ANTIGENIC DETERMINANTS OF CROSS-LINKED BETA-LACTOGLOBULIN HYDROLYSATES AFTER IN VITRO DIGESTION

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Beta-lactoglobulin (β-Lg) has important technofunctional and nutritional properties, however it is resistant to digestion, especially to pepsin, and highly antigenic. Enzymatic hydrolysis is a strategy for the reduction/removal of antigenic determinants - epitopes - in milk proteins, and previous reports have indicated the enzyme transglutaminase (TG) as a means of modifying the allergenic properties of proteins. In the present study the association of hydrolysis with Alcalase and polymerization with TG was investigated as an alternative to reduce the β-Lg epitopes. β-Lg (donated by Davisco Inc.) was hydrolyzed with Alcalase (3% β-Lg w/w in distilled water; 25 U enzyme g⁻¹ of substrate, pH 7.5, 240 min, pH stat method) and then polymerized by TG (donated by Ajinomoto Co.) under the following conditions: 7% hydrolysate, 10 U TG g⁻¹ protein, 50 °C/180 min. The samples were submitted to in vitro digestion, simulating both gastric and duodenal conditions, and the peptides released were identified by RP-HPLC-tandem mass spectrometry (RP-HPLC-MS/MS). The untreated β-Lg was highly resistant to pepsin, and after gastrointestinal digestion, three IgE-binding epitope fragments - Tyr42-Leu54, Val41-Lys60 and Ala67-Ile78 - were detected. The cross-linked hydrolysate was completely digested and no epitopes were detected in this sample after gastrointestinal digestion. The results suggested that structural changes occurring in the protein after the hydrolysis and polymerization processes, resulting in the cleavage of the peptides with antigenic properties during the simulated digestion.

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