NATIVE PLANT PROTEASES AS NOVEL COAGULANTS FOR MILK CLOTTING

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Presentation*

Scientific background*
Milk clotting can be achieved by a number of proteolytic enzymes from various sources, such as different animal and microbial species. Plant coagulants are of growing interest, as the use of animal rennet may be limited for religious reasons (e.g., Judaism and Islam), diet (vegetarianism), or consumer concern regarding genetically engineered foods (Egito et al., 2007). Milk-clotting enzymes from plant sources have been identified in *Ananas comosus* (bromelain), *Ficus* spp. (ficin) and *Carica papaya* (papain). However, information are still scarce concerning the kinetic of milk coagulation by proteases of plant origin.

Aims
In order to delineate the milk clotting potential associated with different vegetable proteases (bromelain, ficin and papain), the milk-clotting index (MCI) was calculated by measuring the caseinolytic (CA) and milk-clotting activities (MCA), both assay using skim-milk powder and milk from different farm animals (buffalo, cow, goat and sheep) as substrates. The traditional animal rennet (chymosin) was used as reference coagulant.

Methods
Materials: The milk from buffalo (CbM), cow (CM), goat (GM) and sheep (SM) were purchased from rural dairy farm. The 1% (w/v) casein solution and the reconstituted skim milk (33%, w/v) was prepared in sodium acetate buffer pH 5.5 (AB).
Methods: The caseinolytic activity (CA) of proteases (bromelain, ficin and papain) and chymosin was assayed using casein and CbM, CM, GM, SM as substrate. The milk-clotting activity (MCA) was determined by adding the enzyme solutions (30 mg/ml) to the reconstituted skim milk or CbM, CM, GM and SM (Ahmed et al., 2009). From the ratio of MCA *vs* caseynolitic activity (CA), the milk clotting index (MCI) was calculated.

Results
The milk clotting potential of vegetable proteases and chymosin, expressed as CA, MCA and MCI, were reported in Fig. 1. For all caseinic substrates, proteolytic activity of vegetable proteases were always higher than that chymosin exhibited (Fig. 1A). In particular, the ficin showed the highest caseinolityc activity while bromelain and papain had lower and similar values. This confirms the already known drawback of vegetable enzymes that, while possessing high caseinolytic activity, produce undesired flavour and cheese texture (Anusha et al., 2014).
Thus, it is important to adequately evaluate the degradation patterns of casein when choosing a potential plant rennet substitute. For this reason, in our study, the MCA and MCI were determined as complementary parameters for the caseinolytic activity in order to evaluate the milk clotting potential.
MCA determined for all enzymes, under tested conditions, were comparable to those previously reported in literature (Anusha et al., 2014). Interestingly the results, reported in Fig. 1B, demonstrate that the highest value of milk-clotting activity was revealed by bromelain while ficin showed the lowest one. The influence of milk composition on the clotting time was observed for all enzymes tested whereas the skim-milk reconstituted was the substrate much less hydrolyzed. The different clotting aptitude of milk from different mammalian species suggests the need of technology modifications according to the milk and vegetable rennet characteristics.
Finally, the ratio of milk clotting *vs* caseinolytic activity (MCI) has been considered crucial for quality cheese production ranging from 0.68 to 9.58 (Cristina et al.,2012). In the present MCI values of bromelain, ficin and papain ranged from 0.1–4.25 (Fig. 1C). By comparing all data, bromelain exhibited a

promising MCA/CA ratio comparable to the value obtained for chymosin. On the contrary ficin resulted to be the less suitable as coagulant for all kind of milk tested.

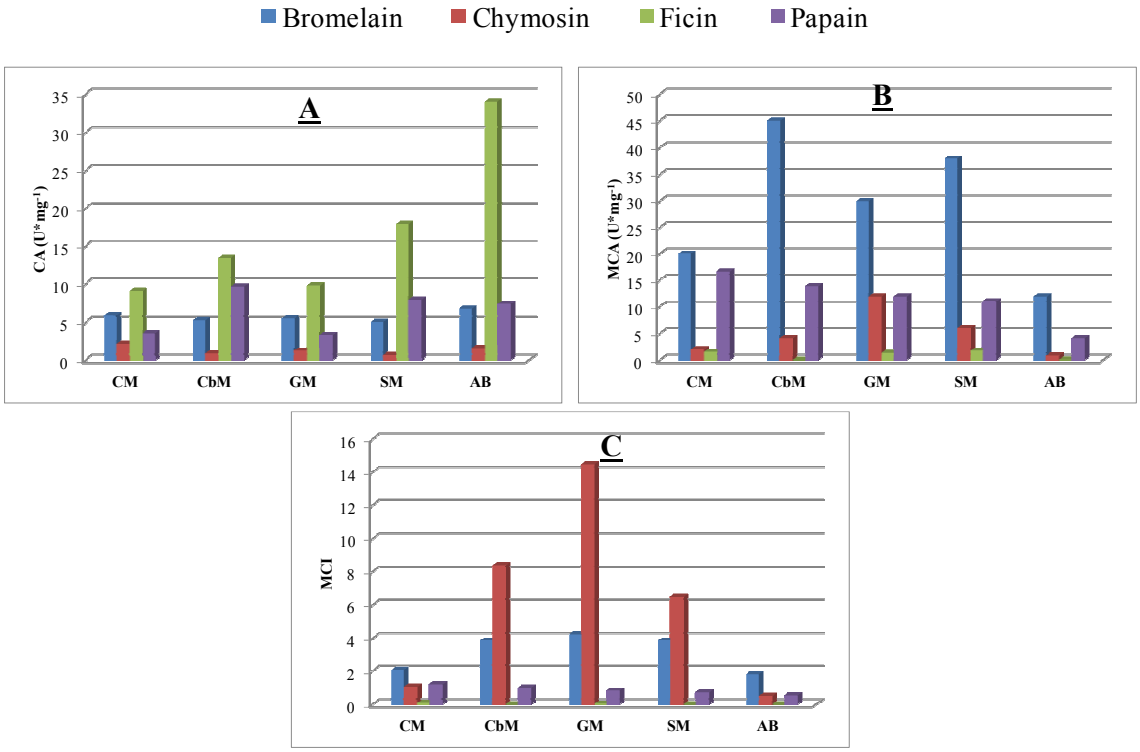

Figure 1: Caseinolytic and milk-clotting activity (CA and MCA, respectively), measured for vegetable proteases and chymosin, were assayed in acetate buffer pH 5.5 (AB) and in cow (CM), buffalo (CbM), goat (GM) and sheep (SM) milk. The resulting milk clotting index (MCI) was reported.
Caseinolityc activity (CA) was espressed as U*mg^{-1}, one unit was defined as the amount of enzyme (mg) that liberate 1 mg of tyrosin per min under standard assay
The MCA was expressed as U*mg $^{-1}$, where one unit was defined as the amount of crude enzyme or protein extracted (mg) needed to coagulate a volume (ml) of reconstituted skim milk powder in 40 min at 37° C.

Conclusion

Findings from current study reveal bromelain to be similar to chymosin in milk coagulation pattern and it therefore seems suitable as vegetable coagulant, also for the production of the new and innovative cheeses.

For these reasons, further study relative to the bromelain purification and immobilization needs to be done to improve the quality of milk coagulum and to confirm its usefulness in the dairy industry.

References

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