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Investigation of raw and thermally treated peanut major allergen post-translational modifications (PTMs)

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Introduction. Peanut allergy affects a large portion of world population causing reactions ranging from mild to severe. Major peanut allergen IgE epitopes are well characterized but little is known about their post-translational modifications (PTM) and how they are affected by thermal treatment. PTM profile may differ between raw and thermally treated peanut, which could affect its allergic potential depending on type, size and position of modifications.

Objective. Our aim was to analyse and compare PTM profiles of 4 major peanut allergens - Ara h 1, Ara h 2, Ara h 3 and Ara h 6, as well as their amounts in raw and roasted samples using bottom-up proteomics methods.

Methodology. Full peanut protein extracts (both thermally treated and non-treated) were digested in gel and in solution, and analysed by a Top10 nLC-MS/MS method by LTQ Orbitrap XL (Thermo Fisher Scientific Inc., Germany). Within the extracts major allergens - Ara h 1, Ara h 2, Ara h 3 and Ara h 6 were identified, label free quantified (LFQ) and searched for PTMs by Peaks X software (Bioinformatics solutions Inc.I, Canada). Epitope sequences were acquired from the Immune Epitope Database (IEDB www.iedb.org).

Main findings. LFQ results show that there is no significant change in the amounts of any of the studied allergens between raw and roasted extracts. Out of the 4 allergens Ara h 6 is modified in the highest portion, with respect to the protein size: 15% and 12% of its positions are modified in raw and roasted sample, respectively. Total of 21 modifications were quantified between the two preparations, with oxidation (M), methylation (K,R) and dethiomethylation affecting the largest number of peptides.

Conclusions. Peanut allergen epitopes are indeed carriers of PTMs that differ in pattern and quantity between treated and non-treated extracts. The *in silico* discovered PTMs could affect protein digestibility and allergenicity. Further investigation is necessary in order to fully understand the impact protein modifications could have on their allergenic potential.

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