

# #111 Food Flour Proteins with Defined Allergen Composition for Use as Reference Materials

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## RATIONALE

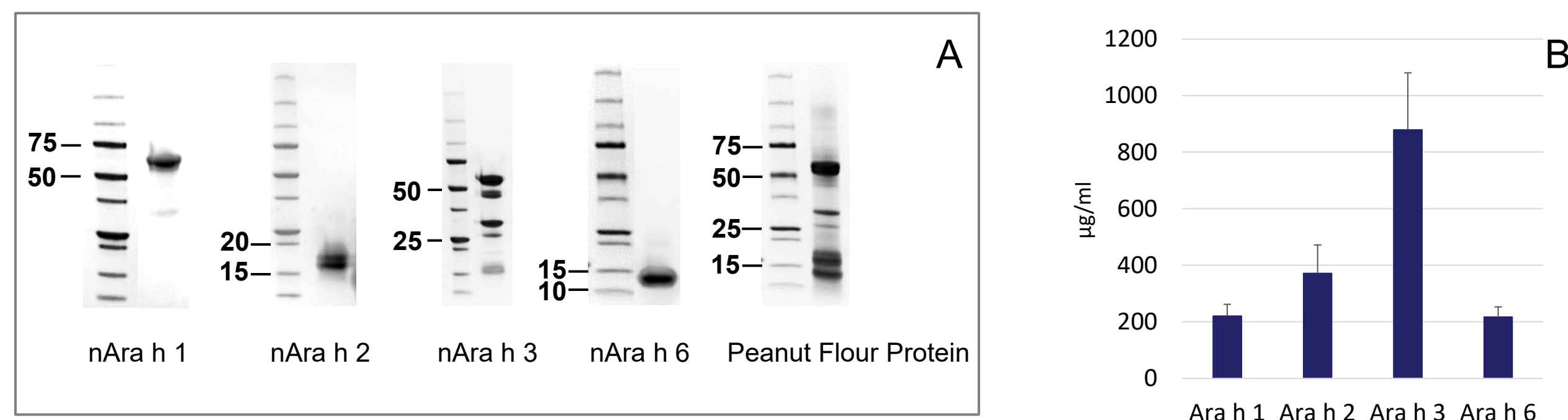
Allergen measurements are widely used for validation of molecular allergy diagnostics and allergy therapeutics. However, few standardized food allergen reference materials have been developed. While NIST and MoniQA food standards are characterized extensively for biochemical and nutritional composition, data on allergen content are lacking. The aim was to produce standardized food-flour-proteins with defined allergen content that could serve as reference materials for allergy diagnostics or therapeutics.

## METHODS

Food-flour-proteins were prepared from defatted peanut-, hazelnut-, pistachio- and soy-flour using optimized aseptic extraction conditions. Allergen composition was analyzed using validated allergen-specific ELISA's or mass spectrometry (LC-MS/MS). Real time stability data were collected from frozen allergens.

## RESULTS

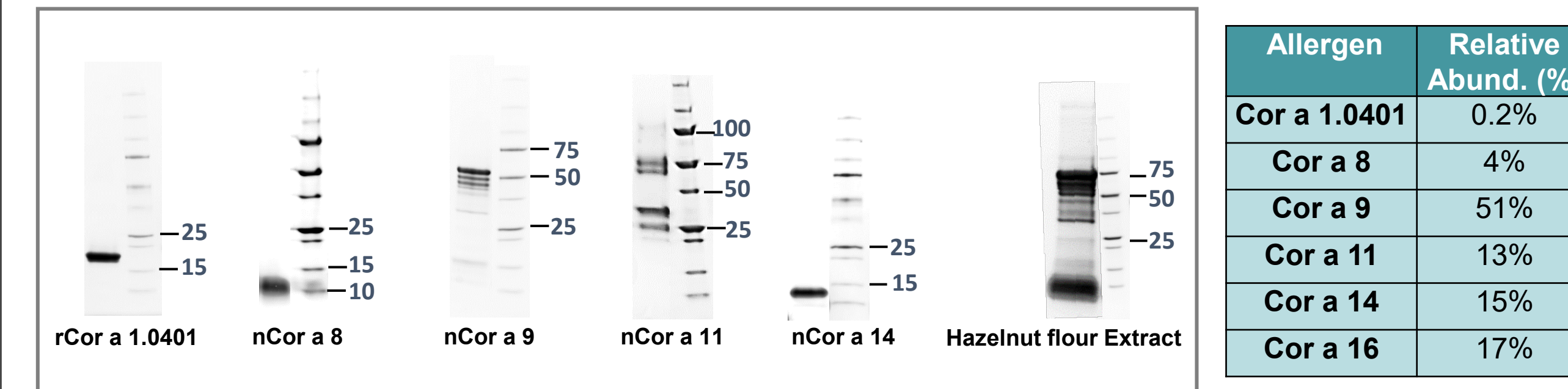
**Peanut** flour was extracted in PBS, 1M NaCl pH 7.4 . Natural Ara h 1, Ara h 2, Ara h 3, and Ara h 6 were purified from blanched or roasted peanuts (Fig.1A). Peanut allergens Ara h 1 (220 µg/ml), Ara h 2 (371 µg/ml), Ara h 3 (879 µg/ml) and Ara h 6 (217 µg/ml) were quantified in peanut-flour-protein by ELISA in quadruplicate (Fig.1B). Endotoxin levels were < 0.03 EU/µg.



