Co-mingling of allergens in spices

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Overview

- Introduction to FARRP
- Introduction to food allergies
- Recent occurrences of allergens in spices
  - Peanut in cumin
  - Peanut in garlic
  - Almond in paprika and cumin
- Conclusions and recommendations

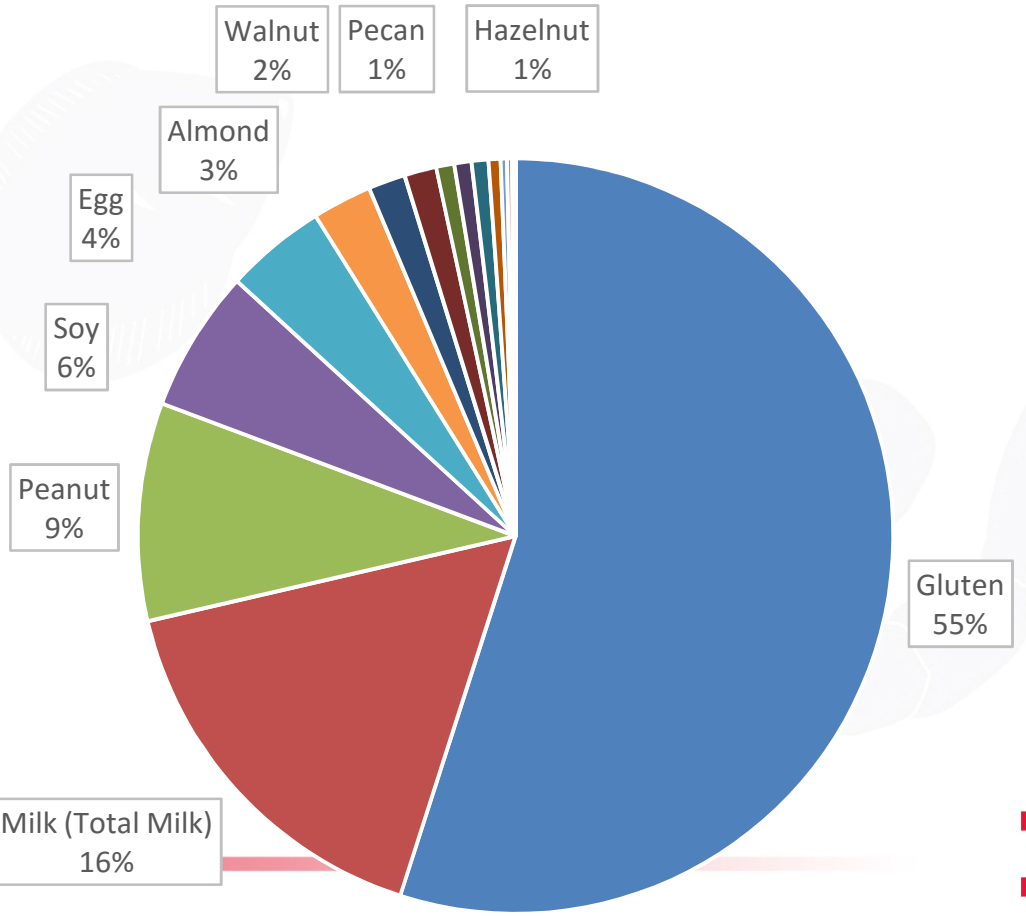
FARRP (www.farrp.org)

- Food Allergen Research and Resource Program
- Industry-oriented research and outreach program (>90 member companies)
- Sits within the Department of Food Science & Technology at the University of Nebraska, Lincoln
- Analytical lab (ISO 17025), expert advice, research

FARRP Analytical Laboratory

- Almond
- Buckwheat
- Cashew
- Clam
- Crustacea
- Egg
- Gluten
- Hazelnut
- Histamine
- Lupine
- Milk (Total Milk, β-Lactoglobulin, Casein)
- Mustard
- Peanut
- Pecan
- Pistachio
- Sesame
- Soy
- Walnut

