Allergenicity Assessment

Natural variability of endogenous allergens - Digestibility of proteins

November 9, 2017

Allergy Safety Assessment

Determine if a GM crop is as safe as its non-GM counterpart

Allergy Assessment

Introduced Protein

Determine if introduced protein is a known allergen or similar to one

- Source organism
- Bioinformatics
- Exposure
  - Expression
  - **Pepsin resistance**

- HESI PATC digestibility project - Paper under preparation

Endogenous Allergens

Determine if the insertion has changed endogenous allergen levels

- **Comparative assessment** between GM and non-GM varieties

- Hill *et al.* 2017. J. Agric. Food Chem. 65: 5531-5544.
- Geng *et al.* 2017. J. Agric. Food Chem. 2017, 65, 463−472
- Hill *et al.* 2017. Reg. Toxicol. Pharmacol. 89:7-73.

Agenda

Part 1

Natural variability of endogenous allergens

- Rationale for testing endogenous allergen levels
- Validated detection methods
- Natural variability of endogenous allergens in non-GM and GM crops
- Conclusion:
  What is the value of monitoring endogenous allergen levels?

Rationale for testing endogenous allergen levels

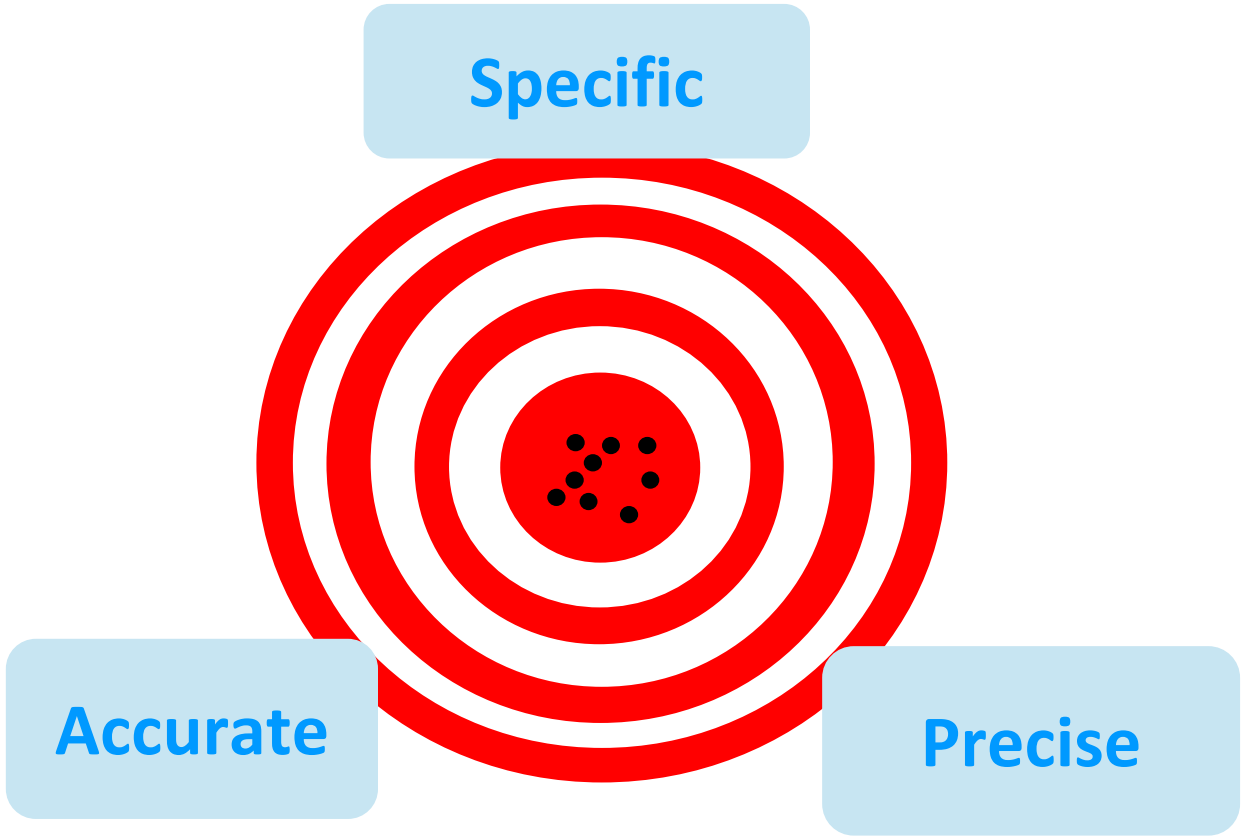
According to IR503/2013, conclusions of the allergenicity assessment should indicate…