**Fig. 1:** A: SDS-PAGE of purified peanut allergens and peanut flour protein under non-reducing conditions followed by Coomassie staining. B: Allergens in peanut food flour protein were quantified by peanut-allergen specific ELISA.

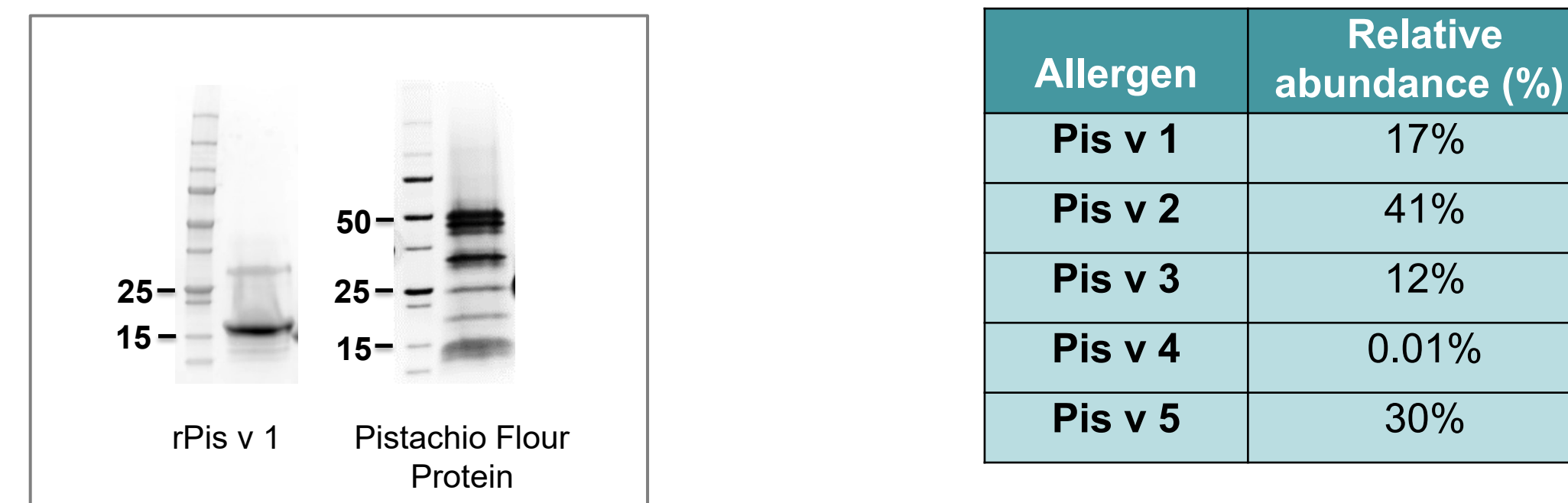
## RESULTS

**Hazelnut** flour was extracted in PBS, pH 7.4 . Hazelnut allergens Cor a 1, Cor a 8, Cor a 9, Cor a 11, and Cor a 14 were purified from hazelnuts or expressed as recombinant proteins (Fig.2). LC-MS/MS analysis of hazelnut-flour confirmed the presence of Cor a 1, Cor a 8, Cor a 9, Cor a 11, Cor a 14 and Cor a 16 (Table 1).



**Fig. 2:** SDS-PAGE of purified hazelnut allergens and hazelnut flour protein under non-reducing conditions followed by Coomassie staining. **Table 1:** LC-MS/MS of allergens in hazelnut flour protein.

