

Allergens in spices workshop - ELISA Assays



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Science
for a safer world



Cumin & Paprika recalls – Referee Cases



- 1 • 31 Jan '15 FSA recall cumin - almond not listed on the label
- 2 • 10 Feb '15 FSA refer cumin sample to Government Chemist
- 3 • Elisa Testing
- 4 • March '15 supplier "...mahleb gives positive ELISA for almond"
- 5 • Protein by Mass Spectrometry
- 6 • 30 April '15 Canada rescinds recalls " ... mahleb false positives"
- 7 • DNA Testing
- 8 • 26 June '15 Govt Chemist confirms cumin contains mahleb
- 9 • 29 June '15 FSA rescinds cumin recalls "mahleb present; not almond"
- 10 • 13 Aug '15 FSA refer sample of paprika to Government Chemist
- 11 • 9 Nov '15 Govt Chemist confirms paprika contains almond

Cumin recalls



- Oct 2014 Canadian Food Inspection Agency random tests for allergens revealed undeclared peanut & almond protein in products containing cumin
- Widespread recalls of cumin products in N America (42 to 19 Feb 2015)
 - > one for **peanut and almond** starting in Autumn 2014, and a second larger one for **peanut only**, starting in December 2014
 - > Salsas, spices inc paprika & mixes, seasonings → meat products, hummus,
 - > None of the N American recalled products distributed in UK

Sayers *et al.*, 2016, How Much of a Problem Is Peanut in Ground Cumin for Individuals with Peanut Allergy? *J Allergy Clin Immunol*, 137, AB99, February 016, Abstract 467

Sources – Patrick Bennett, Allergic Living, USA, <http://allergicliving.com/2015/02/14/inside-the-peanut-tainted-cumin-recalls-what-happened/>

Seasoning and Spice Association Q&A

More + UK & Europe recalls



- Jan 31 2015 FSA recalled ground cumin with almond protein, on a precautionary basis
- February 2015 FSA issued two further recalls re undeclared almond protein in fajita meal/dinner kits and seasoning mixes
- Batch of paprika was the likely source (Santa Maria)
- Denmark, Sweden & Norway issued alerts / recalls
- None of the tests have detected peanut proteins at levels that would require allergen labelling (FSA)

Undeclared allergens RASFFs 1.1.15 – 1.3.15



24/02/15	GB	Peanut 41.3 mg/kg milk chocolate biscuits from UK
17/02/15	GB	Almond >18 mg/kg spice mix from Sweden, via UK
14/02/15	GB	Almond >18 mg/kg fajita dinner kit from Sweden
13/02/15	GB	Almond 306 mg/kg ground cumin from Turkey
12/02/15	GB	Almond 270 mg/kg fajita meal from Sweden
12/02/15	IT	Gluten in gluten-free milk chocolate bar from Italy
30/01/15	IT	Milk ingredient and celery in meat sauce from Italy
28/01/15	DK	Hazelnut and almond in peanut butter from the Netherlands
20/01/15	DK	Wheat in noodles from Thailand
13/01/15	SK	Soya >100 mg/kg candies from Hungary

Almond allergenic proteins – 8 ID'd & characterised



- PR-10 (Pru du 1),
- TLP (Pru du 2),
- prolamins (Pru du 2S albumin, Pru du 3),
- profilins (Pru du 4), 60sRP (Pru du 5), and
- cupin (Pru du 6, Pru du γ -conglutin), Amandin, primary storage protein Costa *et al.*, J Ag Food Chem 2012 60 (6), 1337-1349
- Although only a few tested for reactivity with almond-allergic sera
- **Amandin recovery from spices was typically low**, results suggest that food matrix effects as well as extraction conditions need to be carefully evaluated when developing immunoassays for amandin detection and quantification, Rashmi *et al.*, LWT - Food Science and Technology, 43, 2010, 675-683