FY 2014
43,942 Analyses Performed



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Food allergy

- Affects **1-3%** of the adult population of Western nations
- Reactions can occur at **low (mg) levels of allergen**, can be **fatal**
- Caused by particular **proteins** in foods
- No cure – **avoidance** only 'treatment'
- **Allergen labeling laws** in effect in many locales (including USA and EC). Foods on labeling lists vary but are all based on an initial Codex Alimentarius list

The ‘Big 8’

- Foods that contain or are derived from :

Milk	**Tree Nuts**
Eggs	**Peanuts**
Fish	**Wheat**
Crustacean Shellfish	**Soya**

Must be labeled as such in the US

- Account for most (**not all**) allergic reactions

The Risks of Uncontrolled Allergens

- **Regulatory risk** – undeclared allergens can lead to product recalls, FDA audits, etc.
- **Business risk** - loss of customers, law suits, failed audits (SQF, etc.), cost of product recalls, loss of consumer confidence, loss of retail space for products with your ingredients, allergen control/sanitation, down time, etc.
- **Health risk** – undeclared allergens can cause consumers to have reactions (some of which can be severe and even fatal).

Peanut in Cumin

November 2014 - current

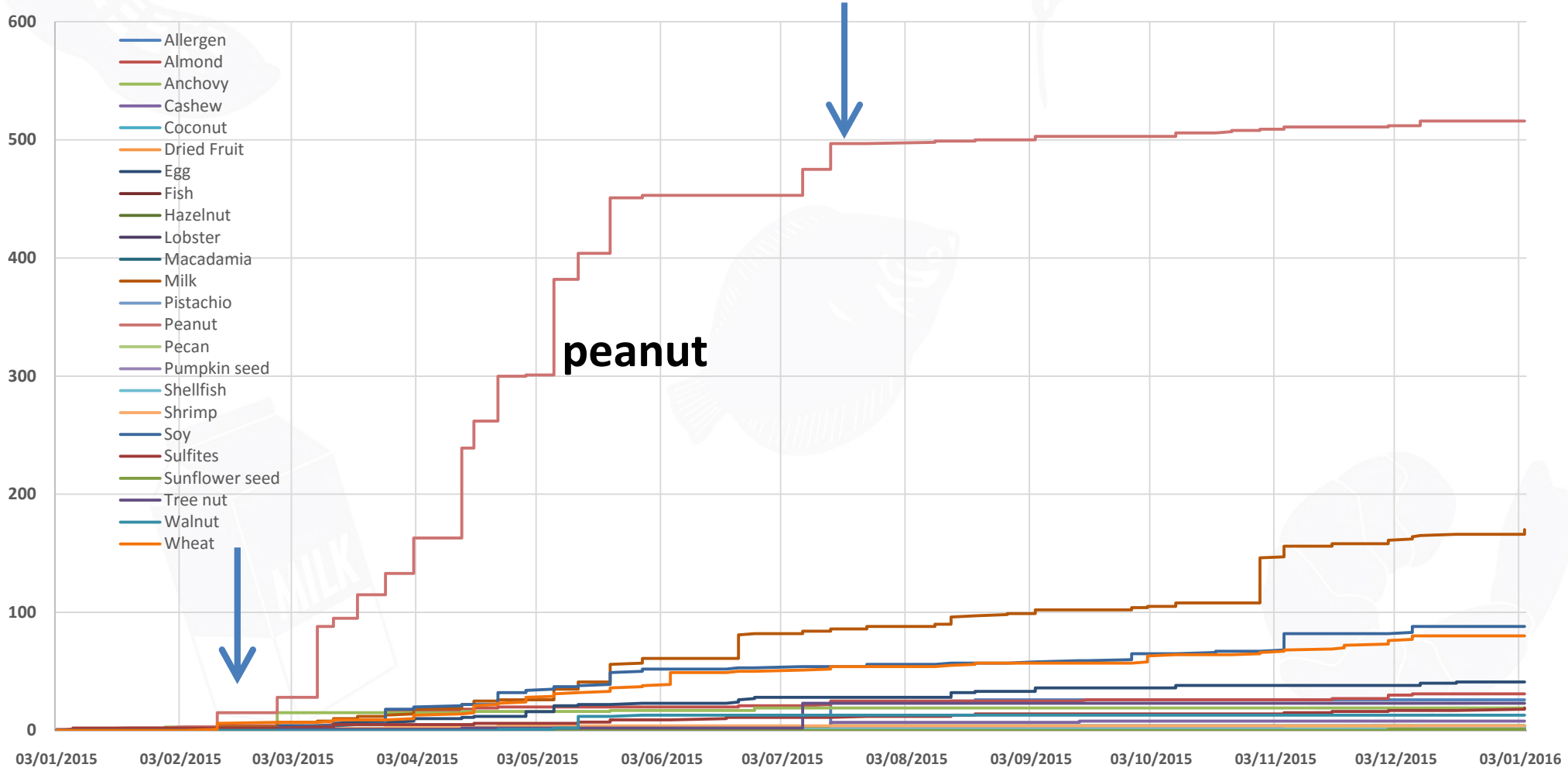
Initial Peanut in Cumin Situation

- **In November 2014** – a random retail analysis (CFIA) of a **taco seasoning product** was positive for peanut (and almond).
- FARRP analyzed retained samples of taco seasoning
  - Concentrations of peanut ranged from **1000 to >5000 ppm peanut** using several ELISA kits
  - Individual ingredients were then analyzed, cumin was found to be positive for peanut (>5000 ppm)
