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The use of a culture independent method as LH-PCR overcame traditional agar plate and culture dependent method limitations. Moreover, the modality of LH-PCR samples preparation allowed to evaluate both the entire and lysed cells evolution during cheesemaking and ripening

L. helveticus and L. delbrueckii subsp. lactis were the dominant species until the second month of ripening, even if an increasing number of them underwent to autolysis process. One month after brining, at least two new species were able to grow in the cheese: L. rhamnosus or L. casei or L. plantarum, and P. acidilactici or L. parabuchneri. After six months of ripening, the same species were found even if no one of them seems to be dominant. Interestingly, in this stage of ripening, also L. rhamnosus or L. casei or L. plantarum which seems to increase, undergo to autolysis process. From the sixth to the twentieth month of ripening any microbial evolutionary change were observed.

Monitoring both entire and lysed cells through LH-PCR allows to coming aware of the importance of this two fractions in PR cheese production and ripening. This approach opens perspectives for insight microbial evolution in fermented food environment.

Keywords: Parmigiano Reggiano, LH-PCR, microbial evolution

## Characterisation of Proteolysis in Castelmagno PDO Cheese

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Castelmagno is a semi-hard cheese produced in the province of Cuneo (Piedmont-Italy), made from raw milk without the use of starter. After cutting, the curd is left 24 hours at room temperature and then putted under acidified whey for 3 days. Then, the curd is crumbled, salted, putted into cylindrical moulds and pressed strongly. Finally the cheese is ripened in natural caves for at least 2 months. The commercial product has a cylindrical shape (20 cm high, 20 - 23 cm in diameter) and a weight of 4 - 6kg.

Although Castelmagno PDO cheese plays an important role in Piedmont dairy economy, there is a lack of knowledge on its evolution during manufacture and ripening. Therefore the aim of this work was to define the proteolysis during production and ripening of 3 batches of Castelmagno PDO cheese. For each sample two layers were analysed: under the rind (3 cm thick, located 2 cm below the rind) and the core (4 cm thick, located in the centre of the cheese).

Proteolysis was assessed at 1, 2, 5 days of manufacture and after 1, 30, 60, 90 and 150 days of ripening by urea-PAGE of the pH 4.6-insoluble fractions from the cheeses, by RP-HPLC of the pH 4.6-soluble fractions and amino acid analysis therefrom.

All cheeses showed a higher degradation of  $\alpha_{s1}$ -casein compared to  $\beta$ -casein and this was superior in the layer under the rind. The higher degradation of  $\alpha_{s1}$ -casein started in the cheese at 1 day of ripening as a consequence of leaving the cheese under acidified whey for 3 days. The principal free amino acids detected in all the samples were Leu, Glu, Phe, Val and Asp, while the greatest level of all amino acids was found in the cheese at 150 days of ripening in the layer under the rind.

Keywords: Castelmagno PDO cheese, proteolysis, cheesemaking, ripening