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Titel des Beitrags:
Formation of Kokumi-Enhancing gamma-Glutamyl Dipeptides in Parmesan Cheese by Means of gamma-Glutamyltransferase Activity and Stable Isotope Double-Labeling Studies

Abstract:
Recently, gamma-glutamyl dipeptides (gamma-GPs) were found to be responsible for the attractive kokumi flavor of Parmesan cheese (PC). Quantitation of gamma-GPs and their parent amino acids in 13-, 24-, and 30-month ripened PC samples by LC-MS/MS and stable isotope dilution analysis (SIDA), in-cheese (13)C-labeling studies, followed by analysis of the gamma-glutamyl transferase (GGT) activity revealed gamma-GPs to be generated most efficiently after 24 months of ripening by a GGT-catalyzed transfer of the gamma-glutamyl moiety of L-glutamine onto various acceptor amino acids released upon casein proteolysis. Following the identification of milk as a potential GGT source in PC, the functionality of the milk's GGT to generate the target gamma-GPs was validated by stable isotope double-labeling (SIDL) experiments. Therefore, raw and heat-treated milk samples were incubated with L-glutamine-[U-(13)C] and acceptor amino acids (X) and the hetero- (gamma-Glu-[(13)C5]-X) and homotranspeptidation products (gamma-Glu-Gln-[(13)C10]) were quantitated by LC-MS/MS-SIDA using gamma-Glu-Ala-[(13)C3] as the internal standard. High GGT activity to generate the gamma-GPs and preference for L-phenylalanine and L-methionine as acceptor amino acids...
acids were found in raw milk and milk samples heat-treated for 10 min up to a maximum of 65 degrees C. In comparison, GGT activity and SIDL studies performed with inoculated Lactobacillus strains, including Lactobacillus harbinensis and Lactobacillus casei identified in PC by means of 16S rRNA gene sequencing, did not show any significant GGT activity and unequivocally demonstrated unpasteurized cow's milk, rather than microorganisms, as a key factor in gamma-glutamyl dipeptide generation in Parmesan cheese.