- A **recall** of taco seasoning and sauce was initiated

Initial Peanut in Cumin Situation

- **Late December 2014** – a second series of FDA and USDA-FSIS recalls initiated involving **well over 500 products and 30+ companies**
- Concentrations of peanut ranged from 100 to >5000 ppm peanut in final product, meaning levels of **50,000 to 105,000 ppm** peanut in the cumin.
- Ground cumin from sourced from Turkey was implicated in both instances
- FDA did receive **consumer reports of alleged allergic reactions** from peanut-allergic individuals
- FDA advised peanut allergics to **avoid cumin** and cumin-containing foods.

FDA 2015 Recall data



The Ongoing Cumin Situation

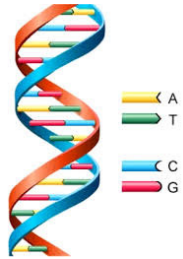
- Since the initial series of recalls involving cumin, many companies are testing for peanut residue in cumin and other spices.

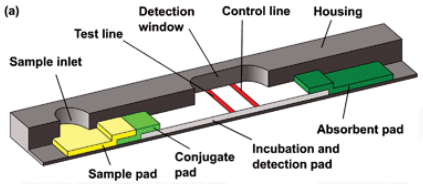
- **Random low level positives** have been found in whole cumin seed with no visible sign of whole or parts of peanut
  - Generally ranging between 5 and 25 ppm peanut

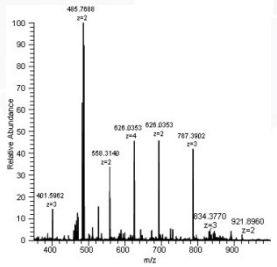
- Likely due to **incidental cross-contact due to agricultural commingling**

Testing for allergens in foods

- **ELISA** (Enzyme Linked Immuno Sorbent Assay use **antibodies** to detect the presence of one or more **proteins** specific to the allergenic food
- **PCR** (Polymerase Chain Reaction) use specific DNA primers to amplify and detect particular regions of **DNA specific** to the allergenic food.
- **LFD** (Lateral Flow Devices) 'Dipsticks' use **antibodies** to detect **protein** from the allergenic food
- **LC-MS/MS** or MS (mass spectrometry) – use MS to detect **peptides and peptide fragments** specific to the allergenic food.







