Effects of Food Processing on Allergenic Potential of Food Proteins

Clare Mills

Manchester Institute of Biotechnology,

Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences

The University of Manchester

EuroPrevall

FAAM

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Declaration of interests

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ReactaBiotech Ltd

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Food processing has enabled humans to access safe and nutritious food over thousands of years

Beginning in the Stone age with cooking

Salting and pickling in Mesopotamia

Brewing and baking in ancient Egypt

Cheese making and fermented foods like yogurt

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Processing helps to form the structural elements of foods – the food matrix

Foods comprise mixtures of different structural elements which are formed from polymers (proteins, starch and non-starch polysaccharides) and oils/fats.

Droplets[1] and micelles

Crystals[3]

Gels[4]

Granules[2]

Foams[5]

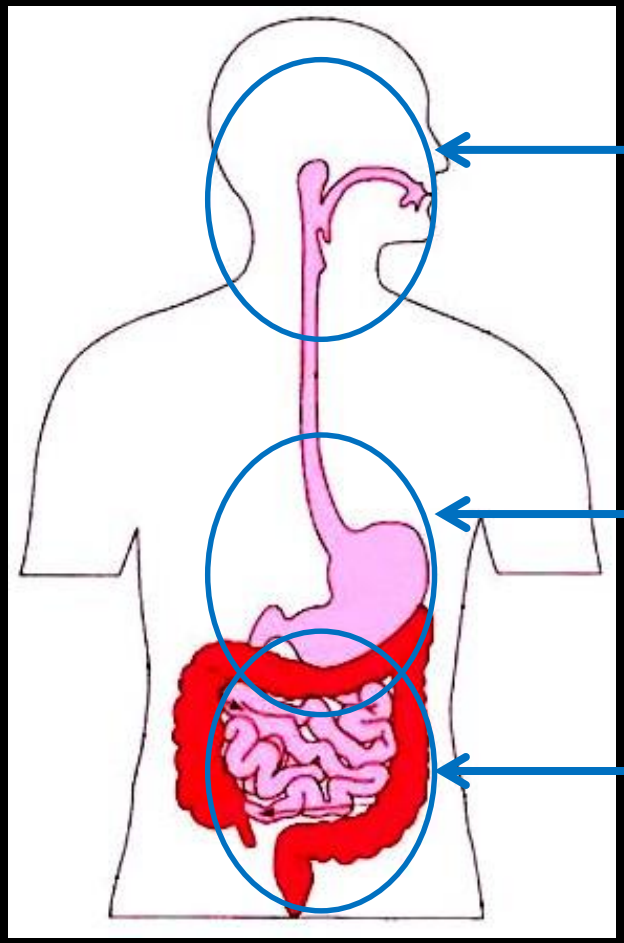
(1) Mackie et al. J Agric Food Chem. 2007 55:5611-9; (2) Liu et al Carbohydr Polym. 2013 97:458-68; (3) Marangoni TIFS 2002 13: 27-47; (4) Macierzanka et al Food Chem. 2012 134:2156-63; (5) Glenn et al *J. Agric. Food Chem.*, 2002 *50*; 7100–7104

How does the food matrix modify allergens?

The formation of the food matrix can affect allergens by

- **Structural modification resulting from**
  - Unfolding, aggregation and cross-linking of different types of proteins
  - Chemical modification such as
    - hydrolysis to give peptides,
    - deamidation,
    - reactions with other food components such as sugars (Maillard modification)
- **Entrapment within a food structure affecting bioaccessibility**
  - Solubility in biological fluids
  - Susceptibility to digestion

The food matrix and impact on bioaccessibility in the GI tract



Oral : Simulated saliva fluid, amylase, lysozyme 37˚C, 2min

Gastric: Simulated gastric fluid, pH2.0-2.5, pepsin, lipase homologue, 37˚C up to 120 min

Duodenal/Intestinal: Simulated pancreatic fluid, bile acids, trypsin, chymotrypsin, amylase, pancreatic lipase and co-lipase 37˚C up to 120 min

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Natural plant tissue matrices can be protective of allergenicity

Thermostability of peach LTP Pru p 3

Peach LTP is thermostable up to 90°C but thermal treatments of 121°C (equivalent to canning) cause the protein to unfold…..

