

Analytical Methods

Thermal processing of peanut impacts detection by current analytical techniques



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ABSTRACT

Recalls of spice containing products due to undeclared peanut have highlighted the importance of analytical methods in these foods. We examined the performance of peanut detection methods in cumin and garlic, focusing on quantitative ELISA. Although suitable for qualitative detection, accurate quantitation proved difficult. Roasting of peanut contaminants influenced ELISA results, with raw peanut over-detected (3.9-fold) and roasted peanut under-detected (3.5-fold). Further investigation demonstrated the importance of protein targets for ELISA. The kit which gave the least variable results was based on detection of 2S albumin proteins. Additionally, we show that these proteins are more efficiently extracted from roasted peanut. We conclude that current methods are largely suitable for the qualitative detection of peanut in cumin and garlic. Quantitation relies on assumptions as to the state of thermal processing of peanut. We suggest that analytical method providers address robust detection by target selection, including identifying targets by MS.

1. Introduction

Food allergy is increasingly prevalent in Westernized economies (Branum & Lukacs, 2009; Prescott & Allen, 2011). There are currently no standardized treatments available for food allergy, and thus an avoidance diet is necessary for those affected (Zeiger, 2003). The practice of avoidance is facilitated by national regulations governing the labelling of foods known to be problematic allergens, such as the Food Allergen Labeling and Consumer Protection Act (FALCPA) in the U.S. and EC Regulation 1169/2011 in the European Union (EU). The ability of the food industry to comply with such regulations is dependent on the proper control of allergens within the food manufacture and supply chain (Jackson et al., 2008). Measures for the control of allergens are often validated through the use of appropriate analytical techniques to detect specific allergenic foodstuffs. The most commonly used techniques are immunological, such as enzyme-linked immunosorbent assay (ELISA) and lateral-flow devices using antibodies raised to one or more allergenic proteins within the foodstuff in question. Polymerase chain reaction (PCR) is also sometimes used to detect the presence of deoxyribonucleic acid (DNA) from the offending allergenic food (Iniesto et al., 2013; Ozdal, Capanoglu, & Altay, 2013).

It has long been known that methods of allergen analysis vary in effectiveness depending on the type of food being analyzed (Abbott

et al., 2010). This has led to calls for standardized materials for the verification of detection methodology within often encountered or problematic food matrices (Taylor, Nordlee, Niemann, & Lambrecht, 2009). Although cumin powder, as a dry minimally processed powder, may at first appear to be a simple matrix in which to analyze for allergens, the chemical constituents of cumin may contribute to difficulty in protein analysis. Cumin contains large amounts of polyphenols, 7.45 mg/g dry seed (Dua, K Gupta, & Mittal, 2012), of which gallic acid, quercetin and kaempferol appear to dominate. Similarly, garlic is high in polyphenol content and also contains high levels of organosulfur compounds, such as allicin (Martins, Petropoulos, & Ferreira, 2016). Polyphenols are known to react with proteins both non-covalently and covalently, under some circumstances causing chemical cross-links or protein aggregation, and have also been observed to cause changes in protein secondary structure (Ozdal et al., 2013). Any or all of these polyphenol-protein interactions have the potential to effect the detection of proteins by ELISA or other methods such as protein mass spectrometry (MS) (Gallo, Vinci, Graziani, De Simone, & Ferranti, 2013). Equally, PCR detection can be deleteriously affected by the presence of plant-derived polyphenols such as those found in green tea (Koonjul, Brandt, Farrant, & Lindsey, 1999) and apple (Ogunjimi & Choudary, 1999).

At the end of 2014, recall notices for products containing cumin

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began to appear due to the detection of undeclared peanut material, with many products been withdrawn due to the presence of peanut in cumin. The observed scale of the contamination led to the Food and Drug Administration (FDA) issuing advice that individuals highly allergic to peanut should avoid consuming cumin containing products (FDA, 2015). Due to potential difficulties in analyzing for peanut in cumin powder we initially hypothesized that cumin and garlic affect detection of peanut using commercially available detection methods. To investigate this we prepared quality control materials of commercial cumin powder and garlic powder spiked with commercial roasted peanut flour at concentrations ranging from 0 to 200,000 mg.kg⁻¹ whole peanut. Initially, the performance of quantitative ELISA and qualitative lateral-flow devices and PCR methods was examined using roasted peanut (likely to occur as a result of cross-contact in a manufacturing facility). In light of the results obtained, we further hypothesized that observed differences in response to thermal processing by detection methods were due to the selection of analytical target protein. Therefore, we evaluated the impact of thermal processing on peanut detection by MS to explain the molecular basis of observed effects.

2. Material and methods

2.1. 1 Sourcing and validating ingredients

Ground cumin was a generous gift from SpiceTec-USF, Carol Stream, IL. Powdered garlic was purchased from Spices Inc. (2E 6th St., Bloomsburg, PA 17815, product code SKU-100117010). Lack of peanut in the cumin was verified by analysis of 20 aliquots (each 5 g) taken from the original 10 kg of cumin using the Neogen Veratox for Peanut ELISA kit. All 20 aliquots returned below the limit of quantitation (BLQ) (< 2.5 ppm whole peanut). The cumin was thus judged to be a suitable material into which peanut could be spiked.

Peanut flour “light 12%” was obtained from Byrd Mill (Byrd Mill Co., Ashland, VA 23005, USA). The flour was manufactured by the Golden Peanut Company, LLC, Alpharetta, GA 30022, USA and is a lightly roasted mechanically defatted peanut flour with a minimum 50% (w/w) protein.

Raw (unroasted) red-skin peanuts (Wor-Fung blanched peanuts, distributor- K.N.T.C, South El Monte, CA 91733, USA) were bought from a local market. Any remnant skin was removed prior to washing peanuts 5 times with dH₂O and drying. Peanuts were ground using a Magic Bullet (Homeland Housewares, LLC) grinder/blender. Ground peanut were defatted by hexane (1:5 w/v peanut : hexane) for 30 min three times, filtered and allowed to dry. Defatted peanut flour was further ground using the grinder/blender.

2.2. Preparation of spiked samples

To obtain different levels of peanut spikes (0, 2, 4, 10, 20, 100, 200, 500, 1000, 2000, 20,000 and 200,000 mg.kg⁻¹ whole peanut) in cumin or garlic powder, the initial 200,000 mg.kg⁻¹ whole peanut sample was generated by weighing 50 g of peanut flour and 450 g of ground cumin or garlic (using a conversion of whole peanut = peanut flour × 2 as described below) and mixing for 30 min using a Kitchen Aid Artisan Ultra Mixer (medium speed setting), with hand mixing (spatula) every 5 min. Serial dilution of this 200,000 mg.kg⁻¹ sample was then performed using the ground cumin or garlic as diluent. For each dilution, mixing was repeated as described above. All mixing equipment was extensively washed between peanut spike levels to prevent carryover. Each dilution was transferred into a clean Beltran zip bag, sealed and stored at 4 °C prior to analysis. To obtain whole peanut only diluents, peanut flour was extracted (2 000,000 mg.kg⁻¹ whole peanut) and serially diluted in the ELISA manufacturers buffer to similar levels that the peanut spikes were generated.

