Allergenicity risk assessment of novel proteins in food: Case study and future improvements

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Background

Sustainable food production > Alternative protein sources >

What It Takes To Make A Quarter-Pound Hamburger

- **feed**
  - Pounds of grains and forage
  - 6.7

- **water**
  - Gallons for drinking water and irrigating feed crops
  - 52.8

- **land**
  - Square feet for grazing and growing feed crops
  - 74.5

- **fossil fuel energy**
  - Blus for feed production and transport. That’s enough to power a typical microwave for 18 minutes.
  - 1,036

Credit: Producers: Elisa Barlay, Jessica Stoller-Conrad; Designer: Kevin Uhrmacher/NPR
General food law
(EC regulation No 258/97 and EU recommendation 97/618)

• The law requires that safety is assured for all food ingredients placed on the market.
• Responsibility of the producers

• Novel food law: Comprehensive food safety assessment for novel foods introduced after 1997

<table>
<thead>
<tr>
<th>Nutrition Facts</th>
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<tbody>
<tr>
<td>Serving Size 172 g</td>
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<tr>
<td>Amount Per Serving</td>
</tr>
<tr>
<td>Calories 200</td>
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<tr>
<td>Total Fat 1g</td>
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<tr>
<td>Trans Fat 0g</td>
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<tr>
<td>Sodium 75mg</td>
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<tr>
<td>Dietary Fiber 11g</td>
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<tr>
<td>Protein 13g</td>
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Microbial | Toxicological | Allergenic

![Nutritional Diagram]
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)?
Current strategy (EFSA/GMO)

- Weight of evidence approach

- **Source of gene**
  - No reports on allergy
  - Allergy common

- **Sequence alignment**
  - < 35% identity over 80 aa
  - > 70% identity over full length

- **IgE tests**
  - No specific IgE
  - Specific IgE to allergen

- **Pepsin digestion**
  - Digested > 90% in < 2 min
  - Stable for 60 min
Yellow Mealworm (Tenebrio molitor)

- Larval stage of the Yellow mealworm beetle
- Originally produced as feed for animals such as fish, reptiles and birds.
- Commercially available for human consumption (Australia, UK, NL and Belgium)
Mealworm related to shrimp and house dust mite

Verhoeckx & van Broekhoven Food and Chemical Toxicology (2014)
sIgE from shrimp allergic patients react with mealworm proteins

Patient 1

Patient 2

Patient 3

Immunoblot

Basophil activation

Verhoeckx & van Broekhoven Food and Chemical Toxicology (2014)
Tropomyosin and arginine kinase are mildly stable in pepsin resistance test

Arginine kinase

Tropomyosin

SRN1 Soluble proteins (tris)
SRN3 Difficult to solubilize proteins (ureum)
New protein (source)

- History of human exposure (work/food)
- Relationship: Taxonomy, Homology
- Identification proteins in source
- Information on usage: raw, matrix, processing

Research material: Extract(s), GMP

Research protocol, METC

Cross reactivity

Sera known allergy with new protein
- Immuno-blot
- ELISA
- CAP/ISAC

Identification proteins

Functional Cross reactivity
Sera known allergy with new protein

- Basophil activation test
- Skin prick test
- Food challenge

Cross reaction
Sera new allergy with known allergen
- In vitro
- In vivo

New allergy
Sera new allergy with new protein
- In vitro
- In vivo

Sensitising potency

History Sensitisation
Screening (interview and/or serology)
- Working population
- Targeted people with symptoms

Identification reactive proteins in source (potential new allergens)

New allergies & Cross reactivity

No history TNO®RAPT (under development)

Intrinsic properties
- Digestion
- Physical chemical
- Biological
Conclusions cross reactivity

- All shrimp allergic patients (n=15) were sensitized to mealworm based on SPT, BAT, Immunoblot and CAP mealworm

- 87% of the Shrimp allergic patients had a positive DBPCFC to mealworm

- Shrimp allergic patients are at risk when eating mealworm
Conclusions De novo sensitisation

› All subjects (n=4) were atopic and sensitized to mealworm according to SPT, BAT, Immunoblot and CAP mealworm.

› Two subjects had a positive DBPCFC to mealworm, but were not allergic to shrimp.

› Test population was too small to draw conclusions.

› New strategies are needed to assess De novo sensitisation.
Food for thought

- Static vs Dynamic digestion
- Matrix
- Bioactivity after digestion
- Bioactivity after transport
TNOs Intestinal model (TIM)

You Tube: hJlmi6iK3gQ
Bio-accessibility after digestion: Static vs Dynamic digestion (TIM)
Digestion kinetics of peanut flower is different from peanut extracts in TIM
Digested Ara h 1 and 3 still able to activate basophils
Ara h 1 and 3 lose reactivity after transport
Research focus

DC
PBMC
Basophils

Immune models
Protein panel

Physical chemical
Protein panel

Hydrophobicity
Lipid binding
Aggregation
glycosylation
Size
stability
Processing/matrix

Digestion

Transport/processing

Epithelial cells

Sensitisation
Elicitation

Data-integration model

Protein/extract

fragments

Protein

fragments

Hydrophobicity
Lipid binding
Aggregation
glycosylation
Size
stability

Processing/matrix

Digestion

Transport/processing
Improvement Allergy Risk assessment initiatives

- TNO Shared Research Program Food Allergy
- COST Action ImpARAS
Goals

Towards a Food Allergy Free World

- Protect the allergic consumer
  - 4-6 years
- Prevent introduction of strong allergenic foods
  - 6-8 years
- Preventive & curative strategies
  - 10-15 years

Program Lines

1: Allergen & allergy management

2: Allergenicity assessment of (novel) food proteins

3: Effect assessment and markers to improve diagnostics, prognostics and monitoring of immune health interventions
Aim:
To build an interdisciplinary European network of scientists with a broad range of expertise to discuss, with an out-of-the-box view, new ideas and more predictive models and approaches to improve the current allergenicity risk assessment strategy.
Website: www.imparas.eu

COST Action 1402:

Improving Allergy Risk Assessment Strategy for New Food Proteins

Aim:
To build an interdisciplinary European network of scientists with a broad range of expertise to discuss, with an out-of-the-box view, new ideas and more predictive models and approaches to improve the current allergenicity.

Due to the continuing growth of the world population from 7 billion today to 9 billion in 2050, we will face a shortage of protein sources for human consumption in the near future. For this reason, Horizon 2020 included the topic “Sustainable European bio-economy; bridging the gap between new technologies and their implementation” within their research program. Food safety assessment is an important requirement before new products can be brought to market. Such assessments include the investigation of microbiological and toxicological hazards as well as the risk of food allergy.

From an industry perspective, there is a need for: a) relatively cheap, easy and reliable tools for screening for allergenicity of new or modified food proteins, b) early risk based decision-making during product development; and, c) an improved understanding of the interactions between the food matrix and allergens.
1st International Conference
Improving Allergy Risk Assessment Strategy for new food proteins

November 24-26, 2015
University of Belgrade
Belgrade – Serbia

For more information please visit: http://imparas.eu/meetings/

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Thank you for your attention

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