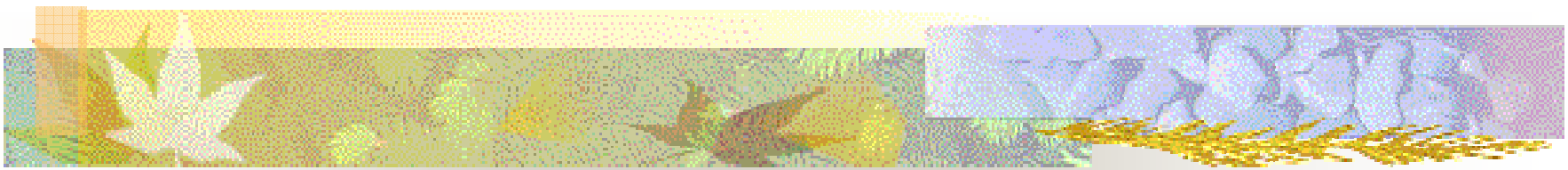
NCFST Allergen Research and Protein Digestibility Studies



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Food allergen research

- Successful management of food allergy can only be achieved by **strict avoidance of allergenic foods**.
- Identify and characterize allergenic proteins in foods
- Develop strategies to prevent inadvertent introduction of allergens in foods
  - Allergenicity assessment of novel proteins
  - Allergen control during food preparation and manufacturing

Allergenicity assessment of novel proteins

- Comparison of the properties of transgenic proteins with those of known allergens
  - Sequence comparison
  - Comparison of physicochemical properties
    - resistance to digestion
    - resistance to heat and other food processing conditions

NCFST Research-

Bioinformatics for food safety

Principal Investigator: Steve Gendel

- Develop high quality data resources-allergen sequence database
  - 64 animal food allergens
  - 390 plant food allergens
  - 645 non-food allergens
  - 38 proteins used in approved bioengineered foods
- Develop and validate methods for applying these resources for protein allergenicity assessment
- Advanced proteomic analysis
  - 3D structures
  - T-Cell and B-Cell epitope analysis

NCFST Research –

Physicochemical properties as criteria for protein allergenicity assessment

Principal Investigator: T. J. Fu

- Comparative studies to evaluate the predictive value of the following parameters as criteria for protein allergenicity assessment
  - **Digestive stability**
  - Acid stability
  - Heat stability

Allergen control during food manufacturing

- Undeclared allergens represent a major cause of all food recalls (36% in 1999)
- Equipment cross-contamination due to inadequate cleaning identified as a major cause of recalls (40%)

(Vierk et al, 2002)

NCFST Research –

Effects of cleaning on removal of allergens from food contact surfaces

Principal Investigator: Lauren Jackson

- Measure the efficacy of different cleaning protocols for removing allergens from food contact surfaces
- Evaluate methods for determining the efficacy of cleaning protocols
- Measure the extent of allergen cross-contact caused by the use of shared processing equipment or cooking media.

NCFST research-

Effect of processing on allergens

Principal Investigator: T. J. Fu

- To examine the effect of thermal processing on the structural and immunological properties of food allergens
- To determine the effect of processing on allergen detection in foods
- To identify treatment methods for the reduction/elimination of allergenic residues from processing equipment and food contact surfaces

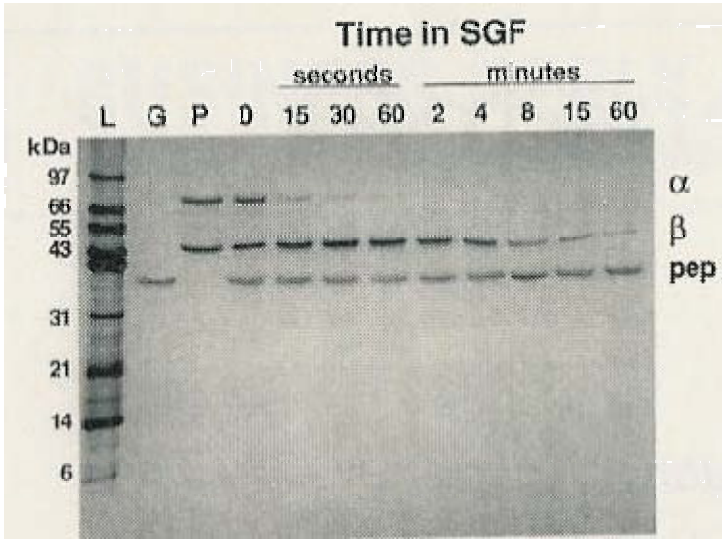
Digestive Stability as a Criterion for Protein Allergenicity Assessment