2.3. Allergen analysis

2.3.1. Elisa

The following ELISA test kits were used: Neogen Veratox for Peanut Allergen (product code 8430) (Neogen (NE) Corp., Lansing, MI, USA), Romer AgraQuant® ELISA Peanut (product code COKAL0148), Morinaga Peanut ELISA kit (Morinaga Institute of Biological Science, Inc. Japan). Each sample was extracted in duplicate and analysed in duplicate.

2.3.2. Qualitative methods

The Lateral Flow Devices used were as follows: Neogen Reveal 3-D for Peanut (product code 901041L), Neogen Alert for Peanut Allergen (product code 8431), R-Biopharm Bioavid (product code BS810-25), Romer AgraStrip® Peanut (product code COKAL0110AS). Cumin and garlic spiked with different levels of peanut flour was provided to laboratories with blinded sample codes. ELISA and lateral flow testing were performed according to the manufacturers' instructions where available. In the case of PCR, the methods used were performed by commercial laboratories using proprietary conditions under ISO 17025 scope. PCR results were blinded in this study. While results represent different testing under differing conditions and methods, this study was not intended as a method-to-method comparison.

2.3.3. LC-MS

2.3.3.1. Protein extraction and LC-MS methodology. The protein from a roasted and a raw peanut flour (described previously) were extracted in duplicate (2 analytical replicates) in a non-reducing buffer. These were generated by suspending 100 mg of each sample in 2 mL of 50 mM Tris-HCl, pH 8.8. The suspension was vortexed before incubation in a shaking water bath at 60 °C for 15 min. The extract was clarified by centrifugation at 15 000 × g for 10 min. The sample was diluted 100-fold and 2.1 µL was reduced by adding 15 µL of 50 mM ammonium bicarbonate, 1.5 µL of 100 mM dithiothreitol and 9 µL of water and incubating at 95 °C for 5 min. The sample was alkylated by adding 3 µL of 100 mM of iodoacetamide and incubated at room temperature in the dark for 20 min. The sample was digested by adding 1 µL of 100 ng.µL⁻¹ Pierce Trypsin Protease, MS grade and incubated at 37 °C for 3 h. A further 1 µL of 100 ng.µL⁻¹ Pierce Trypsin Protease, MS grade was added and samples incubated at 30 °C overnight. The digested sample was loaded on to a Pierce C18 spin column, according to manufactures' instructions. The dried sample was re-suspended in 15 µL of 5% (v/v) acetonitrile/0.1% (v/v) formic acid and 1.5 µL of 200 fmol.µL⁻¹ rabbit glycogen phosphorylase (Waters, Manchester, UK) was added to each sample.

One-dimensional (1D) microscale liquid chromatography separation of tryptic peptides (5.5 µL injection) was performed, in duplicate (2 technical replicates), with an UltiMate 3000 RSL® liquid chromatography (UPLC) system (Thermo Scientific™), equipped with a Javelin™ Direct-Connection Column Filter, 2.1 mm (Thermo Scientific™), a Hypersil Gold aQ C18 1.9 µm, 20 × 2.1 mm pre-column (Thermo Scientific™) and a Hypersil Gold C18 1.9 µm, 100 × 1 mm analytical reversed phase column (Thermo Scientific™). Mobile phase A consisted of water containing 0.1% (v/v) formic acid, whilst mobile phase B was 100% (v/v) acetonitrile containing 0.1% (v/v) formic acid. The sample was injected on-column and peptides were eluted from the analytical column and separated using a gradient of 2–40% mobile phase B over 60 min at a flow rate of 60 µL/min. The analytical column temperature was maintained at 35 °C.

Mass spectrometric analysis utilized a Q Exactive Plus™ Hybrid Quadrupole-Orbitrap™ MS (Thermo Scientific™) in the data-dependent mode with survey scans acquired at a resolution of 70,000 at *m/z* 400, whereas the target value for the fragment ion spectra was set to resolution of 17,500 at *m/z* 400. Up to the top 10 most abundant isotope patterns with charge 2 to 4 from the survey scan were selected with an isolation window of 1.5 Thomson and fragmented by higher energy

collisional dissociation with normalized collision energies of 27. The maximum ion injection times for the survey scan and the MS/MS scans were 100 and 60 ms, respectively, and the ion target value for scan modes were set to 1E6 and 2E5, respectively. Repeat sequencing of peptides was kept to a minimum by dynamic exclusion of the sequenced peptides for 10 s.

2.3.3.2. Analysis of LC–MS data. Thermo Scientific™ Proteome Discoverer™ ver 2.1 SP1 software was used to process all DDA mass spectral data. Protein identifications were obtained by searching a database of peanut allergens and isoforms obtained from PeanutBase (Dash, Cannon, Kalberer, Farmer, & Cannon, 2016) (www.peanutbase.org) (see S2 for how sequences were elucidated, genomic position, and S3 for the complete list of derived sequences in FASTA format) with ORF prediction performed by Augustus software (Keller, Kollmar, Stanke, & Waack, 2011). Quantitation of the most abundant isoforms detected was then performed using Proteome Discoverer™ quantitation with the known amount of rabbit glycogen phosphorylase B (P00489) used to enable label-free normalization. The following data analysis parameters were used: no mis-cleavages; carbamidomethylation as a fixed modification and oxidation of methionine as a variable modification; parent mass error tolerance of 2 ppm, fragment mass error tolerance of 0.02 Da; FDR of 1%; charge states of +2, +3 and +4 accepted. Table 2 shows the peptides used in the exemplar allergome quantitation.

2.4. Data analysis and comparison

Whole peanut (mg.kg^{-1}) was chosen as a standardized unit of peanut concentration for this study. Where necessary, conversion factors were used to convert ELISA kit units to whole peanut (mg.kg^{-1}). These factors were based on the protein content of peanut material and are as follows:

Whole peanut (mg.kg^{-1}) = $2 \times$ Peanut flour (mg.kg^{-1}), based on protein contents of approx. 50% for defatted, roasted peanut flour.

Whole peanut (mg.kg^{-1}) = $4 \times$ Peanut protein, based on protein content of approx. 25% for whole peanut.

ELISA (all levels) and MS experiments were carried out on two separate extractions of the analytes (analytical replicate). Each replicate was further analysed in duplicate (technical replicate). The results of the technical replicates were averaged. The resulting two analytical replicates were used to obtain standard errors and means (as shown in Fig. 1, supplementary figures S1–S4 and supplementary tables S5–S7). Data were analyzed using Excel 2013 (Microsoft) and Prism ver. 4.03 (GraphPad software) and Thermo Scientific™ Proteome Discoverer™ software ver 2.1 ver SP1.

