Food processing and allergenicity
the case of soy

Huub F.J. Savelkoul
Gosia Teodorowicz PhD
Cell Biology and Immunology Group
Wageningen University

Soybean

- Inexpensive
- High protein content (37%)
- Nutritional and health benefits (cardiovascular health, anti-inflammatory and anticancer)
- Increased use in food products

Soy allergy

- Prevalence: 0.6% (Sweden) to 3.6% (Italy) overall 2.1% (general adult population)
- One of the “big 8” most allergenic foods
- Symptoms (mild to systemic)
  - Dermatological
  - Gastrointestinal
  - Respiratory
  - Systemic

Present in:
Bakery ingredients, Beer, Noodles, Prepared meat products, Meat analogs, Breakfast cereals, Food drinks, Baby food, Candy products, Sausage casings, Imitation dairy products, Flavorings, Infant formula, Salad condiments


Major soy allergenic proteins

<table>
<thead>
<tr>
<th>Gly m 5</th>
<th>Gly m 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-conglycinin / 7S globulin</td>
<td>Glycinin / 11S globulin</td>
</tr>
<tr>
<td>150 – 200 kDa</td>
<td>= 350 kDa</td>
</tr>
<tr>
<td>α-subunit (54 – 77 kDa)</td>
<td>5 major subunits (80-72 kDa)</td>
</tr>
<tr>
<td>β-subunit (42 – 55 kDa)</td>
<td>- Acids chain (33, 29 and 27 kDa)</td>
</tr>
<tr>
<td>51% sequence identity with Ara h 1</td>
<td>Acids chain epitopes identical to Ara h 3</td>
</tr>
</tbody>
</table>

Highly thermostable allergens

Gly m 4: cross-reactive allergen

- Bet v 1 homologue (birch pollen allergens)
- 46-53% sequence identity
- 17 kDa
- Heat labile or partially heat stable [?] (unpublished)

Three groups of patients

- Total soy extract is not a good diagnostic marker for soy allergy. In the studied group (n=30), 53.3% of patients would be misdiagnosed based on IgE for total soy only.

Specific IgE levels in two groups of soy allergic patients

Teodorowicz, 2016
### Conclusions

- Total soy extract is not a good diagnostic marker for soy allergy.
- Soy milk (related products) cause allergic symptoms in the majority (56.7%) of soy allergic patients due to cross-reactivity of Gly m 4 with Bet v 1.
- When the diagnosis of Bet v 1 related soy allergy is confirmed, patients may only need to avoid soy milk (related products) rather than all soy products. Birch pollen-allergic patients should be informed about this potential reaction.

### Specific IgE levels in two groups of soy allergic patients

![Graph showing specific IgE levels](image)

### Clinical relevance

<table>
<thead>
<tr>
<th></th>
<th>Gly m 4 positive (P23)</th>
<th>Gly m 5, 6 positive (P2)</th>
<th>Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>Anaphylaxis, angio-edema, laryngeal edema, nausea, vomiting</td>
<td>Anaphylaxis, urticaria, nausea, diarrhea, laryngeal edema</td>
<td></td>
</tr>
<tr>
<td>Product(s)</td>
<td>Alpro Soya milk (&gt;250 ml)</td>
<td>Soy sprouts, soy sauce, cookies</td>
<td></td>
</tr>
<tr>
<td>Hayfever?</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Influence daily life</td>
<td>Avoiding all products containing soy</td>
<td>Always checking labels of products, medication always with me</td>
<td></td>
</tr>
<tr>
<td>ImmunoCAP IgE (kU/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total soy</td>
<td>&lt;0.35</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>Gly m 4</td>
<td>11.8</td>
<td>&lt;0.35</td>
<td></td>
</tr>
<tr>
<td>Gly m 5</td>
<td>&lt;0.35</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>Gly m 6</td>
<td>&lt;0.35</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>Bet v 1</td>
<td>45.1</td>
<td>&lt;0.35</td>
<td></td>
</tr>
</tbody>
</table>

### Processing of soybean

![Diagram showing processing of soybean](image)

### Maillard reaction – soy model

Development of color, aroma, flavor, texture of processed foods. Levels of AGEs correlate with induction of diabetes, cardiovascular disease, renal disease, Alzheimer disease, aging, allergy, chronic inflammation and cancer.