Such thermal treatment made the protein susceptible to gastric digestion…although individuals with peach allergy cannot eat cooked fruit

Can natural plant tissue matrices affect stability to thermal treatment?

- LTP allergens are found in the epidermal tissues of fruit.
- How does this affect thermal denaturation and bioaccessibility
  - Solubility of allergen?
  - Susceptibility to proteolysis?

Sayed Abdullah, Mackie, Rigby Mills unpublished

Peach LTP is resistant to digestion from peach peel

In vitro gastric digestion of peach peel makes Pru p 3

- Soluble in simulated saliva
- Completely resistant to digestion
- Only a proportion is solubilised during digestion

In vitro intestinal digestion shows

- formation of fragment 1-79 at 60 min
- Much of the Pru p 3 remains in the peel matrix

Sayed Abdullah, Mackie, Rigby Mills unpublished

But the matrix increases allergen stability to retorting at 121°C

In vitro gastric digestion of retorted peach peel

- Unlike purified Pru p 3 the peach peel protein remains resistant to digestion
- Again only a proportion is solubilised during digestion
- No digestion resistant fragment 1-79 detected

These data suggest that proteins within a plant tissue matrix are more thermostable than purified proteins

Sayed Abdullah, Mackie, Rigby Mills unpublished

Bioaccessibility of certain milk proteins is altered in processed products

The same patterns are seen for the animal food allergen - the lipocalin allergen β-Lactoglobulin

- The lipocalin fold also confers resistance to thermal processing and stability to digestion to protein β-lactoglobulin from whey

- Can these properties also explain the importance of β-lactoglobulin as a cows milk allergen?

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β-Lactoglobulin - normally resists pepsinolysis in solution but is partially digested as an emulsion

Maceirczenka, Sancho, Mackie, Mills

β-Casein: Emulsification alters the digestion kinetics of giving rise to Mr 6,000 resistant peptides

Digestion of cheese shows the presence of undigested proteins even after extensive gastric digestion

- Large amounts of protein is soluble in simulated saliva
- Much is digested but resistant fragments remain with proteins persisting in gastric and duodenal digestion phases

Not all baked matrices are equal!!
[Allergens are less bioaccessible from bread and muffins than cookies]

Cupin 7S globulin: Gastric digestion of peanut Ara h 1 does not alter its allergenic activity

Eiwegger, Rigby et al Clin Experimental Allergy 2006 6: 1281-1288

Aggregates, but not gels, are readily digested

Ara h 1 aggregates formed by boiling at low concentration are digestible – like the native protein

The gel formed by boiling at high concentration is indigestible

This is because the gel pores are so small pepsin can no longer penetrate the gel ($D=0.49 \pm 0.14\ \mu m^2/s$)

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Prolaimin 2S albumins: Ara h 2/6 has a digestion resistant core

A core of Ara h 6 remains resistant to gastroduodenal digestion – like LTPs and the 2S albumin from Brazil nut Ber e 1

Marsh et al Mol Nutr Food Res 2008 52:S272-S285 Sancho, Marsh, Alexeev et al

Water-continuous and low-water baked matrix does not affect peanut allergens

- *In vitro* gastric digestion for 11 mins shows that peanut allergens are well digested in flour, chocolate dessert and chocolate cookies
- IgE immunoblotting shows Ara h 2/6 reactivity is retained in digests of all foods
- Ara h 2/6 passes into the soluble phase in an almost intact form.