3. Results and discussion

3.1. ELISA, LFD and PCR performance.

Initially, we examined the performance of commercial peanut detection methods on the detection of roasted peanut in spiked cumin and garlic samples. The amount of peanut detected in cumin and garlic, and the recovery of peanut (relative to the known quantity) using three quantitative ELISA methods is shown in Fig. 1. The three test kits used gave average recoveries of 18.3, 84.7 and 45.3% across all of the levels tested for cumin, and 20.2%, 68.6% and 53.8% for garlic. The kit used has great influence on the recovery of peanut from these spices. Recovery of roasted peanut also varied by the level of peanut spiking. When roasted peanut was present in either cumin or garlic at levels below 10 mg.kg^{-1} (where this level could be detected), the % recovery was greater than that for higher levels of known peanut. Interestingly, this effect was not apparent in raw peanut, or in the absence of cumin and garlic (S1–S4). This change in % recovery at $< 10 \text{ mg.kg}^{-1}$ peanut concentrations is apparently linked to both peanut processing and

presence of matrix. At present we cannot explain the cause of this effect.

Performance of the qualitative methods used was also varied. Particularly surprising was the performance of the two PCR methods tested, with one method reporting a positive result between 20 and 100 mg.kg^{-1} roasted peanut in cumin, and the other failing to detect even 1000 mg.kg^{-1} peanut in cumin, as shown in Table 1. The three lateral flow devices examined appeared more sensitive in this scenario, yielding positive results at 2, 10 and 100 mg.kg^{-1} roasted peanut. It is important to note that none of the methods used, whether qualitative or quantitative, gave a positive result in the 0 mg.kg^{-1} peanut samples for either cumin or garlic, suggesting the absence of false positive results. The performance of the methods when used to detect commercial, thermally processed peanut suggest that great care must be taken in selecting detection methods for use in spice matrices, especially where quantitative methods are concerned. The quantitative ELISA methods could detect peanut at low ($< 10 \text{ mg.kg}^{-1}$) levels in spices, albeit with varying recoveries.

3.2. Effect of thermal processing on ELISA detection.

Given considerable disparities, and low average recoveries, of peanut detection methods when analyzing roasted peanut in both garlic and cumin, we proceeded to examine both the effect of thermal processing and the effect of spice matrix on peanut detection to determine which was primarily responsible for observed results. For these studies, we concentrated on the quantitative ELISA methods. Samples consisted of raw or roasted peanut in the absence of a spice matrix or spiked into cumin or garlic, as described previously. Fig. 2 (more in S1) shows the detection of peanut from these samples at a spike level of 100 mg.kg^{-1} peanut. All three test kits examined demonstrated a significant reduction of peanut detection when the sample contained roasted peanut. This reduction was due both to an under-detection of roasted peanut, but also, surprisingly, due to a significant over-reporting of the presence of raw peanut in two of the kits used. For the Neogen and Romer kits, the recovery of roasted peanut (no matrix) was 59.5% and 41.2%, whereas the recovery of raw peanut was 391% and 385% respectively. The Neogen kit calibrant is an extract of a mixture of 22 peanut varieties and processing condition (Neogen Corporation, 2012). It appears that such mixtures cannot adequately represent any one given likely source of peanut contamination when the target (primarily Ara h 3) shows different recovery characteristics with different processing conditions. The differential recovery of raw and roasted peanut is potentially extremely problematic for the evaluation of risk from an allergen containing food. The Morinaga kit performed somewhat more consistently, with 28.5% roasted peanut recovery and 45.6% raw peanut recovery. The Morinaga kit, however, did provide consistently low recoveries in all samples analyzed for our peanut flours and matrices, an observation consistent with those from a previous study using SRM 2387 peanut butter (NIST) (Jayasena et al., 2015). The standard used by the Morinaga kit is an extract of raw peanut which is enriched in prolamin fraction proteins, and contains relatively low amounts of cupins (Akiyama, Imai, & Ebisawa, 2011). A calibration performed with this material, using an antibody which primarily detects prolamins would, therefore, yield higher absorbance values than would an equivalent calibration using a peanut extract containing proteins representative of the peanut seed. Determined amounts of peanut from a source containing distributions of prolamins and cupins similar to that observed in peanut seeds would, therefore, be consistently lower. Surprisingly, the effect of thermal processing was far more noticeable than that of the presence of a spice matrix with a similar pattern of recovery for all kits regardless of whether or not peanut was in a matrix. We postulate that raw peanut contamination in a spice (or other commodity) is more likely to occur due to agricultural co-mingling, whether during field growth, harvest, packaging or transport. The introduction of processed peanut is, however, more likely to occur due to cross-contact in a processing or manufacturing facility. The impact of peanut

