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Effect of High-Pressure Treatment and a Bacteriocin-Producing Lactic Culture on the Proteolysis, Texture, and Taste of Hispánico Cheese

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ABSTRACT

The effects of high-pressure treatment, by itself or in combination with a bacteriocin-producing culture added to milk, on the proteolysis, texture, and taste of Hispánico cheese were investigated. Two vats of cheese were manufactured from a mixture of cow and ewe milk. Milk in one vat was inoculated with 0.5% *Lactococcus lactis* ssp. *lactis* INIA 415, a nisin Z and lacticin 481 producer; 0.5% *L. lactis* ssp. *lactis* INIA 415-2, a bacteriocin-nonproducing mutant; and 2% of a commercial *Streptococcus thermophilus* culture. Milk in the other vat was inoculated with 1% *L. lactis* ssp. *lactis* INIA 415-2 and 2% *S. thermophilus* culture. After ripening for 15 d at 12°C, half of the cheeses from each vat were treated at 400 MPa for 5 min at 10°C. Ripening of high-pressure-treated and untreated cheeses continued at 12°C until d 50. High-pressure treatment of cheese made from milk without the bacteriocin producer accelerated casein degradation and increased the free AA content, but it did not significantly influence the taste quality or taste intensity of the cheese. Addition of the bacteriocin producer to milk lowered the ratio of hydrophobic peptides to hydrophilic peptides, increased the free AA content, and enhanced the taste intensity. The combination of milk inoculation with the bacteriocin producer and high-pressure treatment of the cheese resulted in higher levels of both hydrophobic and hydrophilic peptides but had no significant effect on the free AA content, taste quality, or taste intensity.
Key words: high pressure, bacteriocin, cheese, proteolysis

INTRODUCTION

Ripening of hard cheese varieties is a long and costly process. Therefore, a shortened cheese-ripening period would lead to a considerable reduction in manufacturing costs. During ripening, milk proteins, fat, lactose, citrate, and lactate are broken down or transformed into metabolites that include a high number of flavor compounds. These biochemical changes are carried out by milk enzymes, rennet, starter cultures, and secondary microbiota.

Lactic acid bacteria (**LAB**) contribute to cheese ripening through the production of metabolites that influence cheese texture and sensory characteristics. They are also an important source of enzymes such as proteinases, peptidases, AA catabolic enzymes, and esterases, which transform milk constituents retained in the curd into low molecular weight compounds (Fox et al., 1996). Lysis of starter cells will favor the access of intracellular enzymes of LAB to their substrates and presumably will accelerate cheese ripening.

To enhance the lysis of LAB during cheese manufacture and early ripening, bacteriocin-producing (**BP**) adjunct cultures may be added to milk. Strains of BP-LAB used by different groups have been mostly lactococci, although enterococci were also investigated for this purpose. *Lactococcus lactis* ssp. *lactis* DPC3286, a producer of lactococcins A, B, and M, increased the concentration of free AA (**FAA**) and reduced bitterness scores when used as an adjunct culture in Cheddar cheese manufacture (Morgan et al., 1997). *Enterococcus faecalis* INIA 4, a producer of AS-48 enterocin, accelerated cell lysis, cheese proteolysis, and flavor development when added to milk as an adjunct culture in the manufacture of Hispánico cheese (Garde et al., 1997). Nonprotein and amino nitrogen levels were increased in a semihard cheese when a lacticin 3147-producing *L. lactis* strain was used as a starter culture (Martínez-Cuesta et al., 2001). Addition of *L. lactis* ssp. *lactis* INIA 415, a producer of nisin Z and lacticin 481, increased the release of aminopeptidase activity, rate of proteolysis, and amount of FAA in Hispánico cheese made from milk inoculated with *L. lactis* ssp. *lactis* INIA 415-2, a spontaneous bacteriocin-nonproducing (**BNP**) mutant, and a *Streptococcus thermophilus* culture (Ávila et al., 2005).

Another method used to accelerate the lysis of starter cells and subsequent cheese ripening is high-pressure (**HP**) treatment of the cheese. The permeability of the lactococcal cell membrane is increased by HP treatment (Malone et al., 2002), favoring the release of intracellu-

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lar material such as peptidases to the cheese matrix (Trujillo et al., 2000). On the other hand, HP treatment may induce conformational changes in the casein structure, making the protein more susceptible to the action of proteases (Kunugi, 1993). A faster α_{s1}-casein degradation and an increase in pH 4.6-soluble nitrogen and FAA were reported for Cheddar cheese treated at 50 MPa for 72 h at 25°C (O'Reilly et al., 2000). Treatment of Garrotxa goat milk cheese at 400 MPa for 5 min at 14°C increased the FAA (Saldo et al., 2002). A higher FAA content was reported for ewe milk cheese treated at 300 MPa for 10 min at 12°C than for untreated control cheese and for cheeses treated at 400 or 500 MPa (Juan et al., 2004).

