ENGINEERING OF CAMEL CHYMOSIN FOR IMPROVED CHEESE PROPERTIES

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More than 20 Mio tons of cheese are produced world-wide per year. By improving cheese yield and quality through process optimization, the amount of milk needed for manufacturing can be reduced significantly. Chymosin, an aspartic acid protease, is initiating milk coagulation in cheese manufacturing by cleaving off the glycomacropeptide (GMP) from the surface of casein micelles. Non-specific proteolysis of casein molecules by chymosin during this milk clotting process releases soluble peptides into the whey, resulting in protein losses from the cheese. The ratio between specific clotting activity (C) and non-specific proteolysis (P) of a coagulant can therefore be used as predictor for cheese yield. During ripening of the cheese, remaining coagulant continues proteolytic break-down of the caseins with significant impact on cheese properties. While the main proteolytic activity, the release of N-terminal peptides from alphaS1 casein (alphaS1-N), is associated with cheese softening and loss of firmness, cleavage of the C-terminal end of beta casein (beta-C) contributes to unwanted bitterness of the cheese [1]. The chymosin from *Bos taurus* (bovine chymosin) is traditionally used as milk coagulant in cheese manufacture. However, the homologous enzyme from *Camelus dromedarius* (camel chymosin) has been shown to be a superior alternative for various cheese types, since it reveals higher specific activity (C) and specificity (C/P) for the milk clotting reaction [2], as well as lower alphaS1 and beta casein proteolysis during ripening (Fig. 1).

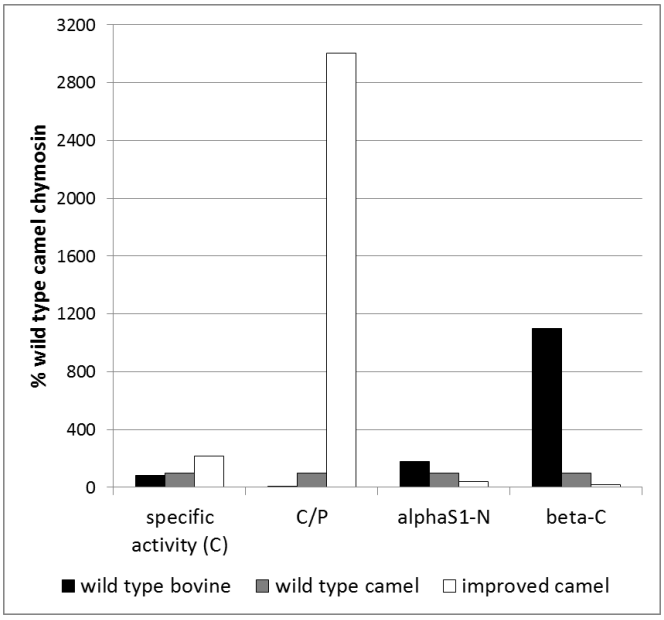

Figure 1 – Properties of wild type chymosins and an improved variant

We have further improved the catalytic properties of camel chymosin by enzyme engineering, applying a combined rational, bioinformatics and directed evolution approach [3]. Mutations selected by structural and sequence comparison of bovine and camel chymosin [4] as well as from multiple sequence alignments of homologous enzymes were evaluated in multiple rounds of diversity generation and functional screening. Gene libraries were designed by DoE principles and the impact of the tested mutations on proteolytic activity and specificity were determined by sequence-function models (ProteinGPS technology provided by ATUM [5]). The resulting high information content of the libraries yielded sufficient improvement of four parameters after analysis of only about 500 variants. This efficiency allowed us to screen the enzyme variants in near-product grade quality in application relevant assays, including an LC-MS/MS-based mapping of casein degradation products in model cheeses. Compared to the wild type starting point, the best camel chymosin variant showed about two-fold increased specific milk-clotting activity, a 30-fold higher C/P ratio, as well as 60% and 80% reduced alphaS1-N and beta-C casein proteolysis, respectively (Fig. 1).

[1] P. L. H. McSweeny, Int. J. Dairy Technol. 2004, 57, 127-144.
[2] S. R. Kappeler et al., Biochem. Biophys. Res. Commun. 2006, 342, 647-654.
[3] Pending patent applications: WO2016207214A1, EP16170409, EP16170411, PCT/EP2016/070468.
[4] J. Langholm Jensen et al., Acta Cryst. 2013, D69, 901-913.
[5] Issued US patents 8635029, 8412461, 8005620 and related pending applications