In Vitro digestibility as a predictive tool for protein allergenicity assessment: Validation

- Are food allergens more resistant to digestion in vitro than non-allergenic proteins?
- Can digestibility be used as a parameter to distinguish food allergens from non-allergenic proteins?
- Is there a ranking order relating protein digestibility to allergenic potential?

Stability of food allergens to digestion *in vitro* (Astwood et al., 1996)

- Simulated gastric fluid (SGF) as described in the United States Pharmacopeia
- SDS-PAGE for measuring protein degradation
- Stability was defined as the last time point for which proteins or proteolytic fragments could be detected



SDS-PAGE analysis of SGF digestion assay.

Soybean β-conglycinin subunits α and β.

Stability

α subunit = 30 sec

β Subunit = 60 min

Stability of food allergens in SGF and SIF (E/S = 10)

FOOD ALLERGENS				
Protein Group	**Protein Source**	**Allergenicity (%)**	**SGF Stability (min)**	**SIF Stability (min)**
Storage Proteins				
α-Casein	Cow's milk	56-100	0	0
β-Lactoglobulin B	Cow's milk	72	120	5 (5)
β-Lactoglobulin A	Cow's milk		0.5	0.5 (0.5)
BSA	Cow's milk	45	0 (120)	120 (120)
α-Lactalbumin	Cow's milk	14	0	-
Ovalbumin	Egg	100	5	5 (120)
Ovomucoid (Trypsin inhibitor)	Egg	62 – 70	0	60
Conalbumin	Egg	51 – 59	0.5 (15)	120 (5)
Trypsin inhibitor	Soybean	20	120	120 (120)
Ara h 1	Peanut	> 95	5	15 (60)
Ara h 2	Peanut	> 95	0.5	0.5 (0.5)
Patatin	Potato tuber	74	0	0.5
Plant Lectins				
Soybean lectin	Soybean	10	5	120 (120)
Peanut lectin	Peanut	50	5	120 (60)
Contractile Proteins				
Tropomyosin	Shrimp	82	0 (5)	0 (0.5)
Enzymes				
Lysozyme	Egg	0 – 44	60	120
Lactoperoxidase	Cow's milk	67	0	120
Papain	Papaya	-	0	120
Bromelain	Pineapple	-	0 (0.5)	120
Actinidin	Kiwi fruit	100	0	-

- Not determined

Fu et al. 2002. *J. Agri. Food Chem.* 50, 7154-7160.

Stability of non-allergenic proteins in SGF and SIF (E/S = 10)

NON-ALLERGENIC PROTEINS			
Protein Group	**Protein Source**	**SGF Stability (min)**	**SIF Stability (min)**
Storage Proteins			
α-Lactalbumin	Human	0	-
Zein	Corn	120	0.5
Trypsin inhibitor	Lima bean	120	Interference
Trypsin inhibitor	Bovine pancreas	120	120
Plant Lectins			
Red kidney bean lectin	Red kidney bean	15	120 (120)
Pea lectin	Pea	5	120 (120)
Lentil lectin	Lentil	0.5	120 (120)
Lima bean lectin	Lima bean	5	120
Jack bean lectin	Jack bean	15 (60)	120 (60)
Contractile Proteins			
Tropomyosin	Bovine	0 (0.5)	0 (0.5)
Tropomyosin	Chicken	0 (5)	0
Tropomyosin	Pork	0 (0.5)	0
Enzymes			
Pepsin	Porcine	120	0 (0.5)
Cytochrome c	Bovine heart	0	60 (60)
Lipoxidase	Soybean seed	0	120
β-Amylase	Barley	0	120
Rubisco[a]	Spinach leaf	0	Interference
Phosphofructokinase	Potato tuber	0	5 (60)
Sucrose synthetase	Wheat kernel	0	0.5 (60)

- Not determined

a D-Ribulose 1,5-Diphosphate Carboxylase

Fu et al. 2002. *J. Agri. Food Chem.* 50, 7154-7160.

