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Analysis and evaluation of potential nutraceutical milk and dairy products derived from the Centrale del latte di Salerno

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Abstract

The main objective of my PhD has been focused to the analysis and characterization of milk and dairy products derived from "*Centrale del Latte di Salerno*" in order to assess their potential use as dietary supplements and functional foods. To fulfill these objectives, in these three years my research group developed innovative analytical methods for the qualitative characterization of the protein fraction, contained in commercial bovine milk products and preserved at room temperature after expiration date.

The chromatographic characterization of caseins is a challenging task due to the tendency of such molecules to form, in aqueous solution, micellar structures, highly stabilized by non-covalent interactions. For this reason, in order to optimize chromatographic resolution, we studied the effects of various solvents and denaturant solutions capable of altering the three-dimensional structure, for a better identification of the individual components.

The best chromatographic separation was obtained by incubation of casein powder, for one hour at room temperature, with a denaturing solution containing 8 M urea, 165 mM Tris-HCl, 44 mM sodium citrate, 0.3% β-mercaptoethanol.

The caseins were, thereafter, characterized by Cap-LC-HRMS experiments, carried out by employing a lab-made monolithic capillary column (Protein-Cap-RP-Lauryl-γ- Monolithic). The newly developed method allowed to study the degradation pattern of the protein fraction in milk samples. Results obtained highlighted the stability of α_S- and κ-casein to proteolysis as well the formation of polypeptides (proteose-peptones) deriving from cleavage of the N-terminal and of the C-terminal β-CN by bovine plasmin.

Moreover, a different protein degradation pattern was observed in different milk samples. We hypothesized that a possible stabilizing role is exerted by the lipid fraction, able in inhibiting casein degradation in whole milk. This effect was, in fact, not evidenced in skim milk and only partially observed in semi-skimmed milk. Similarly, the whey protein stability in semi-skimmed milk samples has been studied, underlining a time-dependent degradation of β-lactoglobulin A and B, associated to the formation of a large number of peptides.

Analysis of soluble peptides fraction four week after the expiry date, was performed by an on-line comprehensive two dimensional liquid chromatography, using the high performance combined with the ultra high performance conditions. Through the use of a differential pHs between the two dimensions and a continuous shifted gradient in 2D, high values of peak capacity, satisfactory selectivity as well as good employment of the 2D separation space were obtained. At the best of our knowledge this is the first time that these chromatographic conditions are applied for peptide separation and analysis.

Finally, peptide sequences corresponding to the β-Casomorphins have been identified by 1D-LC-ESI-IT-TOF analysis. These results confirmed that β-casein is the protein mainly subjected to proteolytic degradation in semi-skim milk samples, stored at room temperature four weeks after expiration date.