‘whether the genetically modified food or feed
is likely to be more allergenic than its conventional counterpart’

According to EFSA 2017, Guidance on allergenicity assessment of genetically modified plants…

‘Endogenous allergens: […] the starting point of the assessment should be the identification of statistically significant differences between the GM plant and its conventional counterpart.
A further evaluation should investigate whether or not the differences observed fall within or outside the **range of natural variation** estimated from the reference varieties included in the field trial, i.e. the equivalence test (IR503/2013)’

Detection methods of endogenous allergen



ELISAs

- Geng *et al.* **2015**. J. Agric. Food Chem. 2015, 63.20: 4947-4953
- Geng *et al.* **2017**. J. Agric. Food Chem. 2017, 65, 463−472
- …

Mass spectrometry

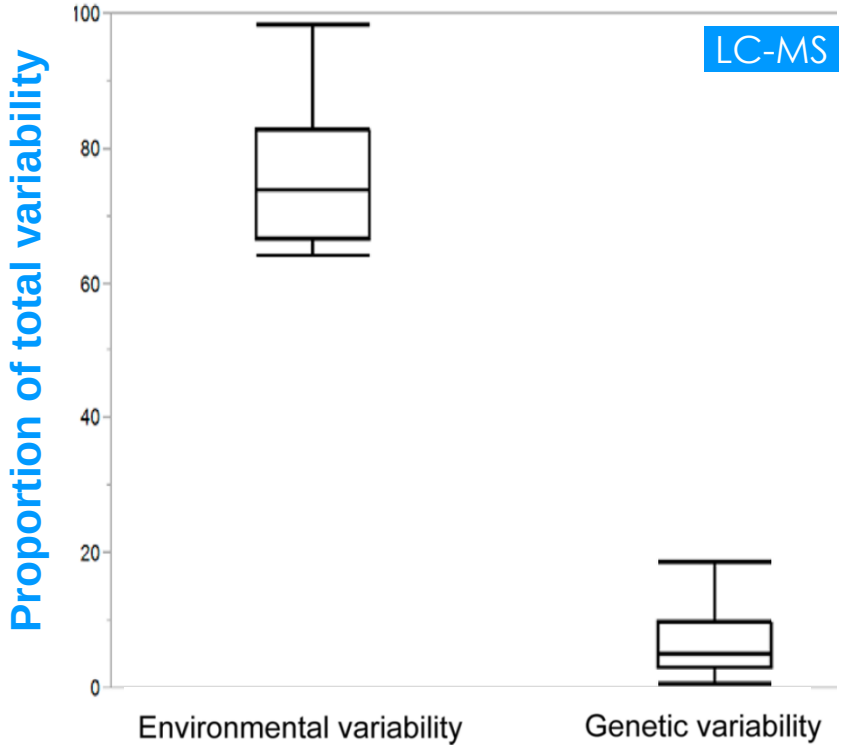
- Houston *et al.*, **2011**. J. Proteome Res. 10, 763–773.
- Stevenson *et al.*, **2012**. Front. Plant Sci. 3, 1–13.
- Hill *et al.* **2017**. J. Agric. Food Chem. 65: 5531-5544
- …

Gels/optical spectrometry

- Rouquié *et al.* **2010**. Regul. Toxicol. Pharmacol. 58, S47–S53.
- Satoh *et al.* **2016**. Biosci. Biotechno. Biochem., 80.11: 2198–2207
- …

