF INDIGENOUS BACTERIA

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## Abstract
It is normal practice to make cheeses using commercial starter cultures but in the last decade, research has underlined the organoleptic impoverishment of these cheese and thus the use of native cultures, able to give characteristics more similar to regional cheeses, has been suggested. As a consequence, there has been a growing interest in the characterisation of wild isolates from artisanal cheeses. Characterisation of the patterns of proteolysis in cheeses made using these strains is very important as proteolysis contributes to texture development due to the breakdown of the protein matrix of the cheese and to the development of flavour and off-flavours by the production of peptides and amino acids.
The aim of this work was to define the effects on proteolysis of 35 strains isolated from an artisanal Italian PDO cheese. Proteolysis was assessed at 60 d of ripening of miniature model cheese by urea-PAGE of the pH 4.6-insoluble fractions from the cheese and by RP-HPLC of the pH 4.6-soluble fractions therefrom. Data obtained from urea-PAGE analysis were analysed using Cluster Analysis and 2 groups were found. Cheeses made using strains belonging to the first group were characterised by a higher degradation of $\alpha_{s1}$-casein compared to strains of the second group that determined a higher degradation of the β-casein. Cluster Analysis was also applied to 48 peaks of the RP-HPLC and highlighted two groups containing nearly the same strains as the groups obtained by urea-PAGE. Cheeses made using strains from the first group were characterized by high concentrations of hydrophilic peptides and the second group by high concentrations of mostly hydrophobic peptides.

Keywords: Toma Piemontese cheese, indigenous strains, proteolysis, casein, peptide, amino acid

## Introduction
In many European countries cheeses are traditionally made using natural cultures obtained by the incubation of milk or whey from the previous day's production under defined conditions. The inoculum is characterised by a well-balanced presence of mesophilic and thermophilic lactic acid bacteria that give uniform products. Nevertheless, they are characterised by a high variability in time and it takes a long time to prepare such starters. It is now a normal practice to make cheese using commercial starter cultures that are easy to use and able to give cheeses that are standardised but with an organoleptic impoverishment. As a consequence, interest has been shown for the genotypic and technological characterisation of wild isolates from artisanal cheeses, consequently it may be possible to select new strains that could be used as defined-strain cultures able to give cheeses having technological and organoleptic characteristics that are more similar to traditional cheese. Nowadays in Italy, these autochthonous starter cultures are used for the production of many PDO cheeses such as Asiago, Bitto, Fontina and Pecorino Toscano but have not been used for Toma Piemontese which is the most important cheese from the North-West Alps. Proteolysis in Toma

Piemontese cheese has not been studied and there has no been research into the use of native single strain cultures in the manufacture of this cheese in order to evaluate their potential. The objective of the present study was to evaluate the effect of thirty-five strains isolated from artisanal Toma Piemontese cheeses on proteolysis of the cheese after 60 days of ripening.

**Method**

After 60 days of ripening pH 4.6-insoluble and –soluble fractions were prepared by a slight modification of the method of Kuchroo and Fox (1982). Urea-polyacrylamide gel electrophoresis (urea-PAGE) (12% T, 4% C, pH 8.9) of the pH 4.6-insoluble fraction of the cheese was performed using a Protean II xi vertical slab-gel unit (Bio-Rad Laboratories Ltd., Watford, UK) according to the method of Andrews (1983) with modifications of Shalabi and Fox (1987). RP-HPLC of the pH 4.6- soluble fraction of the cheese was performed using the method described by Hayaloglu *et al*., (2004).

**Results**

Cluster analysis of the peak area of corresponding urea-PAGE bands after 60 days of ripening, divided the cheeses in two groups. The first group was composed of cheeses made using 18 *Lactococcus lactis* subp. *lactis*, 2 *Lactococcus lactis* subsp. *cremoris*, 2 *Streptococcus macedonicus*, 1 *Lactobacillus paracasei* and 1 *Streptococcus thermophilus*. The second group was composed of cheeses made using 3 *Lactococcus lactis* subsp. *lactis*, 3 *Streptococcus macedonicus*, 2 *Streptococcus thermophilus*, 1 *Lactobacillus fermentum*, 1 *Lactobacillus paracasei* and 1 *Lactobacillus casei* subsp. *rhamnosus*. The cheeses made using strains belonging to the first group were characterised by a higher degradation of the $\alpha_{s1}$-casein contrary to the strains of the second group that determined a higher degradation of the β-casein. Cluster analysis of the 4.6-soluble fractions of the 2-month-old cheeses, based on the height of 48 peaks, grouped the cheeses into two classes that were made up of the same strains except for the cheese made using the strain *Streptococcus macedonicus* TB 1.4 as starter.

**Conclusion**

The data obtained from this research together with further studies should be considered in the development of new defined-strain starter cultures that could be used for the production of Toma Piemontese cheese.

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