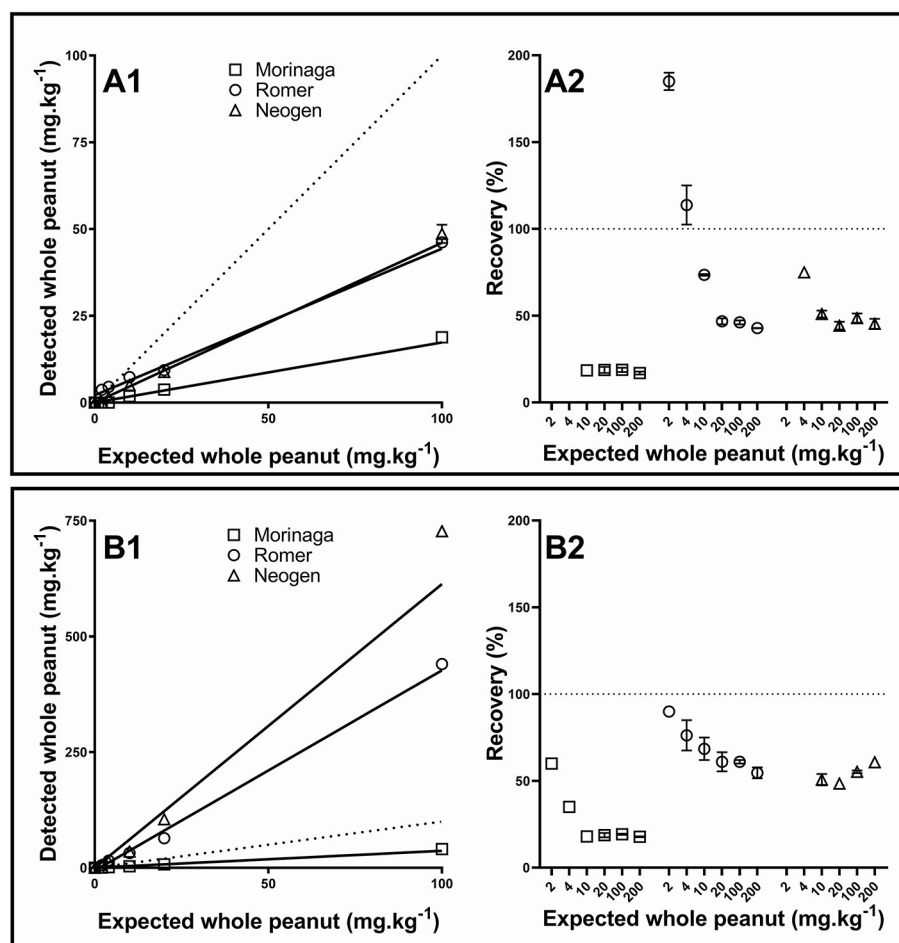


Fig. 1. Detection of roasted peanut in cumin (A1) and garlic (B1), and comparison of % recovery of roasted peanut in cumin (A2) and garlic (B2) using three commercial ELISA kits. Results indicate the mean and standard error of two analytical replicates each of two individual replicates. Recoveries were calculated as a percentage of expected recovery (that initially spiked into the sample). Ideal recovery (100%) is indicated as a dotted line.

Table 1

Detection of roasted peanut in cumin using commercially available qualitative detection methods for peanut. ‘-’ indicates a negative result, ‘+’ a positive result. ¹ indicates four individual samples were tested and results indicate the median response. ² indicates a single set of samples was used for analysis.

Peanut level (mg.kg ⁻¹ whole peanut)	Qualitative method used				
	Neogen 3-D ¹	BioAvid (R-Biopharm) ¹	Romer AgraStrip ¹	PCR 1 ²	PCR2 ²
0	-	-	-	-	-
2	-	-	+	-	-
4	-	-	+	-	-
10	-	+	+	-	-
20	-	+	+	-	-
100	+	+	+	-	+
200	+	+	+	-	+
500	+	+	+	-	+
1000	+	+	+	-	+

roasting on detection was also considerably larger than the effect of peanut cultivar described previously (Koppelman et al., 2016).

3.3. Investigating differential peanut protein recovery due to thermal processing.

We attempted to explain the differential recovery of raw and roasted peanut by the quantitative ELISA methods using protein MS. Label-free quantitation with a spike of a mammalian protein digest of known

Table 2

Peptides used for peanut allergen quantitation. For each major allergen, three peptides were selected for use in quantitation based on their presence in the most abundant identified allergen isoform, and their abundance. Charge state and *m/z* of the precursor ion for each peptide are indicated.

Allergen	Peptide sequence	Charge	Monoisotopic <i>m/z</i>
Ara h 1	SFNLDEGHALR	+3	420.21
	DLAFFPGSGEQVEK	+2	688.84
	NNPFYFPSR	+2	571.27
Ara h 3	FHLAGNQEQEFLR	+3	530.27
	VYDEELQEGHVLVVPQNFVAALK	+3	852.44
	AHVQVVDSDGNR	+3	432.88
Ara h 6	VNLKPCEQHIMQR, C6-Carbamidomethyl	+4	413.96
	ELMNLPPQQCENFR, C9-Carbamidomethyl	+2	775.37
	QMVQQQFK	+2	454.74
Ara h 2	NLPQQCGLR, C6-Carbamidomethyl	+2	543.28
	CCNELNEFENNQR, C1-Carbamidomethyl, C2-Carbamidomethyl	+2	863.85
	ANLRPCEQHLMQK, C6-Carbamidomethyl	+4	406.96

quantity was used to quantify as described previously (Johnson et al., 2016). To avoid issues with quantitation of proteins with many shared or common peptides, we first identified the most abundant isoform of each allergen and selected the most abundant peptides from each to use in label-free quantitation (Table 2). We postulated that the differences in recovery of raw and roasted peanut could be explained by differences

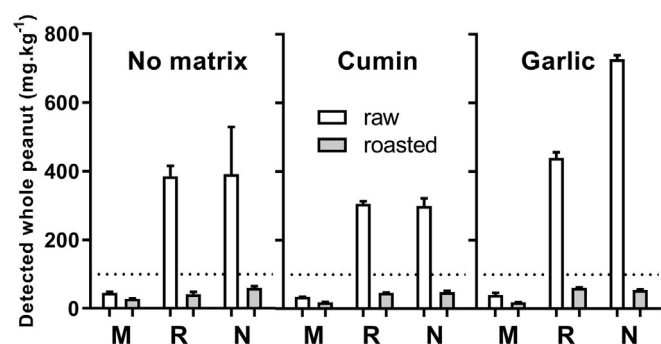


Fig. 2. Effect of thermal processing and spice matrix on peanut detection using three commercial ELISA kits. The amount of peanut detected by analysis of 100 mg.kg^{-1} peanut (either raw or roasted) either with no spice matrix, with cumin or with garlic was assessed using three ELISA test kits (M = Morinaga, R = Romer, N = Neogen). Results indicate the mean and standard error of two analytical replicates each of two individual replicates. Ideal recovery (100%) is indicated as a dotted line.

in extractability of proteins targeted by each ELISA kit. Jayasena *et al* (Jayasena *et al.*, 2015) showed that the Neogen Veratox and Romer AgraQuant antibodies are predominantly reactive to the cupin protein Ara h 3, while the Morinaga antibodies are reactive to the major seed prolamins Ara h 2 and Ara h 6. The prolamin proteins of peanut have been previously demonstrated to be more soluble than other major protein components of the peanut seed (mostly cupins) (Montserrat *et al.*, 2015), and readily leach into water after boiling peanut (Turner *et al.*, 2014). The results of our proteomics experiments are summarized in Fig. 3. The amounts of the major cupin proteins Ara h 1 and Ara h 3 are strongly affected (2.5–4-fold) by peanut roasting. However, the prolamin family of peanut seed storage proteins (including Ara h 2 and Ara h 6) are only minimally affected by roasting. It is apparent that under the conditions examined here, the prolamins are either more apt to be extracted after peanut roasting, or less susceptible to degradation or modification which might affect their detection by MS.

4. Conclusions

Presence of cumin or garlic, and thermal processing clearly affect detection with the methods used here, confirming our original hypothesis. The most significant issue arising from our work was the observation of differences in peanut detection after thermal processing. That a result of 10 mg.kg^{-1} peanut from an ELISA kit may result from the presence of either 20 mg.kg^{-1} roasted peanut or 2 mg.kg^{-1} raw peanut makes knowing the processing state, and thus likely the origin of contaminating allergen necessary for accurate quantitation. However, the absence of false positive results in this study indicate that a qualitative (present or absent) determination is reliable. Even for the qualitative methods, however, we would suggest that food manufacturers or analytical testing laboratories employ a spike/recovery to verify that detection limits are fit for purpose. The likely source of potential contamination should therefore also be considered when validating analytical methods, as this will also effect detection.

