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Characterisation of peanut allergens and possible post-translational modifications (PTMs)

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Background
Peanut allergy is the most common type of food allergy causing severe reactions or even fatal anaphylaxis in sensitised individuals. The major peanut allergens are Ara h 1, Ara h 2, Ara h 3, and Ara h 6 which cause the most severe responses. Their molecular properties have been characterised but possible post-translational modifications (PTMs) that might explain their severe allergenicity are not well understood. The goal of this study was to utilize a combination of nanoLC-Mass Spectrometry (MS)/MS methods and PEAKS Studio 8.0 (Bioinformatics Solutions Inc., Ontario, Canada) program to evaluate PTMs in the major peanut allergens.

Method
Acquired MS data of purified peanut allergens, Ara h 1, Ara h 2, Ara h 3, and Ara h 6 were analysed and identified via hybridized databases obtained from UniProt (www.uniprot.org).
More than 1200 reviewed (Swiss-Prot) and unreviewed (TrEMBL) entries from peanut were combined with common MS contaminants, the Repository of Adventitious Proteins (cRAP), to create a hybridized database. We then focused on Ara h 2 (Conglutin-7) and Ara h 6 (Conglutin) because of their propensity to cause severe anaphylactic reactions. Epitopes found in the Immune Epitope Database (www.iedb.org) were analysed for possible PTMs by matching PEAKS PTM results with mapped positions of epitope sequences.

Results
We identified 37 proteins from the purified peanut allergens. There were 33 peanut proteins and 4 contaminants originating from human keratin and pig trypsin. Ara h 2 had 242 epitopes, 29 potential PTMs and 4 mutations. Eight of the epitopes had up to 8 possible PTMs. Several relevant PTMs were discovered, including tryptophan oxidation to oxolactone in position 25, sulfonation of N-terminus of cysteine in position 116 and oxidation of methionine in position 50 and 125. Notably, all had either a "NNQRCMCEALQ" or "QQIMENQSD" motif, which are linked to Th2 cytokines and T cell proliferation. We observed 8 epitopes, 9 likely PTMs and no mutations for Ara h 6 and half of the epitopes had possible PTMs and a maximum of 4 PTMs was found on one epitope.

Conclusion
The analysis of relevant peanut allergens by nanoLC-MS/MS methods and PEAKS Studio 8.0 program revealed several PTMs, which might have important ramifications due to their influence on allergenicity and digestibility resulting from modification properties by trypsin and other food protein enzymes. These data suggest that PTMs on certain peanut epitopes could be involved in the pathogenesis of severe food allergy to peanuts.