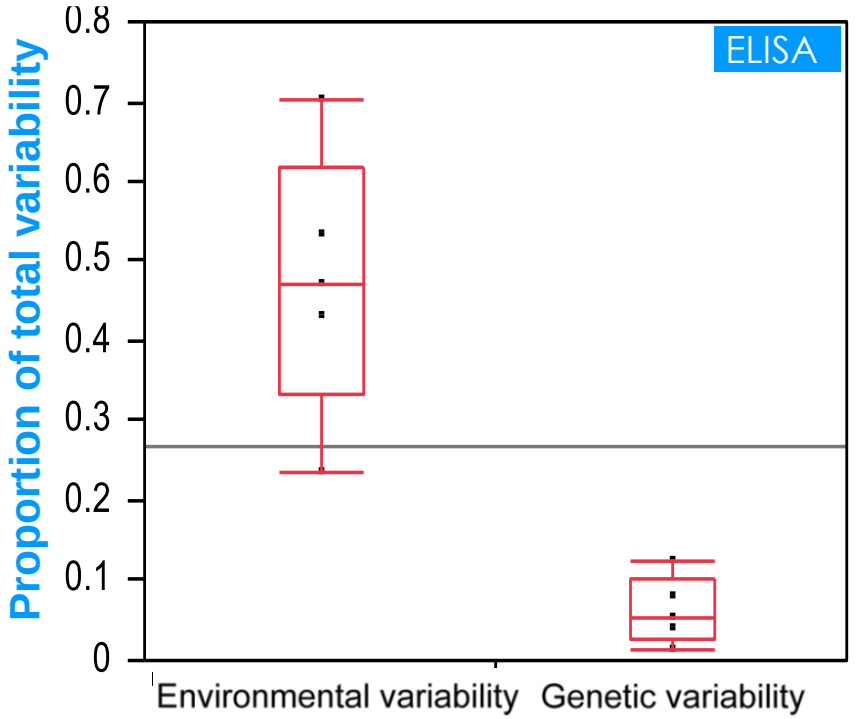
Environmental conditions → principal contributor to variation in non-GM soybean endogenous allergens

21 non-GM soybean - 3 locations – 3 growing seasons



From: Hill *et al.* 2017. **Development, validation, and inter-laboratory evaluation of a quantitative multiplexing method to assess levels of ten endogenous allergens in soybean seed and its application to field trials spanning three growing seasons**. J. Agric. Food Chem. 65: 5531-5544.

37 non-GM soybean – 26 locations – 5 growing seasons



From: Geng *et al.* 2017. **Natural Variability of Allergen Levels in Conventional Soybeans: Assessing Variation across North and South America from Five Production Years**.
J. Agric. Food Chem. 2017, 65, 463−472

Does transgenesis present a greater risk of altering soybean endog. allergen levels compared with traditional breeding?

4 GM soybean lines containing 4 events
- DAS-44406-6
- DAS-81419-2
- DAS-81419-2 x DAS-44406-6
- DAS-68416-4 x MON-89788-1

Matched non-GM isolines

20 non-GM commercial reference varieties

3 multisite field studies conducted over 5 year period

8 endogenous allergens measured by LC/MS/MS

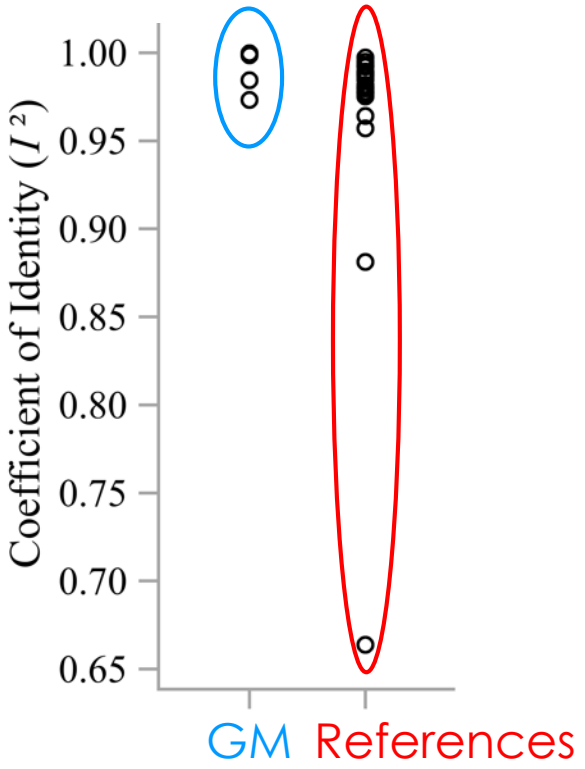
Calculation of coefficient of identity (I^2)

GM ⇔ non-GM isolines

Reference varieties ⇔ non-GM isolines

From Hill *et al.* 2017. **Transgenesis affects endogenous soybean allergen levels less than traditional breeding**. Reg. Toxicol. Pharmacol. 89:70-73.