That the sensitivity of ELISA methods to thermal processing of peanut was due to the method primary target, was confirmed by protein MS. Furthermore analysis of MS data indicated optimal protein targets for avoiding sensitivity to thermal processing. Given our observations, and previous demonstrations of differential solubility (Johnson *et al.*, 2016), we would therefore suggest that Ara h 2 and 6 make superior candidates for ELISA detection than the more abundant proteins commonly used. We also suggest that untargeted MS would be effective in enabling robust target selection, either peptides for MS or proteins for ELISA/LFD, for detection of other allergens in challenging matrices and after processing.

CRediT authorship contribution statement

Justin T. Marsh: Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Shyamali Jayasena:** Investigation, Writing - review & editing. **Ferdie Gaskin:** Investigation, Writing - review & editing. **Joseph L. Baumert:** Funding acquisition, Writing - review & editing, Resources. **Phil Johnson:**

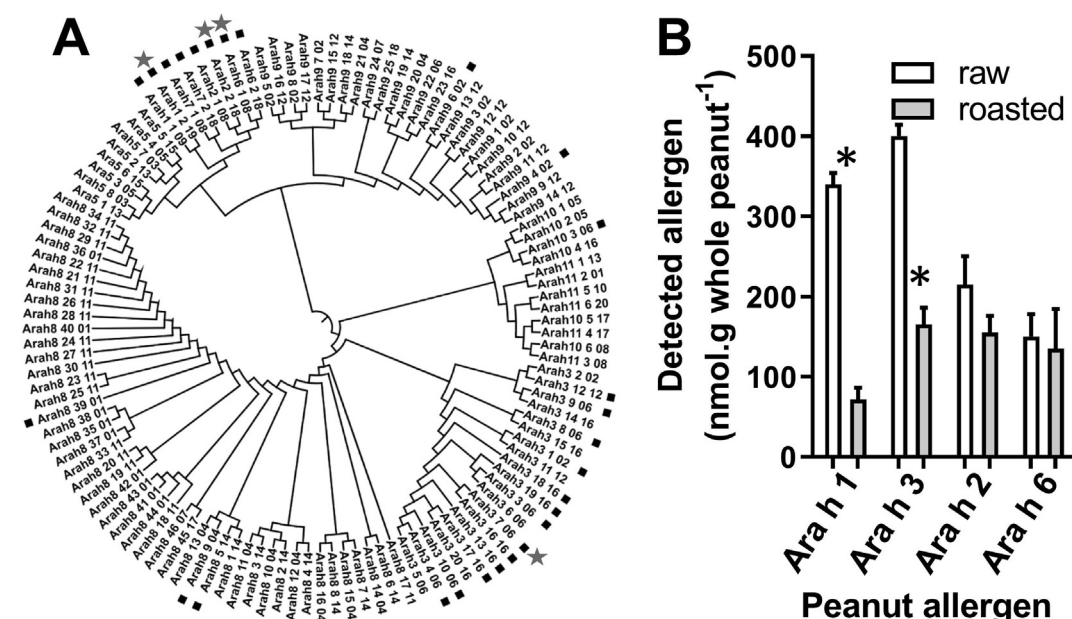


Fig. 3. Mass spectrometry based detection and quantitation of peanut allergens in raw and roasted peanut. Detection of individual allergen isoforms of allergens are shown on an alignment of peanut allergen sequences (A), with isoforms which could be uniquely identified (presence of at least one isoform-specific peptide) denoted by black squares. For each allergen, isoforms which were most abundant were selected (denoted by a star) for label-free quantitation in raw and roasted peanuts (B). Results indicate the mean and standard error of two analytical replicates each of two individual replicates. An asterisk (*) denotes statistically significant differences of $P < 0.01$.

Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2019.126019>.

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S5

peanut level (mg.kg ⁻¹)	Raw Peanut						Cumin and Raw Peanut						Garlic and Raw Peanut					
	Morinaga		Romer		Neogen		Morinaga		Romer		Neogen		Morinaga		Romer		Neogen	
	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)
0	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-
2	nd	-	6.0	6	5.4	10	1.0	0	5.9	0.07	6.8	10	0.6	13	5.6	1	6.3	4
4	nd	-	12.6	2	nd	-	2.2	0	12.3	0.06	12.7	7	1.3	0	15.3	7	13.6	29
5	nd	-	nd	-	16.5	21	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
10	nd	-	30.4	4	33.8	21	3.6	10	29.0	0.05	34.9	2	3.4	6	31.8	1	35.2	11
20	8.9	4	61.0	9	nd	-	7.3	1	62.5	0.06	67.0	7	7.5	1	63.9	0	104.8	3
40	17.1	8	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
50	nd	-	nd	-	196.0	17	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
100	45.6	6	385.1	8	391.0	35	34.8	0	305.5	0.03	298.8	8	40.6	15	440.2	4	727.3	2
200	88.4	12	751.2	7	nd	-	70.9	4	668.8	0.06	640.0	4	71.4	2	854.7	9	1172.0	25

S6

peanut level (mg.kg ⁻¹)	Roasted Peanut						Cumin and Roasted Peanut						Garlic and Roasted Peanut					
	Morinaga		Romer		Neogen		Morinaga		Romer		Neogen		Morinaga		Romer		Neogen	
	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)	Mean (mg.kg ⁻¹)	CV (%)
0	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-	BLQ	-
2	nd	-	BLQ	-	BLQ	-	BLQ	-	3.7	4	BLQ	-	1.2	(1rep+)	1.8	0	BLQ	-
4	nd	-	1.6	0	nd	-	BLQ	-	4.6	14	3.0	(1rep+)	1.4	(1rep+)	3.1	16	BLQ	-
5	nd	-	nd	-	1.2	6	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
10	nd	-	4.0	0	3.9	8	1.9	4	7.4	1	5.1	6	1.8	16	6.9	13	5.1	9
20	6.2	7	8.0	6	nd	-	3.8	13	9.4	5	8.9	7	3.8	9	12.2	13	9.7	0
40	12.3	1	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
50	nd	-	nd	-	29.4	16	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
100	28.5	4	41.5	17	59.5	10	18.8	9	46.2	4	48.7	8	19.3	3	61.0	2	55.3	2
200	58.4	4	88.3	8	nd	-	34.0	7	85.8	0	90.8	9	35.7	2	109.3	8	121.5	0

S7

ELISA kit	Limit of Detection (LoD) (mg whole peanut.kg ⁻¹)	Limit of Quantitation (LoQ) (mg whole peanut.kg ⁻¹)
Morinaga	1.20	1.25
Romer	0.1	1.0
Neogen	1	2.5

S8

Peanut allergen	Most abundant isoform used	Raw Peanut		Roasted Peanut	
		Mean (nmol.g whole peanut ⁻¹)	CV (%)	Mean (nmol.g whole peanut ⁻¹)	CV (%)
Ara h 1	Ara h 1.2	340	4	71.5	21
Ara h 3	Ara h 3.7	400	4	165	13
Ara h 2	Ara h 2.2	215	16	155	14
Ara h 6	Ara h 6.1	150	19	135	37

