Risk assessment of allergens in new food proteins

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Background
Table: The ecological footprint of food

<table>
<thead>
<tr>
<th>Food</th>
<th>Footprint (ha)</th>
<th>Water footprint (m³)</th>
<th>Nitrogen footprint (kg)</th>
<th>Carbon footprint (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer</td>
<td>15,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>5,000</td>
<td>16,000</td>
<td>79</td>
<td>6</td>
</tr>
<tr>
<td>Eggs</td>
<td>3,000</td>
<td>46,000</td>
<td>18</td>
<td>1.8</td>
</tr>
<tr>
<td>Milk</td>
<td>3,333</td>
<td>55,000</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>Rice</td>
<td>1,000</td>
<td>10,000</td>
<td>98</td>
<td>0.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>1,398</td>
<td>34,000</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Beef milk</td>
<td>3,400</td>
<td>130,000</td>
<td>610</td>
<td>1300</td>
</tr>
</tbody>
</table>

The ecological footprint of food

World: Total population

Urgent need for
➢ Sustainable food production
➢ Alternative protein sources

As of 1 January 2018, the new Regulation (EU) 2015/2283 on novel foods (the new Regulation) is applicable. It repeals and replaces Regulation (EC) No 258/97 and Regulation (EC) No 1852/2001 which were in force until 31 December 2017.

The new Regulation improves conditions so that food businesses can easily bring new and innovative foods to the EU market, while maintaining a high level of food safety for European consumers.
‘Novel Food’ means any food that was not used for human consumption to a significant degree within the European Union before 15 May 1997 ...
“Food shall not be placed on the market if it is unsafe “Food shall be deemed to be unsafe if it is injurious to health” Regulation 178/2002 (The Food Law)

“The food does not pose, on the basis of scientific evidence, a risk to human health” Regulation 2015/2283

EFSA Guidance on the preparation and presentation of an application for authorization of a Novel Food

This includes allergenicity “The default assumption for Foods containing proteins is that such Novel Foods have allergenic potential. The allergenic potential of the Novel Food should be explored....”
Improving Allergy Risk Assessment Strategy for New Food Proteins

http://imparas.eu/
Working groups

Working group 1
Physical chemical properties and Analysis

Working group 2
In vitro methods

Working group 3
In vivo methods

Working group 4
Risks assessment and clinical perspectives

http://imparas.eu/
Risk analysis:
A process for controlling situations where an organism, system, or (sub)population could be exposed to a hazard. The three components of risk analysis are the risk assessment, risk management and risk communication
(WHO IPCS Risk Assessment Terminology, 2004)

Hazard: Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub)population is exposed to that agent.

Risk: The probability of an adverse health effect and the severity of that effect, consequential to a hazard.
Risk analysis

What about food allergy?
Risk assessment: food allergy

Risk assessment : food allergy

Allergy risk assessment strategy

• Is the novel protein able to elicit an allergic reaction in a food allergic population (cross reactivity)?
• Is the novel protein able to induce a new allergy (sensitization)?
Guidance and how to predict allergy risk

Allergenicity

Food allergens are mostly proteins.

The **default assumption** for novel foods containing proteins is that **they have allergenic potential**. The allergenic potential of the novel food should be explored by considering its composition, particularly its protein(s), its source (including taxonomic relationships), the production process, and available experimental and human data, including information on cross-reactivity.

**Protein analysis**

- Protein content
- Immunological tests (e.g. Western Blotting)
- Molecular weight of potentially allergenic protein, heat stability, sensitivity to pH, digestibility
- Degree of sequence homology with known allergens

**Human studies**

- Detection of specific IgE antibodies
- Skin prick testing
- Double blind placebo-controlled food challenge studies

Ultimate conclusion is based on Weight of Evidence
Current risk assessment approach for GMO risk assessment

Novel food

Protein extracts

MS analyses
Primary sequence
Identification & sequence homology

Specific serum screens
Protein stability testing
Structural homology

Allergenic risk assessment of novel foods focusing on allergenic cross reactive structures.
MS based protein sequencing methods

Bottom-UP approach

LC-MS

Sequenced organism mandatory
Protein identification by MS/MS sequencing

MS = Proteolytic peptides

MS/MS = peptide fragmentation
MS based protein sequencing methods

<table>
<thead>
<tr>
<th>MW</th>
<th>ion</th>
<th></th>
<th>ion</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>b₁</td>
<td>S</td>
<td>y₉</td>
<td>1080</td>
</tr>
<tr>
<td>145</td>
<td>b₂</td>
<td>SG</td>
<td>y₈</td>
<td>1022</td>
</tr>
<tr>
<td>292</td>
<td>b₃</td>
<td>SGF</td>
<td>y₇</td>
<td>875</td>
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<tr>
<td>405</td>
<td>b₄</td>
<td>SGFL</td>
<td>y₆</td>
<td>762</td>
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<tr>
<td>534</td>
<td>b₅</td>
<td>SGFLE</td>
<td>y₅</td>
<td>633</td>
</tr>
<tr>
<td>663</td>
<td>b₆</td>
<td>SGFLEE</td>
<td>y₄</td>
<td>504</td>
</tr>
<tr>
<td>778</td>
<td>b₇</td>
<td>SGFLEED</td>
<td>y₃</td>
<td>389</td>
</tr>
<tr>
<td>907</td>
<td>b₈</td>
<td>SGFLEEDE</td>
<td>y₂</td>
<td>260</td>
</tr>
<tr>
<td>1020</td>
<td>b₉</td>
<td>SGFLEEDEL</td>
<td>y₁</td>
<td>147</td>
</tr>
</tbody>
</table>

