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# Influence of a bacteriocin-producing lactic culture on proteolysis and texture of Hispánico cheese

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**Abstract**

Hispánico cheese was manufactured in duplicate experiments, each consisting of two 50-L vats, and ripened for 75 days. Lactic cultures for experimental cheese were 0.5% *Lactococcus lactis* subsp. *lactis* INIA 415 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain), a bacteriocin-producing ($Bac^+$) strain harbouring the structural genes of nisin Z and lacticin 481, 0.5% *L. lactis* subsp. *lactis* INIA 415-2, a $Bac^-$ mutant and 2% TA052, a commercial *Streptococcus thermophilus* culture. Lactic cultures for control cheese were 1% *L. lactis* subsp. *lactis* INIA 415-2 and 2% TA052. *S. thermophilus* counts were lower, and levels of cell-free intracellular aminopeptidases higher, from day 1 in cheese made with the bacteriocin producer, indicating early lysis of the thermophilic culture. Experimental cheese showed reduced proteolysis of $\alpha_s$-casein and lower levels of hydrophilic and hydrophobic peptides than control cheese. However, proteolysis as estimated by the *o*-phthaldialdehyde method and total level of free amino acids were in experimental cheese 1.38- and 2.47-fold, respectively, those in control cheese on day 25, and 1.49- and 2.34-fold, respectively, on day 75. Higher values of fracturability, elasticity and hardness were recorded from day 50 for cheese made with the bacteriocin producer, which were related to its higher residual $\alpha_s$-casein content. The use of a bacteriocin-producing culture, though retarding $\alpha_s$-casein proteolysis and softening of texture, enhanced considerably secondary proteolysis during cheese ripening.


*Keywords:* Cheese; Proteolysis; Bacteriocin; Lysis; Texture

## 1. Introduction

The main sources of proteolytic enzymes acting in cheese are milk, rennet or other milk coagulants, lactic starter cultures and other microorganisms, which are added during manufacture or spontaneously contaminate milk, curd or cheese. The proteolytic system of lactic acid bacteria (LAB) consists of proteinases, which hydrolyse caseins, and peptidases, which are responsible for the formation of small peptides and free amino acids (FAA), an essential step in cheese ripening (Kunji, Mierau, Hagting, Poolman, & Konings, 1996; Lane & Fox, 1997). Since peptidases are located in the interior of the cell, the lysis of LAB will favour the access of intracellular peptidases to their substrates and presumably will accelerate cheese ripening (Morgan, Ross, & Hill, 1997; Garde, Gaya, Medina, & Nuñez, 1997), leading to a reduction in production costs.

One of the approaches to enhancing the lysis of LAB during ripening is the inoculation of milk with bacteriocin-producing adjunct cultures. *Lactococcus lactis* subsp. *lactis* DPC3286, a producer of lactococcins A, B and M which have a bacteriolytic effect on sensitive strains of lactococci, was used as adjunct culture in Cheddar cheese manufacture, increasing concentrations of FAA and reducing bitterness (Morgan et al., 1997).

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*Enterococcus faecalis* INIA 4 (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain), a non-virulent haemolysin-negative enterocin AS-48-producing strain, accelerated cell lysis and flavour development when used as adjunct culture to a commercial mixed-strain LD-type starter in the manufacture of Hispánico cheese, a semi-hard variety manufactured in Spain from a mixture of cows' and ewes' milk (Garde et al., 1997). Levels of non-protein and amino nitrogen in cheese increased when a lacticin 3147-producing *L. lactis* strain was used as starter culture (Martínez-Cuesta, Requena, & Peláez, 2001). When comparing the effect of different inocula of *E. faecalis* INIA 4 on cheese ripening, most FAA and some volatile aroma compounds reached their maximum levels in cheese made from milk inoculated with 0.1% *E. faecalis* INIA 4, which exhibited the highest scores for flavor quality and flavor intensity throughout ripening (Oumer et al., 2001).

The dairy industry is reluctant to the use of enterococci in cheese manufacture. For this reason, *L. lactis* subsp. *lactis* INIA 415, a strain harbouring the structural genes of nisin Z and lacticin 481 (Garde, Rodríguez, Gaya, Medina, & Nuñez, 2001) was used as bacteriocin-producing adjunct culture in the manufacture of Hispánico cheese from milk inoculated with a mesophilic starter culture and a thermophilic starter culture (Garde, Tomillo, Gaya, Medina, & Nuñez, 2002). Extracellular aminopeptidase activity, proteolysis and total level of FAA increased and flavour formation was accelerated in cheese with the bacteriocin-producing adjunct culture.

