

Potentialities of *Onopordum Tauricum* (Willd.) as Milk Clotting Agent

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Abstract : Proteases from herbs, woody plants, and trees are exploited for cheesemaking in several countries, especially in South Europe and West Africa. Particularly, “thistles” belonging to several genera within the Asteraceae family (*Cynara*, *Silybum*, *Centaurea*, *Carlina*, *Cirsium*, *Onopordum*) are traditionally used in Mediterranean countries for clotting raw ewe’s and goat’s milk. For the first time, the clotting performance of an aqueous extract from flowers of *Onopordum tauricum* Willd. (Taurian thistle, bull cottonthistle) were tested in milk of different origin (cow, goat, ewe). The vegetable material was collected in the Central Apennines range, between the Marche and Umbria regions. A response surface methodology (RSM) approach was used to study the effect of the curdling variables (temperature, pH, amount of enzymatic extract) on the technological performance of the thistle extract. A three-step procedure for the purification of the enzyme (ammonium sulphate precipitation, gel filtration and ion-exchange chromatography) was also carried out. The milk clotting activity (MCA) of *O. tauricum* crude extracts was strongly affected by temperature, pH and by the interaction between these two variables, according to a second-order response surface model, while the milk/coagulant ratio did not affect in a significant way the clotting properties. Experimental data showed that the addition of 10 mM CaCl₂ reduced the clotting time of ewe’s, goat’s, and cow’s milk by about 3-fold, 8-fold, and 14-fold, respectively, at 35°C and pH 6.7-6.8. After purification, an enzymatic preparation very close to homogeneity was obtained, which showed a major band at about 30 kDa when analyzed by SDS-PAGE. The identity of the enzyme as an aspartic protease was confirmed by inhibition studies. Cheese-making trials were carried out to check the scale-up (1 to 5 L of milk; 37 °C; 10 mM CaCl₂ fortification) and set the recipe: 35-45% of curd yields were recorded, according to curd cutting and pressing.

Keywords : milk clotting activity, *Onopordum tauricum*, plant proteases, vegetable rennet

Conference Title : ICDSP 2020 : International Conference on Dairy Science and Processing

Conference Location : Venice, Italy

Conference Dates : November 12-13, 2020