The influence of the food matrix on allergenicity* 

Christal Bowman  
National Center for Environmental Assessment  
US Environmental Protection Agency  
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Food matrix issues can affect:

- Detection and quantitation of proteins
- Processing alterations
- Digestibility assays in vitro
- Digestion and absorption in vivo
- Interactions with the immune system
Matrix effects: Digestibility
Enzymatic digestion

Complete digestion (labile)

Not a good target for immune system

“immunologic ignorance”

Semi-stable

Available for processing by antigen-presenting cells

Protein (antigen)

Stable

Dong et al., TOXICOLOGICAL SCIENCES 73, 8–16 (2003)

Clinical Effects
Asthma, rhinitis, bronchospasm
Eczema, urticaria, rash, edema
Nausea, vomiting, diarrhea
Anaphylactic shock

Mediators

Mastcell
Basophil

B-cell

T-cell

IL-4, IL-13

APC

IgE

Protein content

- High protein meals can overwhelm digestive enzyme levels
- Matrix may contain proteins with specific enzyme inhibition activity
Plant structures

- Cell walls may not be broken down in the upper GI tract
- Nut protein bodies – dry, relatively insoluble – hydration status may be deciding factor in allergenicity of some of these
Lipids

• In vitro pepsin digestion of $\alpha$-lactalbumin slowed by phosphatidylcholine (abundant surfactant in milk and produced by the stomach) – Moreno et al. 2005 J Agric Food Chem

• Phosphatidylcholine did not affect pepsinolysis of $\beta$-lactoglobulin, but protected from duodenal enzymes – Mandalari 2009 Mol. Nutr. Food Res.
Polysaccharides

- Gum arabic, pectin, or xylan at 50 wt% enhanced peptic digestion of $\beta$-lactoglobulin. Interaction with polysaccharides reduced protein aggregates/increased solubility – Mouecoucou et al. 2004, BBA

- Affects the size of peanut peptides obtained by two-step hydrolysis – Mouecoucou et al. 2004, Clin Exp Allergy
Polysaccharides cont’d

Pectin forms a gel at low pH

Effects of 3% or 6% pectin on fruit protein digestibility: -- Polovic et al. 2007 Clin. and Exp. Allergy.
In vitro digestion of crude and dialyzed kiwi extracts

Crude extract (CE) and extensively dialyzed extract (DE) of kiwi fruit in simulated gastric fluid

Polovic et al. 2007 Clinical and Experimental Allergy
The presence of a food matrix significantly impedes the transepithelial transport of allergens in vitro.

- no hazelnut extract
- with hazelnut extract

(aqueous extract)

Proteins in blood of rats after feed with and without hazelnut extract

- black: no hazelnut extract
- grey: with hazelnut extract

Lipids: long chain fatty acids enhance antigen absorption

Palmer et al. 2012 Brit Med J. epi study
Enterocoated beads release contents only at pH > 5.

Encapsulating protein protects against digestion; low hydration.

Method used with OVA to sensitize mice (Michael et al., Vaccine 1996)
Oral tolerance

• Immunologic hyporesponsiveness to specific antigen encountered via feeding
• Inhibits allergic responses to food
• Generally thought that soluble protein/antigen is better for inducing tolerance
Oral tolerance

1 week

Tolerance generated

Anergy or deletion = less T cell help for IgE response
Generation of Tregs = active suppression of Th and B cells

Bowman and Selgrade 2008 Tox Sci
Oral tolerance is no longer induced when ovalbumin is encapsulated.

Also demonstrated by JG Michael et al. 1996 Vaccine.

Bowman and Selgrade 2008 Tox Sci
Matrix pH may affect protein characteristics and therefore tolerance induction.

Chemical and heat denaturation also abrogate oral tolerance (Peng 1998).

Ovalbumin was aggregated and did not induce oral tolerance.

Bowman and Selgrade 2008 Tox Sci

OD 450 nm

0.0 0.1 0.2 0.3

NAIVE  OVA IP  5 MG OVA Oral,IP

in water, pH 3.0

no tolerance
Peanut becomes orally tolerizing with sodium bicarbonate treatment

Increased solubility?
or digestion inhibition?

Bowman and Selgrade 2008 Tox Sci
Matrix effects: stimulation of the immune system

- Saponin adjuvants; increased antigen uptake
  - Maharaj et al., 1986 but also act at a distance
- Plant sterols
- Liposomes, LPS, other lipids
- Lectins
The influence of lipids on IgE production in an injection hazelnut allergy mouse model

Mice exposed ip to partially purified hazelnut extract or recombinant (lipid-free) Ber e 1

Dearman et al. 2007; Clin and Exp Allergy
The influence of lipid fractions on IgE production in an injection hazelnut allergy mouse model

Dearman et al. 2007; Clin and Exp Allergy
Whole peanut extract stimulates an increase in cell number after footpad injection in mice

Processing

- Protein aggregates are generally more immunogenic
- Glycosylation via heating with matrix-supplied sugars
Detection

Need to understand exposure; relies on accurate determination of specific protein content

- Compatibility with standardized reference materials
- Matrix ‘quenching influence’ in immunoassays – varies with protein of interest and matrix tested
  - Van den Bulcke 2007 Eur Food Res Technol
- Potential cross-reactivity between assay antibody and other proteins
Recap of food matrix considerations

- Detection and quantitation of proteins
- Processing alterations
- Digestibility assays in vitro
- Digestion and absorption/handling in vivo
- Interactions with the immune system

Discussion

sugars  pH  fats  structure  other proteins  adjuvants