SGFLEEDELK
MS based protein sequencing methods

Applications:
- Protein identification → Homology with know allergens?
- Protein characterization → Homology with know protein modifications impacting allergenecity?
- Protein quantification → Trace analysis in food?
Methods relevant for allergenic risk assessment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Read out</th>
<th>Limitation</th>
<th>Food extract, novel protein, and processed protein</th>
<th>Needs</th>
<th>Relevance for allergenic risk assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid sequence homology</td>
<td>MS; bioinformatics</td>
<td>Primary sequence; Homology to known sequences</td>
<td>Access to updated allergen / sequence databases</td>
<td>Pure protein, processed protein</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>Structural Similarity</td>
<td>CD FTIR, X-ray based crystallography, and NMR</td>
<td>Secondary and tertiary structure analyses</td>
<td>Shared structure not always linked with allergenicity</td>
<td>Pure protein, processed protein</td>
<td>Scientific evidence of certain structures linked with allergenicity</td>
<td>✓</td>
</tr>
<tr>
<td>Aggregation</td>
<td>SEC, SAXS analysis</td>
<td>Monomers versus oligomers versus polymers</td>
<td>Limited knowledge of aggregation of proteins in processed foods</td>
<td>Pure protein, processed protein</td>
<td>Scientific evidence of link between aggregation &amp; allergenicity</td>
<td>✓</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>LC-MS</td>
<td>Glycosylation: yes/no N-, O-glycans; Monosaccharide versus branching</td>
<td>Sensitization with and without clinical relevance</td>
<td>Extracts, pure protein, and processed protein</td>
<td>Scientific evidence of link between glycosylation &amp; allergenicity</td>
<td>✓ (only for certain glycan structures)</td>
</tr>
</tbody>
</table>

<table>
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<tr>
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<th>Needs</th>
<th>Relevance for allergenic risk assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific serum screening (IgE binding)</strong></td>
<td>Immunoblot, ELISA, RAST; EAST ISAC ImmunoCAP</td>
<td>Binding to specific IgE</td>
<td>Sensitization with and without clinical relevance; availability of target protein in test systems; and availability of well characterized sera</td>
<td>Extracts, pure protein, and processed protein</td>
<td>Scoring, high throughput; de novo sequencing, when no database available; well characterized patients' sera</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Biological activity testing</strong></td>
<td>BAT and RBL assay, SPT; Food challenges</td>
<td>Functional IgE testing (cellular assay); Food challenge: Symptoms</td>
<td>Availability of test material; legal and ethical limitations</td>
<td>Extracts, foods, pure proteins, and processed proteins</td>
<td>Availability of well-defined sera and patients</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Resistance to gastric and duodenal digestion</strong></td>
<td>Standardized digestion assays</td>
<td>% of digested protein; or residual peptide fragments</td>
<td>Complexity of extracts; lack of standardized methods under physiological conditions; not always predicting allergenicity</td>
<td>Extracts, pure protein, and processed protein</td>
<td>Automatization process (high reproducibility, kinetic digestion indexes) guidance to interpret data. Validation with allergens and nonallergens</td>
<td>✓, Under debate</td>
</tr>
<tr>
<td><strong>Compar. compos. analysis of GM plant &amp; its appropriate</strong></td>
<td>Proteomics; LC–MS; and 2DE</td>
<td>Protein amount</td>
<td>Laborious</td>
<td>Extracts, pure protein, and processed protein</td>
<td>Guidance of qualitative and quantitative allergen testing</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Thermal / chemical stability</strong></td>
<td>CD FTIR, LC–MS; 2DE</td>
<td>Intact protein versus protein fragments</td>
<td>No single type of structure associated with stability</td>
<td>Extracts, pure protein, and processed protein</td>
<td>Guidance to interpret data; Validation with allergens and nonallergens</td>
<td>✓</td>
</tr>
</tbody>
</table>

### Gaps and limitations of current available methods and tools

| **Allergen databases** | Different databases provide different levels of information; Some of them are not regularly updated/curated and therefore relevant information is missing or available information outdated. Inclusion criteria for allergenic proteins vary for individual databases. |
| **Analytical methods** | Highly sensitive and advanced methods available for protein characterization; Sample preparation especially for complex food extracts is sometimes difficult (lack of harmonized protocols). |
| **IgE binding assays** | Well standardized reference assays including reference proteins are missing. In case of novel proteins no reference material is available. If IgE is not available, animal derived antibodies can be used. |
| **Digestion assays** | Lack of harmonized protocols, guidance how to interpret data, and reference material. |
| **Food processing techniques** | The current knowledge on food processing and its impact on allergenicity is incomplete. Lack of knowledge about which methods are the most relevant ones to up- or downregulate allergenicity. |
| **Food matrix** | Analytical methods are established—but limited data are available showing a link of food matrix components to allergenicity. |
| **Biological assays** | Reliable assays to assess de novo sensitization are lacking. |
Conclusions

Increasing need for novel food sources ➔ Assessment of the allergenic risk

- Investigation of primary structure:
  - secondary structure/
  - tertiary structure: Available

- Cross reactivity with known allergens can be assessed but:
  - No high throughput methods available

- Limited experiment-based knowledge about B- and T-cell epitopes/
  - food processing/matrix effect: Harmonization / guidance needed, availability of standards is mandatory

- No single method exists to predict allergenicity of novel food (cross-reactivity):
  - Knowledge database is needed to build predictive model on based bioinformatic, cellular assays and animal models

- Up to date no combination of tests exists to predict or detect novel protein able to induce a new allergy (sensitization):
  - Weight of evidence approach is needed

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Thank you

Questions?