

**Comparison of selective hydrolysis of  $\alpha$ -lactalbumin by Acid Protease A and Protease M as alternative to pepsin: Potential for  $\beta$ -lactoglobulin purification in whey proteins**

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**Short title:** Selective hydrolysis of  $\alpha$ -lactalbumin: Microbial enzymes vs pepsin

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## Summary

The experiments reported in this research paper examines the applicability of the acidic enzymes digestion under low pH on the susceptibility of  $\alpha$ -lactalbumin ( $\alpha$ -La) to enzyme hydrolysis while conferring the resistance of the  $\beta$ -lactoglobulin ( $\beta$ -Lg). Hydrolysis of whey protein by enzymes Acid Protease A and Protease M was performed in order to purify the individual whey proteins as well as compare their selectivity and hydrolysis conditions with pepsin. Since pepsin has a known selectivity to whey proteins and is an acid enzyme it was intended to investigate whether other acid enzymes like Acid Protease A and Protease M possess the same selectivity. The research was performed in order to develop a process for the preparation of pure and native  $\beta$ -Lg while  $\alpha$ -La is depleted. Analysis of the hydrolysis environment show that the pH and temperature play a significant role in determining the best conditions for achievement of above mentioned process. Whey protein isolate (WPI) was hydrolysed using pepsin, Acid Protease A and Protease M by randomized hydrolysis conditions. Reversed-phase high performance liquid chromatography was used to analyse residual proteins. Regarding enzyme selectivity under various milieu conditions, all three enzymes show similarities in the reaction progress and their potential for  $\beta$ -Lg isolation.