Mass Spectrometry Based Allergen Detection: Applicability of Published Peptide Sequences for the Quantification of Different Peanut Varieties and Processed Peanuts Using Triple Quadrupole LC/MS

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Introduction

- Currently food allergy is not curable and the elimination of the culprit food is the only means to avoid unwanted allergic reactions.
  - Allergic individuals have to rely on the list of ingredients and on precautionary labeling.
- Accurate methods for allergen detection are a prerequisite to evaluate the allergen status of food products
- Current state of the art methods for allergen detection: enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR)
  - Both techniques are affected by cultivar dependent variations in protein and DNA content and especially by food processing
- Mass spectrometry (MS) based detection of allergenic foods has been described as powerful technique with the potential to overcome drawbacks of other methods concerning reduced recovery due to processing
  - Indeed only little information about the detectability, meaning the response to different varieties and the response to allergenic foods after food processing is available.
- **Aim of the study**: using the example of peanut (*Arachis hypogaea*), it was investigated if published peptides suggested for peanut detection using a triple quadrupole LC/MS System are applicable for different varieties/ commodities and processed peanuts.

Agilent 6490 Triple Quadrupole LC/MS System with iFunnel Technology

Experimental

Table 1. Peanut varieties investigated in this study

#	Country of Origin	Peanut Type	Roasting Time/ Temperature	Comments
1	USA	*Spanish*	-	
2	China	*Virginia*	-	
3	Argentina	*Runner*	-	
4	Bolivia	*Overo*	-	
5	USA	*Runner*	-	
6	Brazil	*Runner*	-	
7	Bolivia	*Bayo*	-	
8	Bolivia	*Colorado*	-	
9	South Africa	*Natal*	-	
10	USA	*Runner*	140°C/ 25 min	
11	Brazil	*Runner*	140°C/ 25 min	
12	South Africa	*Natal*	140°C/ 25 min	
13	unknown	*unknown*	unknown	Salted peanuts
14	unknown	*unknown*	unknown	Peanut cream used in food industry

Sample Preparation:

- **Homogenization:** Each peanut sample was ground under liquid nitrogen using an analytical mill and stored at -70°C until further use.
- **ELISA:** Ground peanut samples were extracted and measured according to the manual of the „AgraQuant® F.A.S.T. Peanut“ kit.
- **MS:** Ground peanut samples were extracted according to published protocols [1] with slight modifications. The diluted protein extracts were quantified, diluted, reduced, alkylated and digested overnight using trypsin. The acidified extracts were directly injected into the LC/MS/MS system.
- **UHPLC MS/MS parameters:**
  - Agilent 1290 Infinity UHPLC system coupled via an Agilent Jet Stream electrospray ionization source to an Agilent G6490 QQQ system
  - Column: Agilent ZORBAX Eclipse Plus C18 RRHD 2.1 x 150 mm, 1.8 µm @ 30°C
  - Mobile phase A: 0.1% formic acid, mobile phase B: 90% acetonitrile + 0.1% formic acid; flow rate 0.4 ml/min; 0.5 min isocratic at 5% B, linear gradient to 60% B in 10.5 min, linear gradient to 100% B in 1.0 min, 2.0 min isocratic at 100% B, linear gradient to 5% B in 0.1 min. Stop time 15 min.
  - Acquisition in positive electrospray ionization and with dynamic multiple reaction monitoring (dMRM) mode with five to seven MRM transitions per peptide.

Results - MS analysis

pdb|3SMH|A Chain A, Crystal Structure Of Major Peanut Allergen Ara H 1

SRNNPFYFPSRRFSTRYGNQNGRIRVLQRFDQRSRQFQNLQNHRIVQIEAKPNTLVLPKHADADNILVIQQGQATVTVANGNNRKSFNLDEGHALRIPSGFISYILNRHDNQNLR
VAKISMPVNTPGQFEDFFPASSRDQSSYLQGFSRNTLEAAFNAEFNEIRRVLLEENAGGEQEERGQRRWSTRSSENNEGVIVKVSKEHVEELTKHAKSVSKKGSEEEGDITNPIN
LREGEPDLSNNFGKLFEVKPDKKNPQLQDLDMMLTXVEIKEGALVLPHFNSKAMVIVVVNKGTGNLELVAVRKEQQQRGRREEEEDEDEEEEGSNREVRRYTARLKEGDVFIMPA
AHPVAINASSELHLLGFGINAENNHRIFLAGDKDNVIDQIEKQAKDLAFPGSGEQVEKLIKNQKESHFVSARP

