The Potential of Enzymatic Hydrolysis to Improve Immunotherapy and Ingredient Applications of peanut flour.

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Peanut flour is currently being used as the active ingredient in oral immunotherapy applications designed to desensitize peanut allergic patients. This strategy for treating peanut allergy is proving quite promising; however, there is a risk for adverse reactions using this approach. In the current study, enzymatic hydrolysis of peanut flour was investigated as a processing strategy to minimize such reactions and hence improve immunotherapy applications of peanut flour. To investigate this potential, soluble fractions of 10% (w/w) light roasted peanut flour dispersions were hydrolyzed with Alcalase (pH 8.0, 60°C), pepsin (pH 2.0, 37°C), Flavourzyme (pH 7.0, 50°C), or sequentially with Alcalase and Flavourzyme. SDS-PAGE was used to visualize peptide distribution. Immunoreactivity was evaluated by Western blotting, T cell proliferation, and basophil degranulation. Generally speaking, the SDS-PAGE band intensity of major peanut allergens, Ara h 1 and Ara h 3/4 decreased and a series of smaller MW (3-22 kDa) subunits were produced after enzymatic hydrolysis. Sequential and Alcalase hydrolysates retained minimal IgE binding, whereas Flavourzyme and pepsin hydrolysates displayed very clear IgE binding in the region of 0-28 kDa. These peptide fragments were derived primarily from Ara h 2 as demonstrated by Western blotting experiments using anti-Ara h1 and anti-Ara h2. T cell proliferation assays (n=5 subjects) indicated that hydrolysates retained ~50% of the T helper cells stimulation compared to non-hydrolyzed controls. Data for individual hydrolysates was comparable to unhydrolyzed controls in the basophil assay (n=5 subjects). Additionally, two bioactive properties of the hydrolyzed flours were also characterized: angiotensin-converting enzyme (ACE) inhibition and oxygen radical absorbance capacity (ORAC). Hydrolysis generally improved ACE inhibition and ORAC compared to parent proteins, and specific differences were observed across enzyme conditions. These results indicate that enzymatic hydrolysis has the potential to improve both immunotherapy and food ingredient applications of peanut flour.