Detection of Food Allergens: Current Analytical Methods and Future Needs

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Presentation Outline

- Background
- Characteristics of food allergens
- Type of materials that are analyzed
- Methods for detection of allergens
  - Immunochemical methods
  - PCR
  - Mass spectrometry
  - Non-specific methods
- Methods in development
- Conclusions
Food allergies - immunological response to proteins

Food allergies are a major health issue in industrialized countries

- 10-12 million people in the U.S.
- 30,000 emergency room visits due to food allergies
- 150 deaths

Prevalence is increasing

Impact on society
Undeclared Allergens

- Strict avoidance is used by individuals with food allergies
- Food labels are used to indicate the intended presence or absence of allergens
- Undeclared allergens can be inadvertently introduced into a food
  - Ingredient/supplier changes
  - Labeling errors
  - Improper use of rework
  - Cross-contact
Why Test for Allergens?

- Consumer safety
- Ensure accuracy of food labeling
- Cleaning effectiveness
- Consumer complaints
Properties of Food Allergens

- Proteins
- Typically 10-80 kDa
- Most are water soluble
- No commonality in structure or amino acid sequence
- Multiple IgE binding sites
- Resistant to heat
What Do We Test?

- Ingredients or raw materials
- End product testing
- Environmental samples
- Cleaning
  - Food-contact surfaces
  - Rinse-water
  - Push through materials (salt, sugar, first product off line)
Allergen Detection “Toolbox”

- Immunochemical methods
  - ELISA
  - Lateral flow devices (dipsticks)
  - Multiplex

- DNA-based methods
  - PCR
  - Multiplex

- Mass spectrometry

- Generic/non-specific (cleaning)
  - Protein
  - ATP
  - Visual inspection

- Other methods
Choice of Allergen Detection Method

- Purpose
- Type of sample
- Food matrix
- Processing effects
- Turn-around time
- Availability of equipment/cost
Enzyme-Linked Immunoassay (ELISA)

- Antibody-based detection of allergenic protein or other protein in food
- Available in well and lateral flow formats
- Sandwich format most common
- Analysis takes between 1 – 2 h to complete
- Quantitative or qualitative
- Kits available for most of the 8 major allergens
- Used for ingredients, finished products, cleaning fluids, swabs, environmental samples
Lateral Flow Devices (LFD) and Dipstick Tests

- Qualitative (can be semi-quantitative with reader)
- Available for many allergenic foods
- Typically used for environmental sampling, cleaning verification, screening of foods
- True measurement of presence of allergenic food
- Rapid
- Sensitive (DL~ 5 ppm)
Advantages of ELISA

- Sensitive (ppm range)
- Quantitative or semi-quantitative
- Measure amount of offending food component (i.e. proteins)
- Antibody can detect allergenic proteins or marker protein in food
- Fairly rapid
- Equipment needs are minor (plate reader)
- Skill level = low to medium
Limitations of ELISA

- Some training required/adherence to instructions
- Sampling important
- Extraction and immunoreactivity important
- Food matrix important
  - Polyphenols
  - Oils
  - pH
  - Processing

From: Taylor et al. 2009, JFS; 74(5):T46-50
Limitations of ELISA

- Cross-reactivity
- Need to understand what kit detects (e.g. some milk kits detect casein while other detect whey proteins)
- Values obtained from kits do not agree
- Lack of reference materials
- Need to do “in house” validation of ELISA
Immunochemical Methods of the Future: Multiplexed approaches for detecting protein allergens (Bioplex) Eric Garber (FDA/CFSAN) and coworkers

- Beads are labeled with antibodies for selected antigens
- Beads are mixed and exposed to antigens for binding
- Second antigen-specific labeled antibody is added for detection
- Beads flow through optical path of two lasers
- First laser identifies bead by color; correlated with specific antigen
- Second laser determines whether recognition event has occurred and how many
Polymerase Chain Reaction (PCR)

- Detects DNA sequences indicative of allergenic species
- Based on heat stable DNA polymerase amplifies DNA fragment
- Kits available for milk, peanut, soy, walnut, hazelnut, fish, crustaceans
- Useful in cases where ELISAs are not available or results questionable (e.g. hydrolyzed proteins)
- Good method for verifying ELISA or immunochemical assay results
- Equipment are becoming more common
- Very specific
- High throughput
- Multi-screening (multiplex) potential
PCR- Pitfalls

- Detect DNA not protein
- Qualitative
- Sample preparation and analysis require skill
- Cross-contamination possibilities
- Equipment expensive and not available in all labs
- Absence of DNA does not indicate absence of protein
The Future of PCR-Based Analysis:
Multiplexed approaches for detecting allergen DNA
Eric Garber (FDA/CFSAN) and coworkers

1. Quantitation
2. False positives
3. Is the allergenic protein expressed?
Mass Spectrometry

- Detects proteins and peptides
- Involves extraction, cleanup, ionization, separation of ionized protein/peptide, detection
- High degree of sensitivity and resolving power
- Provides protein composition, structure and sequence information
- Protein detection and confirmation in single run
- Peptides detection and quantification easier
Ara h1 as a Marker for Peanut in Foods