In the present work, a more simple combination of bacteriocin-producing and bacteriocin-sensitive cultures was assayed on the aim of accelerating cheese proteolysis and ripening. Changes in proteolysis and texture during ripening of Hispánico cheeses made with or without addition of *L. lactis* subsp. *lactis* INIA 415 from milk inoculated with *L. lactis* subsp. *lactis* INIA 415-2, a spontaneous $Bac^-$ mutant, and a thermophilic commercial culture have been investigated.

## 2. Materials and methods

### 2.1. Lactic cultures and cheese manufacture

*L. lactis* subsp. *lactis* INIA 415, from the INIA culture collection, was used as bacteriocin-producing adjunct culture. *L. lactis* subsp. *lactis* INIA 415-2, a spontaneous nisin- and lacticin 481-resistant mutant not producing bacteriocins, with acid production and proteolytic activities similar to those of the parental strain, was used as mesophilic starter culture. They were maintained at −80 °C in MRS broth (Biolife, Milano, Italy) and subcultured twice in reconstituted skim milk at 30 °C before use in cheese manufacture. Thermophilic lactic culture TA052 (Rhodia, Dangé Saint-Romain, France), consisting of *Streptococcus thermophilus* strains, was subcultured twice in reconstituted skim milk at 37 °C before use in cheese manufacture.

Hispánico cheese was manufactured in duplicate experiments on different days from a mixture of pasteurized cow (80%) and ewe (20%) milk. Each experiment consisted of two 50-L vats. Lactic cultures for vat 1 (experimental cheese) were 0.5% *L. lactis* subsp. *lactis* INIA 415 ($Bac^+$), 0.5% *L. lactis* subsp. *lactis* INIA 415-2 ($Bac^-$) and 2% TA052. Lactic cultures for vat 2 (control cheese) were 1% *L. lactis* subsp. *lactis* INIA 415-2 and 2% TA052. Rennet (6 mL Maxiren, 1:15000 strength, Gist Brocades, Delft, The Netherlands) was added to milk 60 min after lactic culture inoculation. The curds were cut 40 min after rennet addition into 6–8 mm cubes and scalded at 37 °C for 15 min. Whey was drained off and curds were distributed into cylindrical moulds. Three cheeses, approximately 2 kg in weight, were obtained from each vat. Cheeses were pressed for 18 h at 20 °C, salted for 16 h at 12 °C in brine (150 g NaCl $L^{-1}$), and ripened at 12 °C for 75 days.

### 2.2. Microbiological analysis

Viable counts of LAB were determined in duplicate on plate count agar (Liofilchem, Roseto, Italy) with 0.1% skim milk (Biolife, Milano, Italy) added, using a DS Plus Spiral plater (Interscience, Saint-Nom-La-Bretèche, France). Previous trials had shown that on plates incubated aerobically for 24 h at 30 °C only lactococci formed colonies, and on plates incubated aerobically for 24 h at 40 °C only thermophilic streptococci formed colonies. Bacteriocin-producing lactococci were determined on the surface of double-layer APT agar (Biolife) plates, with the lower layer inoculated with 0.1% of a 16 h culture of *Lactobacillus buchneri* St2A as the indicator microorganism; colonies forming a zone of growth inhibition in the lower layer were considered to be *L. lactis* subsp. *lactis* INIA 415. Lactobacilli were determined on Rogosa agar (Biolife) plates incubated anaerobically for 48 h at 37 °C, and enterococci on KF Streptococcus agar (Oxoid, Basingstoke, UK) plates incubated aerobically for 48 h at 37 °C.

### 2.3. Bacteriocin and aminopeptidase activity

For the determination of bacteriocin activity, cheese samples held at −40 °C were thawed and 5 g were homogenized in a Stomacher 400 (Seward Laboratory, London, England) with 10 mL sterile 0.02 N HCl at 50 °C. Homogenates were centrifuged (12,000*g*, 20 min, 4 °C) and pH of supernatants was adjusted to pH 6 with