S9. Assembly of an 'allergome' for peanut from an online, searchable genome

A high quality genome assembly for cultivated peanut (*Arachis hypogaea* cv. Tifrunner) has recently been described (Nagy *et al*, 2012). The genome is searchable at https://peanutbase.org/peanut_genome or <https://blast.ncbi.nlm.nih.gov/Blast.cgi> using the whole-genome shotgun contigs (wgs) database and BioProjectID: PRJNA419393. Using known allergen protein sequences of the major and minor allergens listed in Allergen Online, the genome was searched using tBlastn. Highly similar sequences were identified and the nucleotide sequence was downloaded. Intron prediction was conducted by using the Augustus tool (<http://bioinf.uni-greifswald.de/augustus/submission.php>) (Keller *et al*, 2011). The intron prediction was manually checked and the translated coding region was assigned nomenclature, with the first number indicating allergen type, the second number indicating gene number and third number indicating which chromosome the gene is found. The tables below list 119 genes which were translated and searched against in our MS experiments.

S10. Table of identified allergome genes with chromosomal position.

Ara h 1 (2 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Arah1_1_09	9	110643240	110645304	3
Arah1_2_19	19	157326218	157323943	3
Ara h 2 (2 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Arah2_1_08	8	35990964	35991446	0
Arah2_2_18	18	15347118	15347636	0
Ara h 3 (20 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Arah3.1_02	2	14483592	14486663	7
Arah3.2_02	2	63843355	63840810	3
Arah3.3_06	6	1344886	1348712	3
Arah3.4_06	6	1353574	1351057	3
Arah3.5_06	6	1367137	1369654	3
Arah3.6_06	6	1375706	1371998	3
Arah3.7_06	6	1377729	1380096	2
Arah3.8_06	6	1389129	1386594	3
Arah3.9_06	6	1546102	1547833	3
Arah3.10_06	6	1858274	1856399	3

Arah3.11_12	12	18656009	18659430	6
Arah3.12_12	12	78773397	78770187	3
Arah3.13_16	16	24346514	24348745	3
Arah3.14_16	16	25549444	25547862	3
Arah3.15_16	16	26492911	26495330	3
Arah3.16_16	16	26497881	26500324	4
Arah3.17_16	16	26553007	26550801	3
Arah3.18_16	16	26721318	26719451	3
Arah3.19_16	16	26725973	26722462	3
Arah3.20_16	16	26707930	26710136	3

Ara h 5 (8 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Ara5_1_13	13	34440135	34440624	1
Ara5_2_13	13	34473697	34475345	2
Ara5_3_05	5	95247519	95250006	2
Ara5_4_05	5	95252625	95253842	2
Ara5_5_15	13	150315278	150314058	2
Ara5_6_15	15	150319994	150317008	2
Arah5_7_03	3	32678223	32679864	2
Arah5_8_03	3	32637881	32638496	1
Ara h 6 (2 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Arah6_1_08	8	36038583	36039020	0
Arah6_2_18	18	15355271	15355708	0
Ara h 7 (2 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Arah7_1_08	8	36073558	36074052	0
Arah7_2_18	18	15509772	15510266	0
Ara h 8 (46 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Arah8_1_14	14	16376595	16378203	1
Arah8_2_14	14	16315463	16316006	1
Arah8_3_14	14	16330902	16331897	1
Arah8_4_14	14	16345040	16346017	1
Arah8_5_14	14	16376595	16378203	1
Arah8_6_14	14	16388820	16390462	1
Arah8_7_14	14	16397043	16398220	1
Arah8_8_14	14	16431610	16432688	1
Arah8_9_04	4	15681827	15683597	1
Arah8_10_04	4	15179714	15179178	1
Arah8_11_04	4	15625239	15626238	1
Arah8_12_04	4	15668378	15669337	1
Arah8_13_04	4	15681827	15683597	1
Arah8_14_04	4	15694495	15696341	1
Arah8_15_04	4	15704012	15705222	1

Arah8_16_04	4	15716712	15717697	1
Arah8_17_11	11	19246262	19248392	1
Arah8_18_11	11	142223953	142222905	1
Arah8_19_11	11	142256715	142256161	1
Arah8_20_11	11	142277247	142276578	1
Arah8_21_11	11	143778620	143779180	1
Arah8_22_11	11	143855603	143856165	1
Arah8_23_11	11	143865247	143865814	1
Arah8_24_11	11	143906123	143906683	1
Arah8_25_11	11	143911812	143912379	1
Arah8_26_11	11	143916877	143917441	1
Arah8_27_11	11	143927539	143928101	1
Arah8_28_11	11	143934100	143934667	1
Arah8_29_11	11	143941159	143941719	1
Arah8_30_11	11	143945655	143946222	1
Arah8_31_11	11	143941225	143941719	1
Arah8_32_11	11	143970243	143970804	1
Arah8_33_11	11	144079903	144080467	1
Arah8_34_11	11	144083334	144083894	1
Arah8_35_01	1	100042558	100041998	1
Arah8_36_01	1	100047877	100047317	1
Arah8_37_01	1	100051307	100050743	1
Arah8_38_01	1	100095934	100095374	1
Arah8_39_01	1	100104026	100103466	1
Arah8_40_01	1	100160973	100160413	1
Arah8_41_01	1	101220388	101220967	1
Arah8_42_01	1	101230566	101231187	1
Arah8_43_01	1	101239015	101239838	1
Arah8_44_01	1	101246315	101247166	1
Arah8_45_17	17	6775355	6775837	1
Arah8_46_07	7	5033016	5033498	1

Arah 9 (25 genes)

Gene Nomenclature	Chromosome	Start	End	Introns
Arah9.1_02	2	92828820	92828476	0
Arah9.2_02	2	92845325	92844966	0
Arah9.3_02	2	92856072	92855719	0
Arah9.4_02	2	93168001	93168381	0
Arah9.5_02	2	93184172	93184546	0
Arah9.6_02	2	92888061	92887753	0
Arah9.7_02	2	93178603	93178235	0
Arah9.8_02	2	93185723	93186082	0
Arah9.9_12	12	108420675	108420262	0
Arah9.26_12	12	108440708	108441121	0
Arah9.10_12	12	107914433	107914086	0
Arah9.11_12	12	107939592	107939236	0
Arah9.12_12	12	107976260	107975907	0

