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Quantifying allergenic versus non-allergenic peanut proteins by mass spectrometry

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Peanut (*Arachis hypogaea*) is a common trigger of food allergy, containing both allergenic and non-allergenic proteins. The WHO/IUIS Allergen Nomenclature recognises 17 allergenic proteins, though their clinical relevance differs. This study investigated the abundance of allergenic vs. non-allergenic proteins in four different peanut extracts and addressed the relevance of: 1) extraction method, 2) allergen databases and BLAST inclusion, and 3) clinical relevance of the peanut allergens, on the assessment of the abundancy of allergenic vs. non-allergenic proteins.

A database of allergenic proteins (Ara h 1-18) was built using allergen databases and BLAST (≥70% sequence identity, taxonomy ID 3818) to acquire unique protein accessions. Four protein extraction methods (Tris/HCl, Tris-DTT, Osborne, and GdCl-buffers) were performed on raw defatted peanut flour. Extracts were analysed by LC-MS/MS (Orbitrap Exploris 480) in data-independent acquisition mode and quantified using shared peptides via taxonomy. Relative abundance was calculated using log10-transformed median values. Data were analysed with Spectronaut 19 and GraphPad Prism.

Thirty-five new unique protein accessions for peanut allergens were identified via BLAST, in addition to Allergen.org (27) and Allergome (59). The Tris/HCl extraction had the most accession hits (48) and the greatest proportion of allergenic proteins to total proteins. Within the allergens, Ara h 3 and 1 were the most abundant allergens for all extraction methods. The total relative abundance of allergenic proteins remained high across all samples, decreasing only slightly when neglecting allergens that are not of high clinically relevance for sensitisation, leaving only Ara h 1, 2, 3, and 6 included. These four proteins quadrupled in individual average content, compared to when all 17 allergens were included, indicating that the protein abundance have an impact on not only allergenicity but also clinical relevance.

A curated database including ≥70% BLAST of allergenic proteins enabled accurate identification of allergenic vs. non-allergenic proteins in peanuts. Despite differences between extraction methods and analysis based on quantification of shared peptides via taxonomy analysis, the overall pattern of allergenic vs. non-allergenic proteins was similar and did not impact the identification of the clinically relevant sensitising proteins Ara h 1, 2, 3, and 6, which were shown to be the most abundant proteins as well.