Hispánico cheese is a semihard Spanish variety made from a mixture of cow and ewe milks. It stands as a representative of the varieties made from a mixture of milks from more than one species, which account for more than 50% of the cheese produced in Spain. In the present work, HP treatment of Hispánico cheese after ripening for 15 d, by itself or in combination with a BP adjunct culture added to the milk, was investigated with the aim of accelerating the ripening process. The effects on the proteolysis, texture, and taste of Hispánico during cheese ripening are reported herein.

MATERIALS AND METHODS

Lactic Cultures

Lactococcus lactis ssp. *lactis* INIA 415, a producer of nisin Z and lacticin 481, from the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) culture collection, was used as the BP culture. *Lactococcus lactis* ssp. *lactis* INIA 415-2 is a spontaneous nisin- and lacticin 481-resistant BNP mutant, with acid production and proteolytic activities similar to those of the parental strain. Both strains were maintained at –80°C in de Man, Rogosa and Sharpe broth (MRS broth; Biolife, Milano, Italy) and subcultured twice in reconstituted skim milk at 30°C before use as mesophilic starters in cheese manufacture. Commercial lactic culture TA052 (Rhodia Iberia, Madrid, Spain) consists of *S. thermophilus* strains of high aminopeptidase activity. It was subcultured twice in reconstituted skim milk at 37°C before use in cheese manufacture.

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 was conducted in two 100-L vats. Concentrations of lactic cultures were chosen following laboratory-scale cheese-making trials. Lactic cultures for vat 1 (BNP cheeses) were 1% BNP culture and 2% *S. thermophilus* culture. Lactic cultures for vat 2 (BP cheeses) were 0.5% BNP culture, 0.5% BP culture, and 2% *S. thermophilus* culture. Rennet (6 mL of Maxiren, 1:15,000 strength; Gist Brocades, Delft, The Netherlands) was added to the milk 60 min after lactic culture inoculation. After the milk had coagulated at 33°C for 40 min, the curds were cut into 6- to 8-mm cubes and scalded at 37°C for 15 min. The whey was drained off and the curds were distributed into cylindrical moulds. Six cheeses, approximately 2 kg in weight, were obtained from each vat. The cheeses were pressed overnight at 20°C and 1.5 kg/cm^2 pressure, salted for 24 h at 12°C in 160 g of NaCl/L of brine, and ripened at 12°C and 85% relative humidity for 50 d. Cheeses were coated on d 7 with 2 layers of pimaricine-containing polyvinyl acetate.

HP Treatment

After 15 d of ripening, 3 cheeses from each vat (BNP-HP and BP-HP cheeses) were vacuum-packaged in CN300 bags (Cryovac Grace S.A., Barcelona, Spain) and pressurized at 400 MPa for 5 min at an initial temperature of 10°C, by means of a 100-L capacity discontinuous isostatic press at NC Hyperbaric (Burgos, Spain). Come-up time to reach 400 MPa was 5.9 min, and depressurization time was 1.8 min. The temperature of the water used as a pressure-transmitting fluid did not exceed 14°C during the process. After treatment, the BNP-HP and BP-HP cheeses were unpacked and followed ripening until d 50. The other 3 cheeses from each vat, which were not pressurized (**NHP** cheeses), were not vacuum-packaged.

Microbiological Analysis

Viable counts of LAB were determined in duplicate on plate count agar (Liofilchem, Roseto, Italy) with 0.1% skim milk (Biolife) added, using a DS Plus Spiral plater (Interscience, Saint-Nom-La-Bretèche, France). Previous trials had shown that lactococci were the only colony formers on plates incubated aerobically for 24 h at 30°C, and that thermophilic streptococci were the only colony formers on plates incubated aerobically for 24 h at 40°C. Bacteriocin-producing lactococci were determined on the surface of double-layer APT agar plates (Biolife), 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* ssp. *lactis* INIA 415.

Bacteriocin Activity

For the determination of bacteriocin activity, cheese samples held at –40°C were thawed and 5-g amounts