GM soybean highly similar to isolines



Endogenous allergen levels

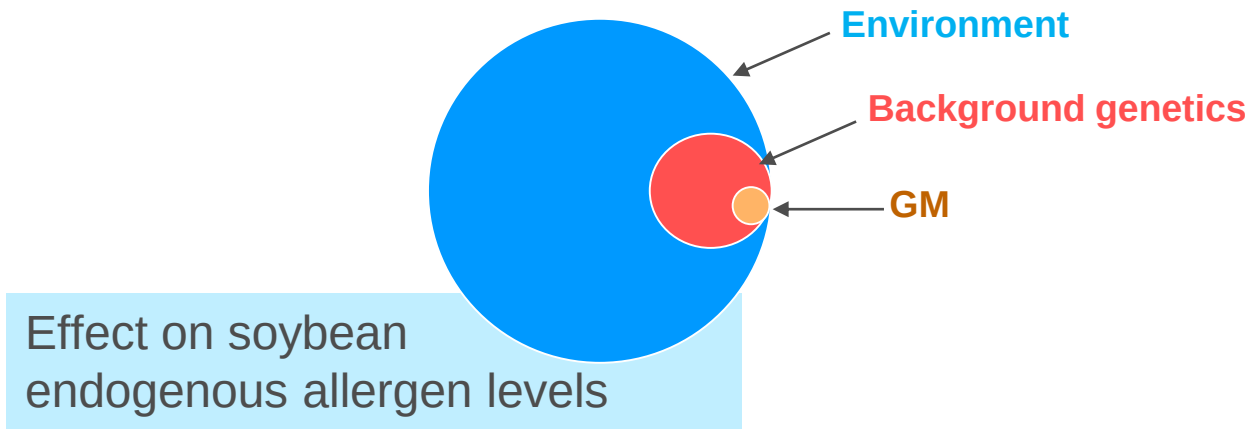
We can observe a wider distribution in the commercial reference varieties due to genetic background diversity

From Hill *et al.* 2017. **Transgenesis affects endogenous soybean allergen levels less than traditional breeding**. Reg. Toxicol. Pharmacol. 89:70-73.

Effects of environment > genotype > GM breeding on endogenous soybean allergens

Genotype association to soybean endogenous allergen levels **minor** compared with **growing environment**

GM breeding and stacking of GM events change soybean endogenous allergen levels **less than traditional breeding**



Part 1 conclusion

There is limited value in monitoring endogenous allergen levels in GM crops as part of the safety assessment

The great majority of variability in endogenous soybean allergen levels is due to growing **environment**, not genotype

Range of soybean allergen expression up to 50-fold change in non-GM soybean

No direct correlation between allergen **exposure** and **incidence** of allergy. In some cases, more exposure is sometimes beneficial

ex: exposure to food allergens at a young age reduces the incidence of allergy

Evaluating the variable allergen levels in non-GM varieties has not been a safety priority

⇨ *Still no hypothesis of testing impact of transgenesis on allergenicity*

Agenda
Part 2

Digestibility of proteins

- Rationale for testing additional conditions for the pepsin resistance test
- Summary of the HESI PATC digestibility project
- Conclusion:
  What is the value of testing additional conditions for the pepsin resistance test?

Rationale for testing additional conditions for the pepsin resistance test

Considerations from EFSA concerning pepsin digestion protocol

‘the EFSA GMO Panel proposes a refined in vitro digestion test that extends the conditions currently used in the classical pepsin resistance test in order to better reflect the range of conditions found in vivo.
This elaborated test includes additional conditions more representative of the gastric environment with regard to pH and pepsin levels, together with an intestinal digestion phase.’

Summary of the HESI PATC digestibility project

Application of a sequential protocol (gastric/duodenal) to pairs of proteins from the same protein family but with different allergenicity