Arah9.13_12	12	108000209	107999862	0
Arah9.14_12	12	108420714	108420262	0
Arah9.27_12	12	108440669	108441121	0
Arah9.15_12	12	108457625	108457257	0
Arah9.16_12	12	108464054	108464425	0
Arah9.17_12	12	108465609	108465968	0
Arah9.18_14	14	119029107	119029472	0
Arah9.19_14	14	119064984	119065379	0
Arah9.20_04	4	108592015	108592404	0
Arah9.21_04	4	108569736	108570101	0
Arah9.22_06	6	104066058	104065663	0
Arah9.23_16	16	135912545	135912150	0
Arah9.24_07	7	48600815	48601156	0
Arah9.25_18	18	107869897	107870238	0
Arah 10/11 (12 genes)				
Gene Nomenclature	Chromosome	Start	End	Introns
Arah10_1_05	5	92473372	92473886	0
Arah10_2_15	15	152600940	152600426	0
Arah10_3_06	6	104906279	104906788	0
Arah10_4_16	16	137133984	137134493	0
Arah10_5_17	17	132778011	132777511	0
Arah10_6_08	8	16296576	16296076	0
Arah11_1_13	13	46302835	46302368	0
Arah11_2_01	1	108522205	108521738	0
Arah11_3_08	8	16296576	16296076	0
Arah11_4_17	17	132778011	132777511	0
Arah11_5_10	10	110745371	110744958	0
Arah11_6_20	20	137183971	137183558	0

S11. Translated coding sequences used for peanut allergen identification ('Allergome')

>Arah1_1_09

MRGRVSPLMLLLGILVLASVSATQAKSPYRK TENPCAQRCLQSCQEPDDLKQKACESRCTKLEYDPRCVYDTGATNQRHPPGERT
RGRQPGDYDDDRRQPRREEGGRWGPAEPREREREEDWRQPREDWRRPSHQQPRKIRPEGREGEQEWGTPGSEVREETS RNPNFYFP
SRRFSTRYGNQNGRIRVLQRFQDQSKQFQNLQNHRI VQIEARPNTLVLPKHADADN ILVIQQGQATVTVANGNNRKSFNLD EGHAL
RIPSGFISYILNRHDNQLNRVAKISMPVNTPGQFEDFFPASSRDQSSYLQGF SRNTLEAAFNAEFNEIRRVLLEENAGGEQEERGQ
RRRSTRSSDNEGVI VKSKEHVQELTKHAKSVSKKGSEEDITNPINLRDGE PDLSNNFGRLFEVKPDKKNPQLQDLDMMLTCVEI
KEGALMLPHFNSKAMVIVVNKGTGNLELVAVRKEQQQRGRREQWEEEEEEDEEEEGSNREVRRYTARLKEGDVFIMPAAH PVA IN
ASSELHLLGFGINAENNHRI FLAGDKDNVIDQIEKQAKDLAFPGSGEQVEKLIK NQRESHFVSAR PQSQSPSSPEKEDQEEENQGG
KGPLLSILKAFN

>Arah1_2_19

MRGRVSPLMLLLGILVLASVSATHAKSSPYQKKTENPCAQRCLQSCQEPDDLKQKACESRCTKLEYDPRCVYDPRGHTGTTNQRS
PPGERTRGRQPGDYDDDRRQPRREEGGRWGPA GPREREREEDWRQPREDWRRPSHQQPRKIRPEGREGEQEWGTPGSHVREETS RN
NPFYFPSRRFSTRYGNQNGRIRVLQRFQDQSRQFQNLQNHRI VQIEAKPNTLVLPKHADADN ILVIQQGQATVTVANGNNRKSFNLD EGHAL
RIPSGFISYILNRHDNQLNRVAKISMPVNTPGQFEDFFPASSRDQSSYLQGF SRNTLEAAFNAEFNEIRRVLLEENAGGE
QEERGQRRWSTRSSENNEGVI VKSKEHVEELTKHAKSVSKKGSEEEGDITNPINLRGE PDLSNNFGKLF EVKPDKKNPQLQDLDMMLTCVEI
KEGALMLPHFNSKAMVIVVNKGTGNLELVAVRKEQQQRGRREEEED EDEEEEGSNREVRRYTARLKEGDVFIMPAAH PVA IN
ASSELHLLGFGINAENNHRI FLAGDKDNVIDQIEKQAKDLAFPGSGEQVEKLIK NQKESHFVSAR PQSQSQSPSSPEKES P
EKEDQEEENQGGKGPLLSILKAFN

>Arah2_1_08

MAKLTILVALALFLAAHASARQQWELQGD RRCSQLERANLRPCEQHLMQKIQRDEDSYERDPYSPSQDPYSPSPYDRRGAGSSQ
HQERCCNELNEFENNQR CMCEALQQIMENQSDRLQGRQQEQQFKREL RNLPQQCGLRAPQRCDLDVESGGRDRY

>Arah2_2_18

MAKLTILVALALFLAAHASARQQWELQGD RRCSQLERANLRPCEQHLMQKIQRDEDSYGRDPYSPSQDPYSPSQDPDRDPYSP
SPYDRRGAGSSQHQERCCNELNEFENNQR CMCEALQQIMENQSDRLQGRQQEQQFKREL RNLPQQCGLRAPQRCDLEVESGGRDRY

>Arah3_1_02

MVIGPFRSLCVCVLVFLT SACFGTRLEESFNECQLDRLNALTPDNRIESQGGITETWNSNHPELRCAGVTLLKRTIFPNGFHLP SY
ANYPQLIFIAQGNVFGVSLPGCPVTYEEAESQ SREDRRQRIVIKRESEQEQQGDSHHKIYHFRQGHLLAIPAGVPYWSFN YGN
EPIVAITLLDTSNLDNQLDPSRRFYLAGNPEEEHPETQQQQPQTRRRHGQHQQDEYGSQGE EGNVL SGFSTQLLAHAFGVDEE
IARILQNPEQTKDQIVRVEGGFRDVISPRWEGEKQYEDLEERQRQPPRRDEQKGQDYDDDRRPRHRQDPYREGDEDDRPRGS
RQGGQGRGYDDDRRPGQYEEGEDRRRPRSSRPKRQGRRHDDDDRRADEDDRRGYDDDERRPDEDDRRGYDDDERRPDDDDRRQGY
DDDDRRPRWSSSRPKQGRNGVEETLCSPTLVEDIARPSRADFYNPAAGRISANSLTFPILRW FQLSAEHVLLYRNGIYSPHWNN
ANSIYGLRGEGRIQVNSQGNVFNGLVREGQILLVPQNF AVGKQAGNEGFEYVAFKTADRASISHLKQVLRGIPADVLINAFGL
RNHQVSALKYNGNQTP LVAAYDSQYGPQR RDQP