Cumin standards & adulteration



Specification	Suggested limit
<i>ASTA cleanliness specifications</i>	
Whole insects, dead (No.)	4
Mammalian excreta (mg/lb)	3
Other excreta (mg/lb)	5
Mould (% by weight)	1
Insect-defiled/infested (% by weight)	1
Extraneous foreign matter (% by weight)	0.5
Ash (% max)	9.5
Acid-insoluble ash (% max)	1.5
<i>FDA DAL</i>	
Volatile oil (% min)	2.5
Moisture (% max)	9.0
Ash (% max)	8.0
Acid-insoluble ash (% max)	1.0
Average bulk index (mg/100 g)	240
<i>USFDA DAL</i>	
Sand and grit (AOAC 975.48)	Average of 9.5% or more ash and/ or 1.5% or more acid-insoluble ash



Adulterants

- Caraway
- Sand, grit & debris
- Milled foliage, straw, wheat & rice husks
- Starches
- Olive stones
- Essential oil can be adulterated with synthetic cumin aldehyde
- Peanut & tree nut by-product?
- ?????

Anjoo Kamboj (2012). Analytical Evaluation of Herbal Drugs, Drug Discovery Research in Pharmacognosy
 Parthasarathy, V. A., Chempakam, B., & Zachariah, T. J. (Eds.). (2008). *Chemistry of spices*. CABI
 Zhu H & Zhao M, 2014, Discourse J Agric Food Sci., 2, 264

Mahleb ...



Canadian Food Inspection Agency

Agence canadienne d'inspection des aliments



- Barts Ingredients Company Ltd, the manufacturer of the UK cumin recalled claimed (early March 2015) that mahleb, a little known nut, was possibly the origin of the ELISA almond positives
- 30 April 2015 CFIA rescinded ~ 25 product recalls ...
 - > "... new evidence regarding the cross-reactivity of mahleb, a spice obtained from a specific species (*Prunus mahleb*) of cherry seeds, with the almond allergen test kit. It is highly likely that the positive sample results for the ground cumin and cumin-containing products were due to mahleb contamination and not almond"
- Had been working on LC-MS/MS for peanut, applied method to almond and mahleb...
- Mahleb (also mahlab, white mahlab ,mahleb, English cherry, Rock cherry, St. Lucie cherry (*Prunus mahaleb* L. of the Rosaceae family, subfamily Prunoideae)

Mahleb (also mahlab, mahaleb, English cherry, etc... (Prunus mahaleb L.)



- Grown, for example in Turkey, where the flesh and seed of mahleb fruit are important.
- Mahlab puree, mahlab vine, mahlab flour and mahlab oil are produced from mahlab fruit. The mahlab seeds form an important source of protein and oil.
- The stones are cracked to extract the seed kernel, which is about 5 mm diameter ...

<http://www.sifali.org/wp-content/uploads/2013/01/mahlep.jpg>

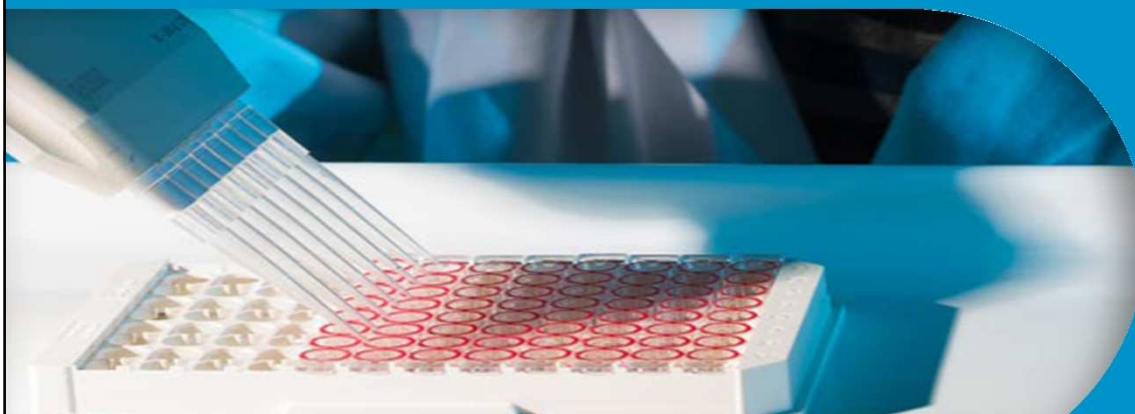
Özbey, A., et al. "Mahlab and mahlab products." *J Agric Faculty of Gaziosmanpaşa University* (2011).