Key question to be answered

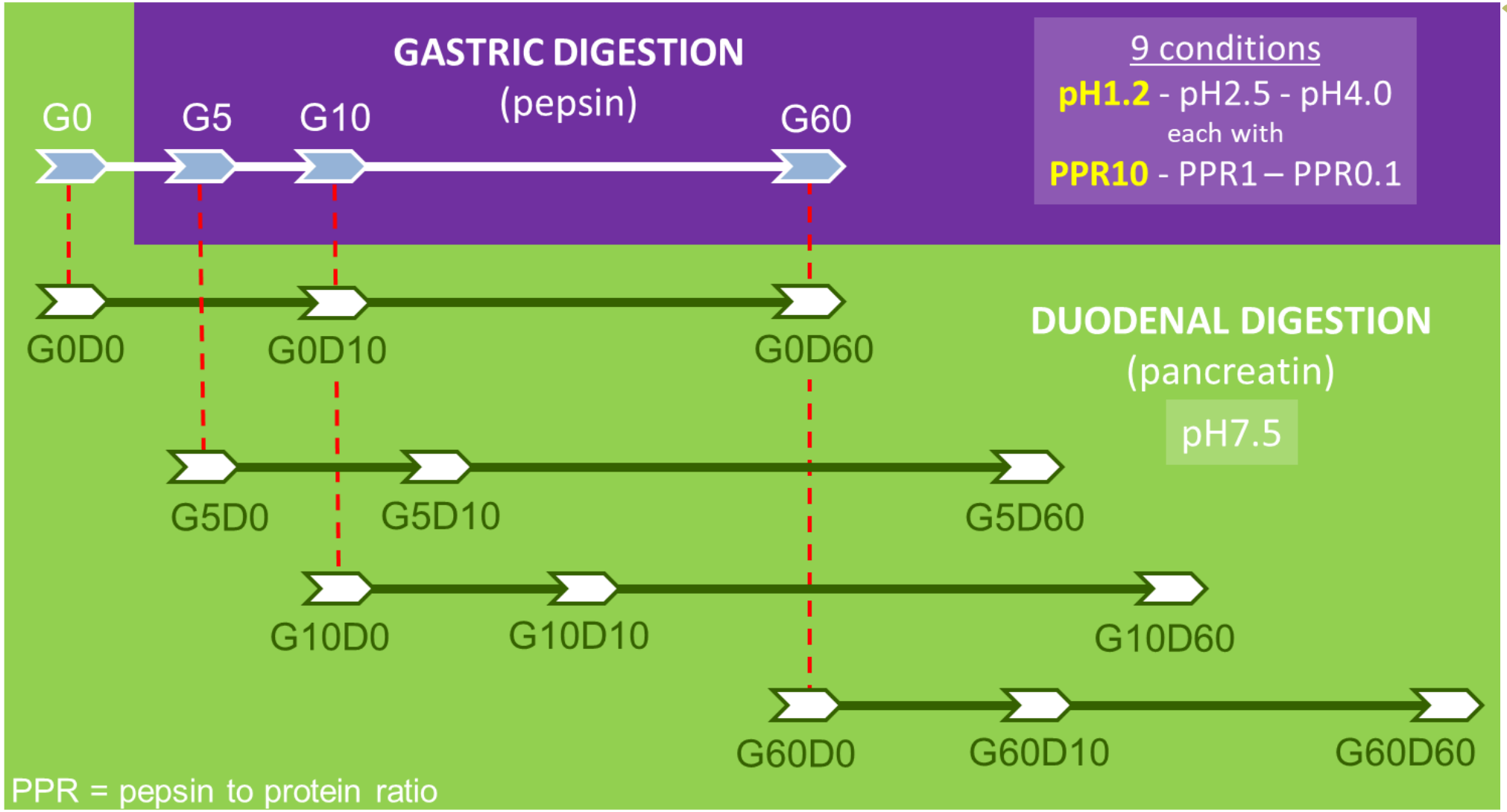
Does the degree of susceptibility to *in vitro* pepsin and pancreatin digestion separate allergens from non-allergens?

Ronald van Ree

Professor of Molecular and Translational Allergology
Academic Medical Center
University of Amsterdam