>Arah3_2_02

MSSSLFLTLAYLV LIERLTCHAQSHGDRIGECRLEHLSVMEPTKRVESEGGAEFWDDKSQQLQCIGVT LIRYTIRPKGLLLPFY
TNAPRIHYILQKGVM EIVVTGCRAMYRSSTKRGMMSSYDEHQIKQISIEQNDAVAVPSSSVHWIYNTGHS DLVLFSLVDVANADN
QLDPTFRNFLQSGNGNGKEEESNNWF IKKKQREAGNVSFGLAETLIGSFNVQREIAEKVQLKDWRGSIILVKEGLDWLSP
EEEEEEGLEKRRKKGVDNGIEETLCSYSFVHPLGEPRMHADKYNPRGGHITSLNTPNMAVLQYLQLGLDRGVLYKDAILVPHYNLN
CHAVIYCTRGSALVQVINENGKRVLD EEVKEGQVLIVPQHYIVVKKAGSDAFDWVAIKTSDNPIINTLAGELSLVRAIPEAVLMSS
YRMERKEARLLKRKGELTIISP LEETH

>Arah3_3_06

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NAPQEIFIYQGSYGFLIFPGCPGTFE EPIQGSSEQFSQRPLDTHQKVHSFREGDLIAVPHGVAFWIYNDQSDSVVAISVLHTNSL
HNQLDQFPRRFNL AGKQEFLRYQQEQENEGGNVFSGFSTEFLLHGFQVNEDI VKNLRGENESEEQGAIVTVKRGLSILVPAERR
QSYQQPGNFNNGIEETICTASVKMNI GKSTADIYNPQAGSVRTVNELDLPILNRLGLSAEYGS IHRDAMFVPHYNMNANSIIYAL
QGGAHVQVVD CNGNRVFEELQEGQSLVVPQNF AVAAKSQSEHFLYVAFKTSRASISNLAGKNSYMWNL PEDVVANSYGLQYEQ A
RQLKNNNPFTFLVPPQDSQMIRTVA

>Arah3_4_06

MAKLLALSVCFCFLVLGASSISFRQQPEENACQFQRLNAQRPDNR IESEGGYIETWNPNNQEFECAGVALSRLVLR RNALRRPFYS
NAPQEIFIQQGRGYFGLIFPGCPSTYEEPAQQGRRHQSQRAPRRFE GEDQSQQQQDSHQKVRRFDEGDLIAVPTGVALWMYNDHD
TDVVAVSLTD TNNNDNQLDQFPRRFNL AGNHEQEF LRYQQQSRRRSLPYSPYSPSQSQRQEEREFSPRGQHSRRERAGQEEENE G
NIFSGFTPEFLAQAFQVDDRQIVQNL RGENESEEEGAIVTVKGGRLILSPDRKRGAD EEEYDEDEYED EEDRRRGRGSRGRGNG
IEETICTASVKKNIGRNRSPDIYNPQAGSLKTANDLNLILRWLGLSAEYGNLYRNALFVPHYNTNAHSIIYALRGRAHVQVVD SN
GNRVYDEELQEGHVLVVPQNF AVAGKSQSDNFEYVAFKTSRPSIANLAGENS IIDNLPEEVVANSYGLPREQARQLKNNNP FKFF
VPPSQQSLRAVA

>Arah3_5_06

MAKLLALSVCFCFLVLGASSISFRQQPEENACQFQRLNAQRPDNR IESEGGYIETWNPNNQEFECAGVALSRLVLR RNALRRPFYS
NAPQEIFIQQGRGYFGLIFPGCPSTYEEPAQQGRRHQSQRAPRRFE GEDQSQQQQDSHQKVRRFDEGDLIAVPTGVALWMYNDHD
TDVVAVSLTD TNNNDNQLDQFPRRFNL AGNHEQEF LRYQQQSRRRSLPYSPYSPSQSQRQEEREFSPRGQHSRRERAGQEEENE G
NIFSGFTPEFLAQAFQVDDRQIVQNL RGENESEEEGAIVTVKGGRLILSPDRKRGAD EEEYDEDEYED EEDRRRGRGSRGRGNG
IEETICTASVKKNIGRNRSPDIYNPQAGSLKTANDLNLILRWLGLSAEYGNLYRNALFVPHYNTNAHSIIYALRGRAHVQVVD SN

GNRVYDEELQEGHVLVVPQNFVAVAGKSQSDNFEYVAFKTDSPSIANLAGENSIIDNLPeeVVANSYGLPREQARQLKNNNPfKFF
VPPSQQSLRAVA

>Arah3_6_06

MIKYIETWNPNNQEFQCAGVALSRFVLRNALRRPFYSNAPQEIFIYQGSYGFLIFPGCPGTFEETIQGSEQFQSQRPLDTHQKV
HSFREGDLIAVPHGVAFWIYNDQSDVVAISVLHTNSLHNQLDQFPRRFNLAGKQEQEFLRYQQEQENEGGNVFSGFSTEFLLHGF
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>Arah3_7_06

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>Arah3_8_06

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>Arah3_9_06

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>Arah3_10_06

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>Arah3_11_12

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>Arah3_12_12

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>Arah3_13_16

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>Arah3_14_16

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>Arah3_15_16

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>Arah3_16_16

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>Arah3_17_16

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>Arah3_18_16

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>Arah3_19_16

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>Arah3_20_16

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>Ara5_1_13

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>Ara5_2_13

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>Ara5_3_05

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>Ara5_4_05

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>Ara5_5_15

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>Arah8_25_11
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>Arah8_26_11
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>Arah8_27_11
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>Arah8_28_11
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>Arah8_29_11
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>Arah8_30_11
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>Arah8_31_11
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>Arah8_32_11
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>Arah8_33_11
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>Arah8_34_11
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>Arah8_35_01
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>Arah8_36_01
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>Arah8_37_01
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>Arah8_38_01
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>Arah8_39_01
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>Arah8_40_01
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>Arah8_41_01
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>Arah8_42_01
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>Arah8_43_01
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>Arah8_44_01
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>Arah8_45_17
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>Arah8_46_07
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>Arah9_1_02
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>Arah9_2_02
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>Arah9_3_02
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>Arah9_4_02
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>Arah9_5_02
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>Arah9_6_02
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>Arah9_7_02
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>Arah9_8_02
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>Arah9_9_12
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>Arah9_10_12
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>Arah9_11_12
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>Arah9_12_12
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>Arah9_13_12
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>Arah9_14_12
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>Arah9_15_12
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>Arah9_16_12
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>Arah9_17_12
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>Arah9_18_14
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>Arah9_19_14
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>Arah9_20_04
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>Arah9_21_04
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>Arah9_22_06
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>Arah9_23_16
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>Arah9_24_07
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>Arah9_25_18
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>Arah10_1_05
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>Arah10_2_05
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>Arah10_3_06
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>Arah10_4_16
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>Arah10_5_17
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>Arah10_6_08

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>Arah11_1_13

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>Arah11_2_01

MADRPTFQTPLSPHHAFRLRLQDHTPNSTQLAGLLTLLITGSILLLLTLTGLTVGTIIAMIFFSPLIIISSPIWVPIGTLFLLITAA
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>Arah11_3_08

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>Arah11_4_17

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>Arah11_5_10

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>Arah11_6_20

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