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How well do the detection methods work for peanut in cumin or garlic ?

- FARRP initiated research as a response to **variable results** for detection of peanut in spices
- Investigated multiple commercially available methods (ELISA, PCR, lateral flow devices)
- Involved **multiple laboratories**, most ISO 17025 accredited.

A common problem with analysis

- Detecting an allergen in a water solution is easy
- The **food matrix** ('background') in which the allergen is present can **greatly effect the ability of our tests to detect allergens**
- Any **food processing** (e.g. heating) can also effect our results
- Spices often contain relatively high levels of **polyphenols** which can interfere with allergen detection
- Test methods are **validated** against certain types of food matrix. Most often these do not include spices.

Aim of the study

- Examine if **methodological variation can account for the diversity of analytical results**
- ***Not a laboratory assessment exercise***
- Publish results – laboratories blinded but methods unblinded (PCR methods often lab specific so will not be identified).
- Thanks to RSSL, IEH, Griffith Labs, Certified Labs, Olam Labs and Eurofins.

Study design

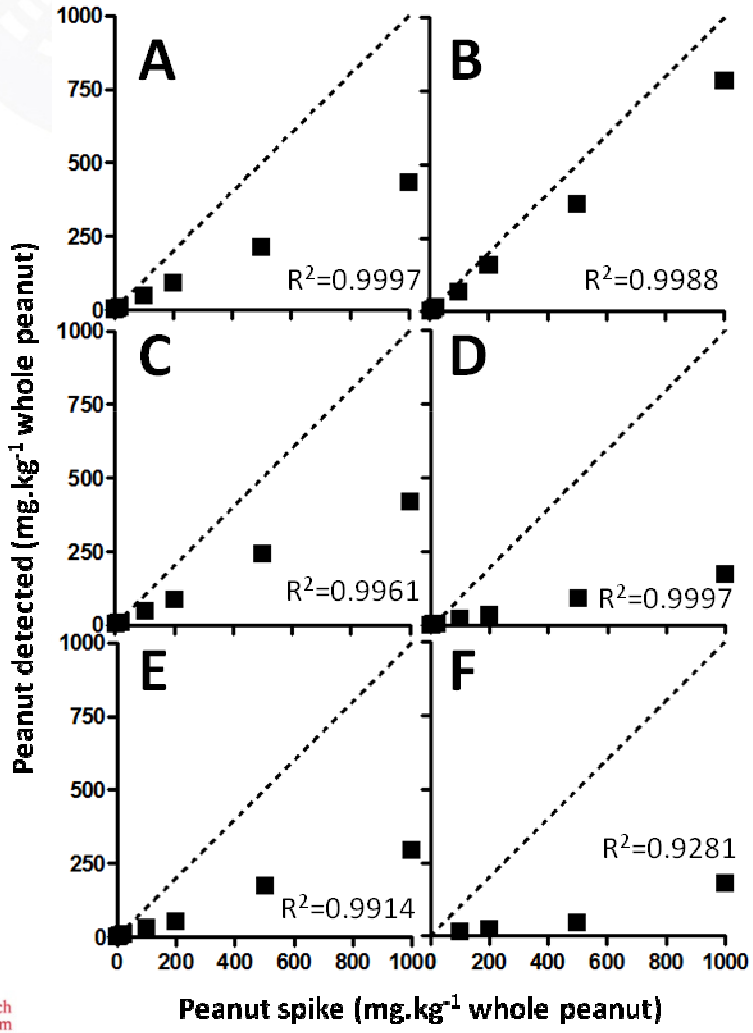
- Generate a series of peanut in cumin spikes containing **known amounts of peanut** (gravimetric).
- Sourced cumin from member company and thoroughly **analyzed to ensure no peanut was present**.
- Design spiking procedure to **maximise homogeneity of samples.**
- Test using a variety of quantitative and qualitative methods (commercial) - **multiple laboratories involved**. Methods represent those frequently used by industry.