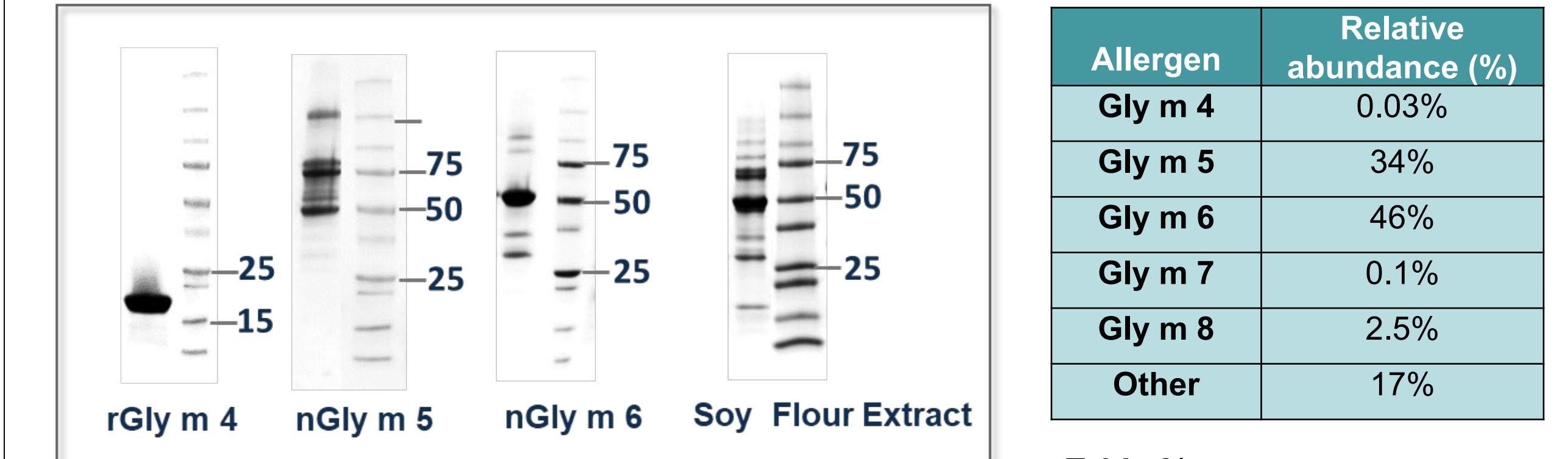
**Pistachio** flour was extracted in 0.1M Potassium phosphate, 0.4M NaCl pH 8.0. Pistachio allergen Pis v 1 was expressed as recombinant protein (Fig.3). LC-MS/MS analysis of pistachio flour protein confirmed the presence of Pis v 1, Pis v 2, Pis v 3, Pis v 4, and Pis v 5 (Table 2).



**Fig. 3:** SDS-PAGE of purified Pis v 1 and Pistachio flour protein under non-reducing conditions followed by Coomassie staining. **Table 2:** LC-MS/MS of allergens in pistachio flour protein.

## RESULTS

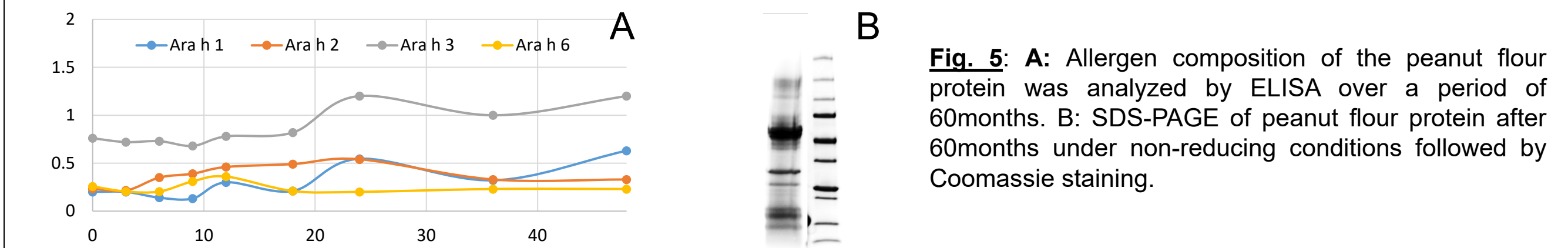
**Soy** flour was extracted in 50mM Tris, pH 8.2. Soy allergens Gly m 4, Gly m 5 and Gly m 6 were purified from soy or expressed as rec. proteins (Fig.4). LC-MS<sup>2</sup> analysis of soy-flour confirmed the presence of Gly m 4, Gly m 5, Gly m 6, Gly m 7, and Gly m 8 (Table 3).



**Fig. 4:** SDS-PAGE of purified soy allergens and soy flour protein under non-reducing conditions followed by Coomassie staining. **Table 3:** LC-MS/MS of allergens in soy flour protein.

## Stability Study of Peanut Flour Protein

Real time stability tests of frozen food-flour-proteins showed consistent protein content by ELISA and no signs of degradation on SDS-PAGE (Example peanut flour protein Fig. 5).



## CONCLUSIONS

Optimized, ISO-9001 compliant, bioprocessing pathways have been established to yield standardized legume and tree nut food-flour-proteins with defined allergen profiles which can serve as food reference materials. The low-endotoxin, stable food-flour-proteins have applications as reference materials for monitoring the composition of allergy diagnostics and therapeutics.

**\*Note:** (1) Relative abundance results are based on label-free quantification of precursor peptide ion intensity (peak area, incl. unique + razor peptides) using Proteome Discoverer 2.2 algorithms. (2) Calculations of relative abundance can be variable and depend on multiple factors such as sample complexity, buffer composition, instrument resolution and analysis software.