Ercisli, Sezai. "A short review of the fruit germplasm resources of Turkey." *Genetic Resources and Crop Evolution* 51.4 (2004): 419-435.

leri, Francesca, Patrizia Pinelli, and Annalisa Romani. "Simultaneous determination of anthocyanins, coumarins and phenolic acids in fruits, kernels and liqueur of Prunus mahaleb L." *Food chem* 135.4 (2012): 2157-2162.



ELISA Assay



ELISA Assays



Kit Company	LoD or lowest non-zero Std	Further info on company website
ELISA Systems	Lowest std 0.5 ppm (?almond)	Further info available on request
R-Biopharm	Lowest std 2.5 ppm Limit of detection: 0.1 mg/kg (ppm) almond Limit of quantification: 2.5 ppm almond	For breakfast cereals, cookies, ice cream and chocolate. Cross reaction to apricot stone is > 100 %. No cross-reactivity to cashew nut, brazil nut, pecan, hazelnut, coconut, macadamia nut, walnut, chest nut, sunflower seeds, sesame and Lima beans.
Romer Labs	Quant. Range: 0.4-10 ppm LoD 0.2 ppm almond Recoveries (various matrices) 71 % - 110 %	Further info available on request, inc a full in-house validation, No cross reactivity to wide range foods inc. cumin, chilli powder, kofta mix, curry mix and tikka mix...
Neogen Europe	Range of quantitation: 2.5–25 ppm Controls provided: 0, 2.5, 5, 10 and 25 ppm almond	Quantitative analysis of almond residue in food products such as cookies, crackers, chocolate bars, ice cream and cereals

RIDASCREEN® FAST Mandel / Almond



- Sample preparation: homogenization, extraction and centrifugation
- Sample preparation..... approx. 20 min
- ELISA (incubation time) 30 mins
- Limit of detection: 0.1 mg/kg (ppm) almond
- Limit of quantification: 2.5 mg/kg (ppm) almond
- Standard Range: 2.5mg/kg – 20mg/kg
- Sample required for analysis: 1g
- Specificity: The antibodies specifically detect proteins from almonds
- Cross reaction to apricot stone

AgraQuant® Almond



- Sample preparation: homogenization, extraction and centrifugation
- Sample preparation..... approx. 25 min
- ELISA (incubation time) 60 mins
- Limit of detection: 0.2 mg/kg (ppm) almond
- Limit of quantification: 0.4 mg/kg (ppm) almond
- Standard Range: 0.4mg/kg – 10mg/kg
- Sample required for analysis: 1g
- Specificity: No Cross Reactivity to: Wheat, Barley, Rye, Oats, Buckwheat, Corn, Rice, Soy, Poppy seed, Sunflower seed, Cashew nuts, Sesame, Peanut, Walnut, Coconut, Brazil nut, Chestnut, Cocoa, Milk, Lecithin, Gelatin, Apricot, Peach, Egg, Plum, Hazelnut, Pistacio, Pecan nut, Cherry

ELISA Systems Almonds ESARD-48



- Sample preparation: homogenization, extraction and centrifugation
- Sample preparation..... approx. 15 min
- ELISA (incubation time) 35 mins
- Limit of detection: ? mg/kg (ppm) almond protein
- Limit of quantification: 0.5 mg/kg (ppm) almond protein (2.4 mg/kg Almond)
- Standard Range: 0.5 – 5.0 ppm almond protein (2.4 – 24 ppm Almond)
- Sample required for analysis: 5g Smaller sample volume may be used as long as the 1(sample) + 10 (extraction buffer) ratio is maintained.
- Specificity: no information given