Performance at 0 ppm peanut - cumin

- **No ELISA (6), lateral flow device (4) or PCR method (3) tested displayed positive (>BLQ) results in the 0 ppm peanut control.**

- In these controlled samples, **no reproducible issue with false positive results** was observed.

Quantitative ELISA



Peanut ELISA kit	Spiked whole peanut in cumin (mg.kg-1)		
	4	1000	200000
1	65.0 (317.5)	43.3 (235)	24.9 (179)
2	91.4	78.6	na
3	113.1	42.1	na
4	19.8	17.3	na
5	52.8	29.4	na
6	nd	18.1	2.2

- **Very kit-dependent recoveries (from 19.8 to 113.1 % at 4 ppm whole peanut).**

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Qualitative methods

Qualitative detection method	Concentration of whole peanut in cumin (mg.kg^{-1})	
	Lowest detected level	**Highest non-detected level**
Immunological methods		
LFD 1	2	0
LFD 2	10	4
LFD 3	200	20
LFD 4	4	2
PCR methods		
PCR 1	20000	2000
PCR 2	100	20
PCR 3	2	0

Peanut in garlic

- End 2015 began seeing positive (**typically 5-50 ppm but some higher**) peanut levels in garlic samples.
- Likely contamination **is not new**, but increased scrutiny of spices has led to detection.
- Affected products **mostly powdered garlic**.
- **Collaborative research** was organized by FARRP – same methodology to that employed for cumin

Peanut in garlic

- **Same experimental methodology** (though fewer analyses)
- **No observed false positive results with ELISA**
- Recoveries displayed **similar kit dependence** as observed with cumin

Peanut ELISA kit	Spiked whole peanut in garlic (mg.kg-1)		
	4	**1000**	**200000**
1	55	47.5	21.9
2	50	89.7	70.0
3	77.5	60.9	55.5
4	5.5	5.4	5.1
5	11.25	10.8	10.3
6	nd	nd	nd

Recovery of peanut from a powdered garlic matrix (%)

Conclusions – allergen detection in cumin and garlic

- **Commercial ELISA kits are capable of effective measurement** of peanut in both cumin and garlic :
  - **No false positive** results
  - Variable but reasonable recoveries

- Care should be taken to **validate qualitative methods** (PCR, LFD)

- **Recommend that labs use an in-house spike to ensure their testing methodology works for their matrix and analyte.**

Almond in Paprika & Cumin

- In November 2014 CFIA found **undeclared almond** in a taco seasoning together with undeclared peanut but peanut became the focus.
- Late December 2014/early January 2015
  - Retail cumin sample in **U.K.** tested positive for almond
  - Paprika tested positive for almond by **several ELISA methods**
  - Levels generally in the **50 to 100 ppm** range
  - Situation first developed in the U.K. but also observed in the U.S. and Canada

Almond in Paprika & Cumin

- **Mahlab** spice a potential source of positive almond results?
  - Made from ground cherry pit seeds
  - Cherry and almond belong to the genus – *Prunus*

- Proteins from closely related species could have sufficient **protein homology to cross-react** in ELISAs
- Mass spectrometry was able to distinguish between almond and cherry, resulting in the rescindment of several recalls.

Conclusions and recommendations

- Allergens in spices is an **ongoing issue**, we still see positive test results at FARRP.
- Current allergen tests work well for peanut in garlic or cumin but **validating your results using blank and spiked samples is best practice.**
- Cross-reaction can be an issue, but generally only when a closely related species is present (**not the case for peanut in cumin garlic**).
- If you test and observe a positive result, **ask for advice** – there have been cases where recalls are not necessary.
- Supplier evaluation is an important part of allergen control and **required under US law (FSMA)**.

Thanks....

- The WSC
- Labs which participated in the research
- FARRP consortium