Academic Co-chair ILSI-HESI PATC

HESI PATC digestibility project
Evaluation of different digestion conditions

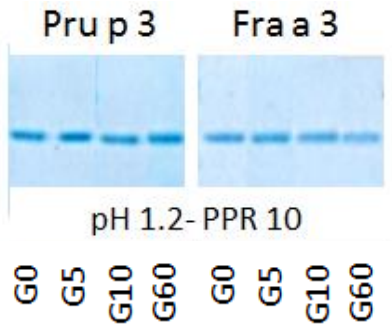


HESI PATC digestibility project
Selection of pairs of proteins

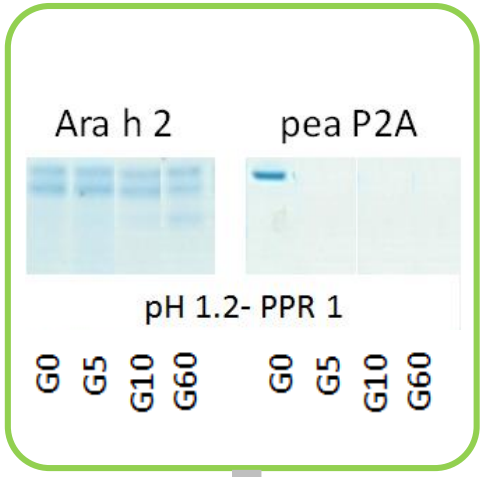
Protein family	Allergenic	Non-/weakly allergenic	% identity
Lipid transfer proteins	Peach Pru p 3 	Strawberry Fra a 3 	66,7
Albumins	Peanut Ara h 2 	Pea PA2 albumin 	5,2
Tropomyosins	Shrimp Pen a 1 	Porcine tropomyosin 	55,0
Collagens	Fish collagen type 1 	Bovine collagen type 1 	55-75
Parvalbumins	Carp Cyp c 1 		NA

Example of results
Gastric phase in optimal conditions

Lipid transfer proteins

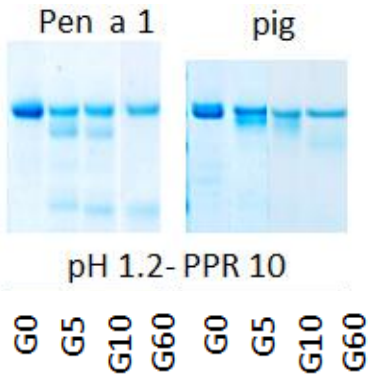


Albumins



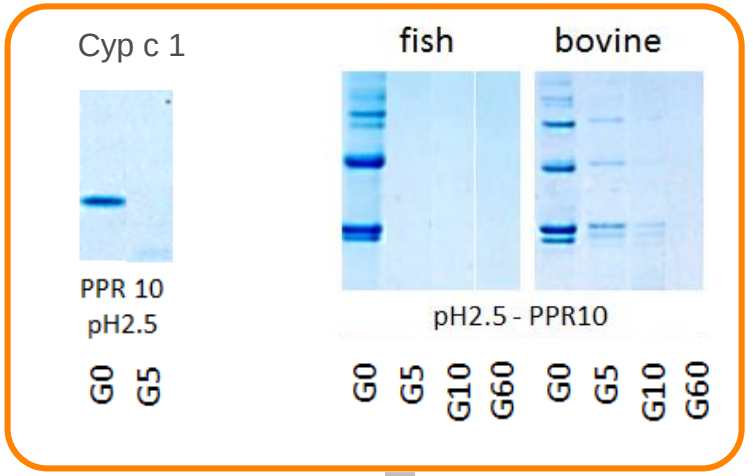
Allergen is not degraded.

Tropomyosins



Parvalbumin

Collagen



Allergens are degraded.

PPR = pepsin to protein ratio

HESI PATC digestibility project
Summary of additional findings

High pH (4.0) and **low pepsin:protein ratios** are **not of added value** to distinguish allergens from non-allergens, despite in some ways being more physiological:

- Most proteins are poorly processed under these conditions, irrespective of being an allergen or not

Combined gastric/duodenal digestion has been tested:

- Ara h 2, Pru p 3 and Pen a 1 were highly susceptible to pancreatin (from 10 min)
- Cyp c 1 was resistant to pancreatin digestion
- **Inconsistent digestion profile** between allergen from non-allergen
  (ex: low allergenic pea albumin was slightly more stable than the peanut protein, Ara h 2)

Part 2 conclusion
HESI PATC digestibility project

This study confirms the fact that there are **exceptions** to the straightforward relation between **resistance** to gastro-intestinal **digestion** and **allergenicity**

- Of the four established allergens tested, fish parvalbumin was highly susceptible to pepsin but very resistant to pancreatin
- For the other three it was essentially the other way around: quite resistant to pepsin but highly susceptible to pancreatin, in particular if preceded by supposedly more physiological conditions for the gastric phase

High pH (4.0) and **low pepsin:protein ratios** were **not of added value** to distinguish allergens from non-allergens

The **addition** of a **duodenal phase did not improve** the power to discriminate allergens from non-allergens

⇨ The current low predictive power of the pepsin digestion assay **cannot be improved** with additional digestibility conditions

⇨ Having said that, it seems useful to **continue** including the pepsin digestion assay, conducted in **optimal conditions** (low pH – high PPR) in the weight of evidence approach

Thank you!