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How models can help to understand and improve the production of protein based bio-ingredients by the lactic acid bacterium *Lactococcus lactis*

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Introduction: Both consumers and industry currently show an increased awareness of healthy and tasty foods. In fermented dairy products lactic acid bacteria (LAB) play an important role in production of bio-ingredients that contribute to both aspects. With a focus on protein metabolism knowledge on balanced degradation of e.g. casein, the formation and subsequent breakdown of peptides, is important in order to (i) produce bioactive peptides that have health beneficial effects and (ii) prevent accumulation of peptides that contribute to a bitter and undesirable taste. Moreover, the proteolysis and peptidolysis of proteins is prerequisite to generate free amino acids, which is the main pathway to flavour formation in cheese manufacture. The hydrolysis of -casein by the cell envelope located PrtPI proteinase of *Lactococcus lactis,* however, is a complex process in which the enzyme is able to cleave multiple peptide bonds. A good mathematical description of the degradation of intact protein is a key step in the understanding of the dynamics of this process.

Methods: To study the extracellular hydrolysis of -casein, without the interference of uptake and degradation of released peptides, *Lactococcus lactis* IM17pLP712 was used. This mutant strain lacks the oligopeptide transport system. Purified bovine -casein was hydrolyzed in a buffer system using non-growing cells and two experimental scenarios: (i) constant enzyme concentration and (ii) constant initial enzyme/substrate ratio. Hydrolysis of the intact -casein was monitored by RP-UPLC.

The experimental data were used to estimate the parameters of four candidate kinetic models.

Results and conclusion: The degradation characteristics of -casein demonstrated that the hydrolysis of intact protein becomes slower at higher initial concentrations. At e.g. an initial concentration of 17.6 M the enzyme had degraded 87% of the intact protein after 30 min. whereas at an initial concentration of 29.7 M -casein, only 60% was degraded. Such a relationship may be caused by a decrease in the accessibility (e.g. micelle formation) of the protein and competition for the active sites of the enzyme.

The obtained data were used to estimate the parameters of four candidate kinetic models: First-order kinetics, n^{th}-order kinetics, Michaelis-Menten kinetics, competitive inhibition kinetics. As the hydrolysis rate was affected by the initial protein concentration, the models were modified to take this relationship into account.

Based on the fit to the experimental data, the modified competitive inhibition model was selected as the best one to describe the hydrolysis of intact -casein by *Lactococcus lactis* cells in a broad range of experimental conditions.