Rao, Baricevic-Jones, Simpson, Sayers, Xue, Bernard, Adel-Patient, Mills et al unpublished

Water-continuous and low-water baked matrix does not affect peanut allergens

IgE reactivity of the bioaccessible peanut allergens by ELISA showed

- Allergenic activity was only reduced for the cookie during gastric digestion
- Was reduced by ~3-fold for the matrices during largely unaltered compared to the peanut flour during gastroduodenal digestion.

Rao, Baricevic-Jones, Simpson, Sayers, Xue, Bernard, Adel-Patient, Mills et al unpublished

Baking reduces the gastric digestibility of gluten proteins

Starch digestion enhances gastro-duodenal digestion of gluten

Smith et al Mol Nutr Food Res. 2015;59(10):2034-43.

Gluten proteins retain immunoreactivity and IgE-reactivity after digestion

- Gluten proteins reactive with antibodies to celiac-toxic motifs survive gastric digestion

Bread digests retains serum IgE binding capacity following digestion

- All of the serum samples from wheat allergic patients (n=12) retained reactivity as determined by inhibition ELISA
- Binding was greater than to an equivalent digest of purified gliadin

Smith, Shewry, Carr, Bartra, Semic-Jusufagic, Simpson, Padfield, Mills un published

Muffin

- Gluten is not digested by pepsin
- A fraction of ovalbumin (Gald 2) is trapped in the matrix and not digested even for 120 min

Biscuit

- Gluten is better digested by pepsin
- Trapped ovalbumin (Gal d 2) is released/ digested after 60 min

Mattar, Padfield, Mills un published

What about the food matrix and eliciting allergic reactions?

Grimshaw et al (2003) – four patients, peanut in 31% vs 23% fat matrix. Higher fat resulted in

- Fewer oral symptoms
- Higher consumption of peanut
- More severe reactions
- Peanut was less available as adjudged by ELISA

Grimshaw et al Clin. Exp Allergy. 2003;33(11):1581-5.

Food structure and composition affects behaviour in the gastrointestinal tract

MRI of chocolate dessert and chocolate bar digestion in health volunteers shows the high-fat bar delays gastric emptying, accounting for differences in development of symptoms between matrices.

Mackie et al Mol Nutr Food Res 2012 56:1708-14

Roasting also increases the threshold dose of hazelnuts

- Reactivity to roasted hazelnut is also reduced such that the threshold increased from 0.1g of “untreated” nut to 0.23g of roasted hazelnut

- However, thresholds doses vary widely and the changes in reactivity are not sufficient to make roasted hazelnuts safe for allergic individuals

Worm et al *Clin. Exp Allergy* 2009;**39**:159-166.

Does the food matrix affect allergenicity?

- *In vitro* digestion studies indicate allergen bioaccessibility is affected by the food matrix
  - Natural plant tissue matrices may be protective for food allergens
  - Processed food matrices are complex structures and not all “baked” matrices are equivalent
- There is a lack of clinical reactivity studies with well defined matrices showing whether *in vitro* behaviour is predictive of *in vivo* behaviour in affecting threshold dose or severity of reaction

The Team

Manchester University: Rebekah Sayers, *Frances Smith,* Phil Johnson, Justin Marsh, Anuradha Balasundaram, Aida Semic-Jusafagic, Angela Simpson, Adnan Custovic, Marina Themis, *Ivona Baricevic-Jones*, Victoria Lee, *Huan Rao,* Daniel Schäffer, *Angela Simpson,* Phil Couch, Iain Buchan, Chris Munro, Bushra Javed, *Hadeer Mattar,* Matt Sperrin

iFAAM collaborators: Sabine Baumgartner, Kathrin Lauter, Gavin O'Conner, Chiara Nitride, *Karine Adel Patient, Hervé Bernard,* Barbara Ballmer-Weber, Montserrat Fernandez-Rivas, Kirsten Beyer, Paul Turner, Audrey DunnGalvin, Jonathan Hourihane, Christine Parker

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