Effect of changing the enzyme/test protein (E/S) ratio on measured stability

	Stability of Test Protein in SGF (min) Pepsin/Test protein ratio (by wt.)		
	10/1	**1/1**	**1/10**
Food Allergens			
β-Lactoglobulin B	120	120	120
Ovalbumin	5	60 (120)	120 (120)
BSA	0	0 (5)	0 (120)
Papain	0	0 (0.5)	0 (120)
Non-Allergenic Proteins			
Zein	60	60 (120)	120
Pea lectin	5	120 (60)	120 (120)
Cytochrome c	0	0.5	0.5 (5)
Sucrose synthetase	0	0	0 (120)

Fu et al. 2002. *J. Agri. Food Chem.* 50, 7154-7160.

How stable is stable?

- Digestibility of proteins as measured by SGF or SIF may be influenced by the ratio of enzyme: test protein used.
- Assessment of the allergenic potential is dependent on the interpretation of the *in vitro* digestion assay results.

The E/S ratio used for allergenicity assessment of bioengineered foods

PROTEIN	TRAIT MODIFIED	ENZYME USED	E / S RATIO	STABILITY MEASURED	REFERENCE
NPTII	Antibiotic resistant marker	Pepsin Pancreatin	1600 : 1 5000 : 1	< 10 s < 5 min	Fuchs et al. (1993)
ACCd	Delayed ripening tomato	Pepsin Pancreatin	800 : 1 2500 : 1	< 15 s < 22 h	Reed et al. (1996)
CP4 EPSPS	Glyphosate-tolerant soybean	Pepsin Pancreatin	1600 : 1 200 : 1	$T_{½}$ < 15 s $T_{½}$< 10 min	Harrison et al. (1996)
CRYIA (b)	Insect resistant Tomato	Pepsin	100 : 1	15 kD fragmen stable for 2 h	Noteborn et al. (1995)
Cry34Ab1 Cry35Ab1	Insect resistant maize	Pepsin	45 : 1 62 : 1	< 20 min < 5 min	Herman et al. (2003)
CRY9C	Insect resistant corn (StarLink)	Pepsin Pancreatin	20 - 64 : 1 60 - 200 : 1	> 2 h > 4 h	Noteborn (1998)

The E/S ratio used in nutrition studies: 1:10 - 1:100.

Interpretation of assay results

Systems	Trait modified	Stability in SGF	Stability in SIF	Conclusion made	Reference
NPTII	Antibiotic resistant marker	< 10 s	< 5 min	Rapidly degraded	Fuchs et al. (1993)
ACCd	Delayed ripening	< 15 s	< 22 h	Rapidly degraded	Reed et al. (1996)
CP4 EPSPS	Herbicide tolerance	$T_{½}$<15 s	$T_{½}$<10 min	Rapidly degraded	Harrison et al. (1996)
Soybean glycinin in transgenic rice	Improved nutrition value	< 10 min	< 30 min	Rapidly degraded	Momma et al. (1999)
CRYIA(b)	Insect resistance	15kD fragments stable for 2 h	Not done	Rapidly degraded	Noteborn et al. (1995)
Cry34Ab1 Cry35Ab1	Insect resistance	< 20 min < 5 min	Not done	Rapidly degraded	Herman et al. (2005)
Cry9C	Insect resistance	> 2 h	> 4 h	Stable	Noteborn (1998)

Summary

- The correlation between *in vitro* digestibility and protein allergenicity is not always present.
- The digestibility, thus the perceived allergenicity, may be greatly influenced by the relative amounts of enzyme and test protein used in the assay.
- A need exists to establish standardized assay conditions so that digestibility results can be compared between different labs.
- Criteria need to be established to relate *in vitro* digestibility to allergenic potential.
- Use of digestive stability for protein allergenicity assessment
  - May screen out stable proteins that may not cause food allergy
  - May miss food allergens that are labile to digestion
- Research is needed to understand the underlying mechanism of food allergy and to understand the relationship between protein digestibility and allergenicity.