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Impact of pH Control on Peptide Profile and Antigenicity of Whey Hydrolysates

Authors : Natalia Caldeira De Carvalho, Tassia Batista Pessato, Luis Gustavo R. Fernandes, Ricardo L. Zollner, Flavia Maria Netto

Abstract : Protein hydrolysates are ingredients of enteral diets and hypoallergenic formulas. Enzymatic hydrolysis is the most commonly used method for reducing the antigenicity of milk protein. The antigenicity and physicochemical characteristics of the protein hydrolysates depend on the reaction parameters. Among them, pH has been pointed out as of the major importance. Hydrolysis reaction in laboratory scale is commonly carried out under controlled pH (pH-stat). However, from the industrial point of view, controlling pH during hydrolysis reaction may be infeasible. This study evaluated the impact of pH control on the physicochemical properties and antigenicity of the hydrolysates of whey proteins with Alcalase. Whey protein isolate (WPI) solutions containing 3 and 7 % protein (w/v) were hydrolyzed with Alcalase 50 and 100 U g-1 protein at 60°C for 180 min. The reactions were carried out under controlled and uncontrolled pH conditions. Hydrolyses performed under controlled pH (pH-stat) were initially adjusted and maintained at pH 8.5. Hydrolyses carried out without pH control were initially adjusted to pH 8.5. Degree of hydrolysis (DH) was determined by OPA method, peptides profile was evaluated by HPLC-RP, and molecular mass distribution by SDS-PAGE/Tricine. The residual α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) concentrations were determined using commercial ELISA kits. The specific IgE and IgG binding capacity of hydrolysates was evaluated by ELISA technique, using polyclonal antibodies obtained by immunization of female BALB/c mice with α-La, β-Lg and BSA. In hydrolysis under uncontrolled pH, the pH dropped from 8.5 to 7.0 during the first 15 min, remaining constant throughout the process. No significant difference was observed between the DH of the hydrolysates obtained under controlled and uncontrolled pH conditions. Although all hydrolysates showed hydrophilic character and low molecular mass peptides, hydrolysates obtained with and without pH control exhibited different chromatographic profiles. Hydrolysis under uncontrolled pH released, predominantly, peptides between 3.5 and 6.5 kDa, while hydrolysis under controlled pH released peptides smaller than 3.5 kDa. Hydrolysis with Alcalase under all conditions studied decreased by 99.9% the α-La and β-Lg concentrations in the hydrolysates detected by commercial kits. In general, β-Lg concentrations detected in the hydrolysates obtained under uncontrolled pH were significantly higher ($p<0.05$) than those detected in hydrolysates produced with pH control. The anti-α-La and anti-β-Lg IgE and IgG responses to all hydrolysates decreased significantly compared to WPI. Levels of specific IgE and IgG to the hydrolysates were below 25 and 12 ng ml-1, respectively. Despite the differences in peptide composition and α-La and β-Lg concentrations, no significant difference was found between IgE and IgG binding capacity of hydrolysates obtained with or without pH control. These results highlight the impact of pH on the hydrolysates characteristics and their concentrations of antigenic protein. Divergence between the antigen detection by commercial ELISA kits and specific IgE and IgG binding response was found in this study. This result shows that lower protein detection does not imply in lower protein antigenicity. Thus, the use of commercial kits for allergen contamination analysis should be cautious.

Keywords : allergy, enzymatic hydrolysis, milk protein, pH conditions, physicochemical characteristics
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