- 68 kDa vicilin seed storage protein

MRGRVSPMLLLLGILVLASVSATQAKSPYRKTEPOCAQRCLQSCQQEP
DDLKQKACESRCTKLEYDPRCVYDTGATNQRHPPGERTRGRQPGDYD
DDRRQPRREEGGRWPAEPREREEREDWRQPREDWRPSPHQPRK
IRPEGREGEQEWGTPGSEVREETSRRNFYFPRSRFSTRYGNQNGRI
RVLRQFDQRQFQNQQNLQRIVQIEARPNTLVPKHADADNILVIQQGQ
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NTPGQFEDFFPASSRDQSSYLLQGFSRNTLEAAAFNAEFNEIRRVLLEEN
AGGEQUEEQRQRRRSTRSDNEGIVKVSVKEHVQELTKHAKSVSKKGS
EEEDITNPINLRDGEPLSLNFGRLEFKIDKNPQLQDLMMLTCVEI
KECALMLPHFNSKAMIVVVKNGTGNNELAVRKEQQQRHREREWEVEEEEDEEEGSNREVRITYARLKEGDVFIMPAAAHPVAINASSELHLLFGI
NAENNHRIFLAGDKDNVIDQIEKQAKDLAFPQSGEQVEKLIKNIQRESHF
VSARPQSQSPSSPEKEDQEEENQGGKGPLLSILKAFN

606.7 $[^{3}\text{H}]^{+}$

686.8 $[^{2}\text{H}]^{+}$

Callahan et al. (FDA/CFSAN)
General Sample Preparation

1. **5K MWCO filter**
   - Add std. digest proteins

2. **Remove low M.W. components**
   - Discard low M.W. interferences

3. **Collect peptides (discard high M.W. components)**
   - Spin filter 10K MWCO

4. **Discard non-proteins**
   - Peptides

Analysis by LC/MS and LC/MS/MS
Low-ppm confirmation of Ara h1 in chocolate

Multiple Reaction Monitoring (MRM)

Sum of 229.1, 300.2, 930.5

DLAFPGSGEQVEK
# Mass Spectrometry

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute identification and quantification of allergens</td>
<td>Requires high level of expertise</td>
</tr>
<tr>
<td>Highly sensitive</td>
<td>High cost of equipment</td>
</tr>
<tr>
<td>Excellent confirmatory method</td>
<td>Time consuming</td>
</tr>
<tr>
<td></td>
<td>Laborious</td>
</tr>
<tr>
<td></td>
<td>Extraction and cleanup needed</td>
</tr>
<tr>
<td></td>
<td>Not useful for routine analyses</td>
</tr>
</tbody>
</table>
Non-Specific Methods: ATP

- Sanitation effectiveness
- Detects ATP from biological sources
- Conventional ATP swabs - hygiene
- Sensitive ATP swabs – detect presence of food soils

Advantages
- Rapid (< 30 sec)
- Less expensive than ELISA
- Test can be performed on site (‘real time’)

Disadvantages
- Limited applicability (wet-cleaned surfaces)
- May pick up ATP from water supply
- Measures presence of ATP, not allergenic food
- May be difficult to detect some food soils
- Need to determine background ATP levels at facility
Non-Specific Methods: Total Protein

- Cleaning effectiveness
- Different companies and formats available

Advantages
- Rapid (< 5 min)
- Less expensive than ELISA
- Measures protein

Disadvantages
- Measures all proteins, not only from allergenic food
- ???
## Detection of Soy Products in Solution

<table>
<thead>
<tr>
<th>Soy Product</th>
<th>Method of Detection</th>
<th>Amount of soy product in solution (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Soy flour</td>
<td>ELISA 1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ELISA 2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Conventional ATP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sensitive ATP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total protein</td>
<td>-</td>
</tr>
<tr>
<td>Soy milk</td>
<td>ELISA 1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ELISA 2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sensitive ATP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Conventional ATP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total protein</td>
<td>-</td>
</tr>
<tr>
<td>Soy-based Infant formula</td>
<td>ELISA 1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ELISA 2</td>
<td>-</td>
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<tr>
<td></td>
<td>Sensitive ATP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Conventional ATP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total protein</td>
<td>-</td>
</tr>
</tbody>
</table>
Visual Inspection

- Most common method for validating/verifying cleaning procedures
- First step in determining if equipment is clean
- Points for inspection
  - Flat surfaces
  - Difficult to clean areas
  - Areas above processing zone

Advantages
- Does not require lab equipment/inexpensive
- Rapid

Disadvantages
- Depends on accessibility, lighting, surface, etc.
- Limited to accessible equipment
- Does visually clean = allergen clean?
Examples of “Visibly Dirty” Surfaces
Visual Inspection - Milk on Stainless Steel Plates

- 1000 µg
- 500 µg
- 100 µg
- 50 µg
- 10 µg
- 0 µg (control)
Methods in Development

- Multiplex DNA and immunochemical methods
- LC-MS and LC-MS/MS
- Spectroscopic methods
  - Mid-IR fiber optics
  - Real time
  - Can be used to detect different organic analytes
- Biosensors
  - Receptor and transducer that results in optical signal
  - Surface Plasmon Resonance (SPR)
  - Real-time, fast, automated
Conclusions

- Many tools are available for detection of allergens or allergenic foods
- Immunochemical methods the most common
- Choice of method depend on specific use, type of food matrix, and other factors
- Need to conduct “in-house” validation
- More than one method may be needed
- More work is needed to understand the chemical properties of food allergens- better extraction and detection
- There is a need for reference standards so that methods can be evaluated and compared