Case study 1: Cumin



Experimental plan



Sample appearance - ground cumin, 21.5 g

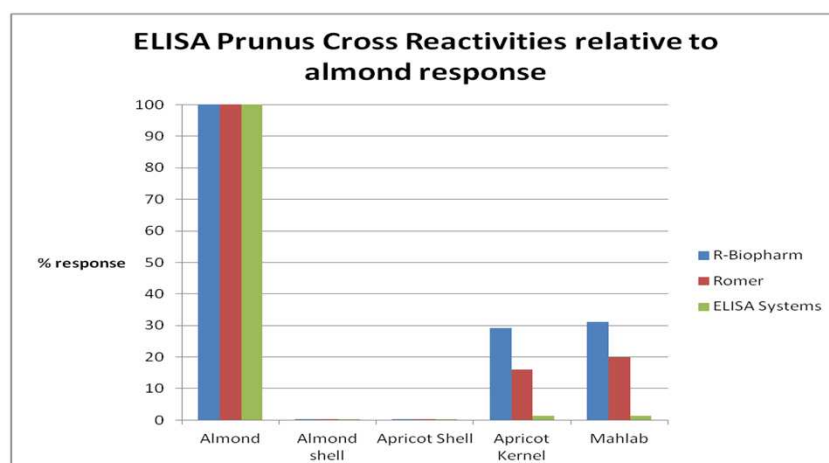
- 3 ELISA Kits
- 3 replicates of sample
- Blank Cumin
- Mahleb
- Cumin spiked with Almond at various levels
- Cumin spiked with Mahleb at various levels
- Almond Shell
- Apricot Shell
- Apricot Kernel

Control Samples



Sample	Brand and Purchased from	Variety	Country of origin	Batch
Organic cumin seeds	Just Ingredients (via Amazon)	Unknown	Unknown	DO-04-2014-H
Mahleb (1) (Ground)	Steenbergs (via Amazon)	Unknown	Lebanon	17023
Mahleb seeds	Supplied by FSA	Prunus mahaleb	Turkey	L131217
Almonds in shell (1)	BuyWholeFoodsOnline (Via Amazon)	Unknown	Australia / USA	Best Before: 13 July 2015
Whole almonds	Neal's Yard Wholefoods, Holland and Barrett	Unknown	USA	15071 09:15 2 90209
Apricots	Asda	Bebeco	South Africa	716975

ELISA cross reactivity



Replicates n =3, on one day, including milled apricot and almond shells

ELISA Results for cumin referee sample



ELISA Platform:	AgraQuant	ELISA Systems	RIDASCREEN
Presumptive Prunus protein mean result mg kg ⁻¹ (n =3) expressed as almond	11.1	1.4	3.3
<i>U</i>	1.1	0.1	0.4
Prunus protein <i>not less than</i> mg kg ⁻¹ expressed as almond	10.0	1.3	2.9
LoQ	0.4	0.5	2.5

Overall ELISA findings in cumin referee sample



Technique	Almond kernel	Mahleb kernel
Commercial ELISAs (n = 3 x 3 = 12)	<i>A Prunus species protein was detected above the limit of quantification of three assay platforms</i>	

Case study 2: Almond in paprika



Cumin / Paprika – almond / mahleb

FSA asked us to look at Paprika

- Formal Sample
- Analysed by Public Analyst using ELISA
- Reported as containing Prunus protein
- Hence retained portion obtained
- Consignment had not entered UK food chain
- Similar experimental plan to cumin ...

Experimental plan



Sample appearance – paprika powder 160g

- 2 ELISA Kits
- 3 replicates of sample
- Blank paprika
- Paprika spiked with almond at various levels
- Paprika spiked with mahleb at various levels
- Paprika spiked with apricot kernel at various levels
- Paprika spiked with peach kernel at various levels

Paprika ELISA results



ELISA Platform:	RIDASCREEN	AgraQuant
Presumptive Prunus protein mean result mg kg ⁻¹ (n =3) expressed as almond	590	390
<i>U</i> *	± 110	Not available
Prunus protein <i>not less than</i> mg kg ⁻¹ expressed as almond	470 [§]	Not available

U: Expanded measurement uncertainty expressed as a 95 % confidence interval

Between-run variation and other associated sources of uncertainty have not been included.

§ Rounded outwards to 2 significant figures

Summary ELISA Paprika Results



Technique	Almond kernel	Mahaleb kernel
Commercial ELISAs (n = 2)	Prunus species protein detected About 400 – 700 mg kg ⁻¹	

<https://www.gov.uk/government/news/paprika-referee-sample-further-testing-identifies-almond-present>

Conclusions



Conclusions



- **ELISA is a good screening technique for Prunus**
- **Cost effective and quick**
- **But confirmation by PCR or LC-MS/MS is required**

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