

C. ORALE 21:

MILK-CLOTTING ENZYMES FROM WILD CARDOON FLOWERS: IDENTIFICATION AND TECHNOLOGICAL CHARACTERIZATION

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Keywords: Wild cardoon, Rennet, Proteomic, Cardosins, Milk gelation.

Abstract: Flowers of *Cynara cardunculus* contains aspartic proteases, mostly used in milk coagulation and cheese making. In this study, proteomic approach was firstly applied to identify milk-clotting enzymes from wild cardoon flowers. The second objective was to optimize extraction conditions of *C. cardunculus* rennet by response surface methodology, in order to maximize its milk-clotting activity (MCA). Then, technological characteristics (G' , G'' , gel firmness, gelation time and water holding capacity) of gels produced by the optimized extract and chymosin were investigated. Proteomic analysis revealed that 46 % of the total analyzed spots, in 2-D gel, resulted in a protein identification by mass spectrometry MALDI-TOF. Four cardosins (A, E, G, and H), which have become a subject of great interest in dairy technology, were identified. The absence of the other cardosins could be an advantage, as it reduces the excessive proteolytic activity that causes bitter flavors and texture defects, during further cheese making. Concerning enzymes extraction, results of the central composite design showed that the optimum extraction conditions, corresponding to maximum MCA (9.550 CAU/mL), were selected as follows: Grinding time 30 min, pH 3, Extraction time 50 min and Solid to liquid ratio 15 g/100 mL. According to technological properties, it can be concluded that *C. cardunculus* extract exhibits an excellent efficiency on raw skim milk, in term of dynamic moduli (G' , G'') and gel firmness. A good gelation time and water holding capacity were also obtained. Consequently, the optimized *C. cardunculus* rennet has the potential to be employed as an efficient milk-clotting agent and a good alternative to animal rennet in the production of cheese.