pdb|3C3V|A Chain A, Crystal Structure Of Peanut Major Allergen Ara H 3

ISFRQQPEENACQFQRLNAQRPDNRIESEGGYIETWNPNNQEFECAGVALSRLVLRRNALRRPFYSNAPQEIFIQQGRGYFGLIFPGCPSTYEEPAQQGRRYQSQRPPRRLQEE
DQSQQQQDSHQKVHRFNEGDLIAVPTGVAFWLYNDHDTDVVAVSLTDTNNNDNQLDQFPRRFNLAGNHEQEFLRYQQQSRQSRRRSLPYSPYSPQSQPRQEEREFSPRGQHSRR
ERAGQEEEHEGGNIFSGFTPEFLAQAFQVDDRQIVQNLRGENESEEQGAIVTVRGGLRILSPDRKRGADEEEEYDEDEYEYDEEDRRRGRGSRGSGNGIEETICTATVKKNIGR
NRSPDIYNPQAGSLKTANELNLLILRWLGLSAEYGNLYRNALFVPHYNTNAHSIIYALRGRAHVQVVDSNGNRVYDEELQEGHVLVVPQNFAVAGKSQSDNFEYVAFKTDSRPS
IANLAGENSVIDNLPEEVVANSYGLPREQARQLKNNNPFKFFVPPSQQSPRAVA

Figure 1A and 1B. Identication of peanut is based on six peptides. Figure A (left hand side): three tryptic peptides (highlighted in different shades of red) can be assigned to Ara h1 and Figure B (right hand side): three petides (highlighted in different shades of blue) are tryptic fragments of Ara h3.

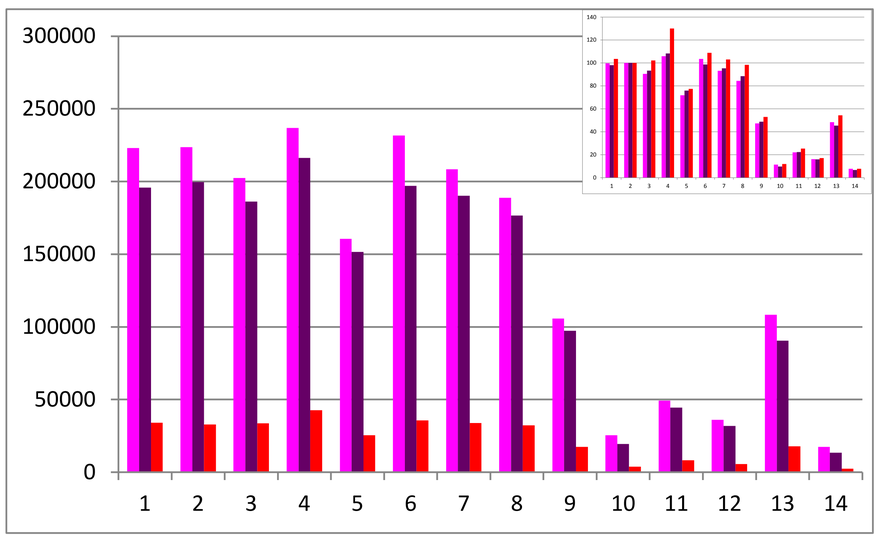

Figure 2. Accumulated MRM signal intensities of peptides specific for Ara h1 from peanuts listed in Table 1. The colour of the bars matches the highlighted peptide sequences in Figure 1A. The picture in the picture shows the relative signal intensity between peptides from Ara h1 by normalization of intensities against peanut #2.

⟹ The signal intensity is dependent from peanut variety and peanut processing; the ratio of signal intensities between peptides is similar

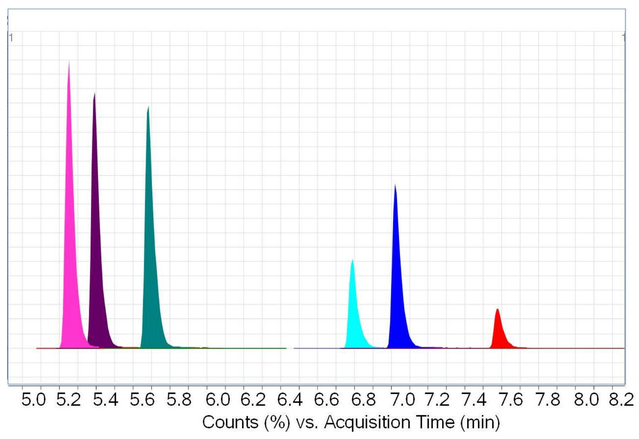

Figure 3. Exemplary Chromtaogram of peanut #2 (Table1) showing the most intense MRM transition of each peptid

