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The Contribution of Lactococcal Starter Proteinases to Proteolysis in Cheddar Cheese

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The contribution of the lactococcal proteinase to proteolysis and flavor development in Cheddar cheese was investigated using the starter strains *Lactococcus lactis* ssp. *lactis* UC317, its proteinase-negative derivative FH041, and variants of UC317 modified in proteinase production, location, and specificity. *Lactococcus lactis* ssp. *lactis* FH041 was transformed by electroporation with plasmids pCI3601, pCI3602, or pNZ521. Plasmids pCI3601 and pCI3602 harbor the cloned proteinase genes of *L. lactis* ssp. *lactis* UC317 on a high copy number vector and, as such, encode an increased concentration of cell wall-associated and secreted enzymes, respectively. Plasmid pNZ521 contains the cloned proteinase genes from *Lactococcus lactis* ssp. *cremoris* SK11. Assessment of proteolysis and flavor development in Cheddar cheese made with these strains revealed that starter proteinases are required for the accumulation of small peptides and free amino acids in Cheddar cheese. Proteolysis was not enhanced by an approximately three-fold increase in concentration of the lactococcal proteinase. The strain in which the proteinase remained attached to the cell wall appeared to contribute more to proteolysis than the strain that secreted the enzyme. Water-soluble peptides unique to *Lactococcus lactis* ssp. *cremoris* SK11 and *L. lactis* ssp. *lactis* UC317 were detected by PAGE and HPLC, respectively. Sensory evaluation showed that the flavors of all cheeses made with proteinase-positive starters were similar, but cheeses made with proteinase-negative starters lacked flavor.

Key Words: starter proteinases • cell wall-associated proteinase • secreted proteinase • cheese ripening

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