![Diagram showing Maillard reaction](image)

### Advanced stages of MR

![Diagram showing advanced stages of MR](image)

![Diagram showing AGEs](image)
Maillard reaction and protein allergenicity

- Epitope formation at surface
- Blocking of epitope
- Formation of neo epitope or hidden epitope

Aggregation by heating and/or Maillardation

- Enhanced or increased allergenicity

Effect of heat on protein structure

<table>
<thead>
<tr>
<th>Temperature zone</th>
<th>Loss of tertiary structure</th>
<th>Loss of secondary structure</th>
<th>New interactions, S-S bonds</th>
<th>Aggregation, polypeptide chain reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-70</td>
<td>75-80</td>
<td>85-90</td>
<td>90-100</td>
<td>100-110</td>
</tr>
</tbody>
</table>

Measurement:
- Furosine (HPLC)
- CML (HPLC, ELISA)
- N-(carboxymethyl)lysine
- Melanoidins (OD)

AGE-OVA is taken up more efficiently by DC than native OVA

Especially at low allergen doses!

And is thus a more potent T-cell activator at low allergen doses! (OTII mice)

Possible link to allergy

- Hilmenyuk et al. 2009
- AGE-OVA (allergen ovalbumin) was created by heating OVA and glucose for 6 weeks at 50°C.
- IL-6 production → Th2 cytokine production
Dry roasted (DR) peanut is a more potent Th2 inducer than raw peanut.

Moghaddam et al. 2014

Effect of thermal treatment on cytokine induction capacity

HC (n=7) PA (n=5)

Vissers, 2011

IgE capture inhibition curves

Native heated in the presence of glucose or in the absence of glucose

Vissers, 2011

β-hexosaminidase release by RBL cells

Peanut allergen processing

Blocking capacity of processed Ara h 1

IL-8 by Caco-2 cells

Inhibition of bacterial adhesion to Caco-2 cells by treated Ara h1

Teodorowicz, 2014

SPE - Identification with pentosidine

Western blot: Identification of immunoreactive fractions with patients sera

Teodorowicz, 2016

The Basophil Activation Test

Allergenicity testing:
RAGE/HEK 293 cells:
Reporter assay (Luciferase activity)

AGE cross-linking

NF-κB

Luciferase

Cytokines: TNFα, IL-1, IL-6

Inhibition ELISA-based RAGE binding assay

Pre-incubation

Process results into percentage of inhibition:

Detection

UV measurement

Extendedly glycated SPE
Resorcinol soluble RAGE
Glycated or heated SPE
Anti-RAGE and detection antibody

Inhibition RAGE-ELISA

Glycated versus heated samples for 25 μg/ml

Conclusions

• Soy processing with sugar provokes the formation of Maillard-type neoallergens.
• MR neoallergens have higher allergenic potential (stronger basophil activation and higher immunoreactivity) than soy proteins heated without sugar.
• Longer heating of the SPEs with glucose leads to enhanced binding capacity to sRAGE and membrane RAGE.

Improving the diagnosis of soy allergy

Total soy extract is not reliable in the diagnosis of birch related soy allergy (Gly m 4) by BAT.
The quality of protein extracts used in BAT for food allergy diagnosis is of high importance.

Clinician

Allergic consumer

Soy processing industry

Processing matters. Patient may be sensitized against raw and/or processed soy proteins.

BAT is a promising tool to study the changes of proteins’ allergenicity and may be helpful in optimization of processing conditions.