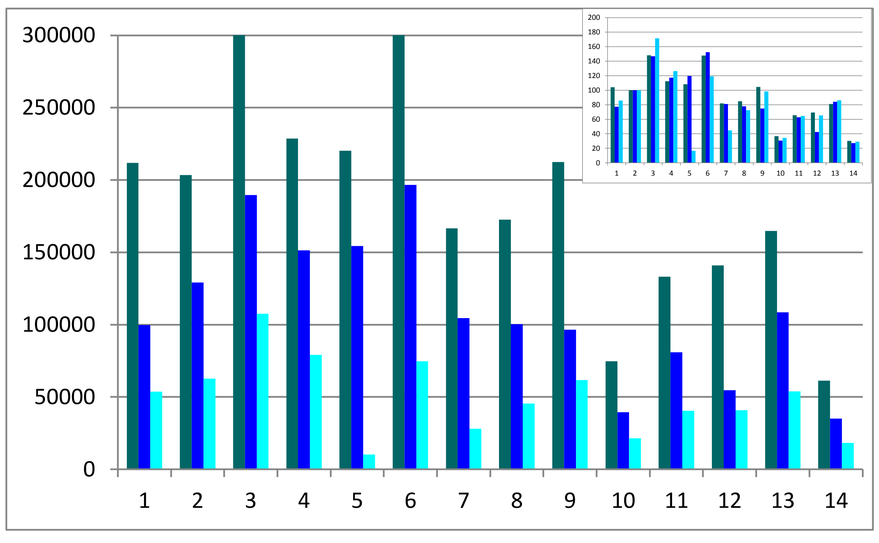

Figure 4. Accumulated MRM signal intensities of peptides specific for Ara h3 from peanuts listed in Table 1. The colour of the bars matches the highlighted peptide sequences in Figure 1B. The picture in the picture shows the relative signal intensity between peptides from Ara h1 by normalization of intensities against peanut #2.

⟹ The signal intensity is dependent from peanut variety and peanut processing; the ratio of signal intensities between peptides is similar

Results - ELISA analysis

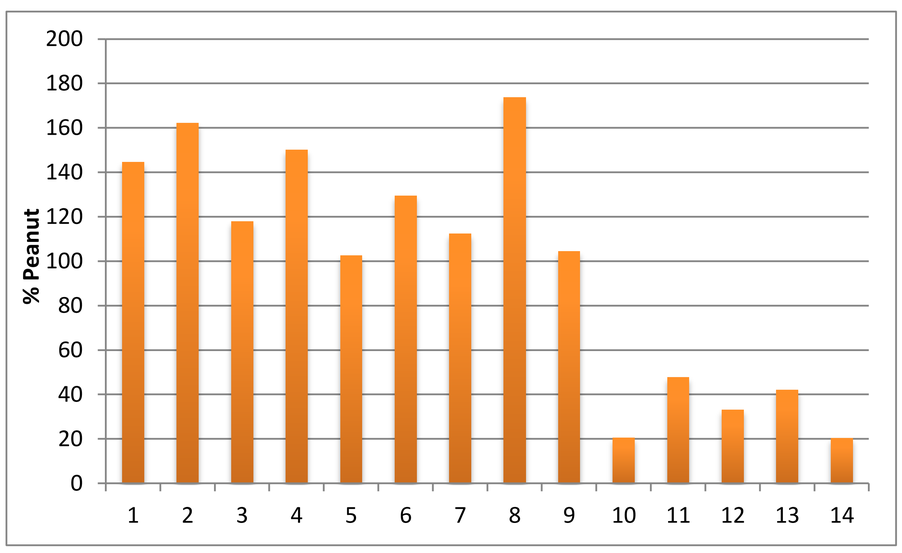

Figure 5. ELISA results of investigated peanuts listed in Table 1.
⟹ The quantitative results are dependent from peanut variety and especially from peanut processing

Conclusions

For MS investigations:

- Published peptide sequences for MS based peanut detection can be used for peanut detection
- In native peanut varieties the signal intensity may vary by a factor of two
- Processing (roasting) seems to affect the proteins individually
  ⟹ Ara h3 (factor 6) is less affected than Ara h1 (factor 14)

For ELISA investigations:

- In native peanuts the quantitative ELISA results varied by a factor of two and considering additionally processed peanuts, the maximum response factor was eight

Final Conclusion

- Mass spectrometry based detection methods based on suggested peptides is not less prone to reduced recoveries due to processing.
- The reduced recovery of processed peanuts by LC/MS seems to be comparable to currently available ELISA
- Even if the selected peptides are chosen in a way that they are not modified or are not affected by the Maillard reaction reduced recovery due to processing has to be considered.

Literatur: [1] Heick et al. JAOAC, 2011, 94 (4), 1-9