

IMMUNOASSAYS FOR PEANUT DETECTION IN FOOD SAMPLES

Anders Eriksson, Birgitta Kruse, Linda Strömbeck, Ingrid Malmheden Yman
National Food Administration, Box 622, SE 751 26 Uppsala, SWEDEN

Introduction

Peanut proteins account for more than one third of the most severe allergic reactions to food in Sweden. Typical foods where peanuts might cause a problem for the allergic individual are bakery products, chocolate and ice cream. Recently also Asian foods account for a growing number of reactions to peanuts. Accurate and sensitive methods for peanut are thus of major concern in food quality control programmes.

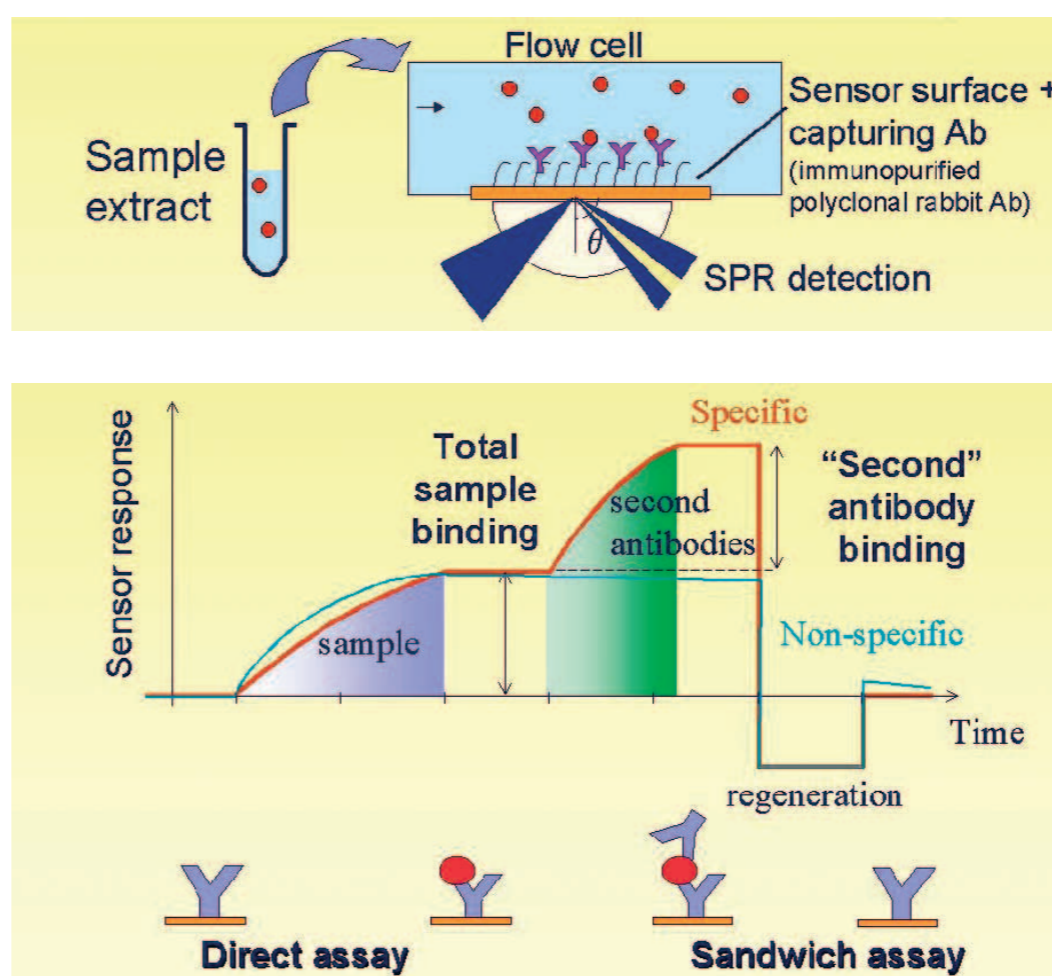
Peanuts in food are normally detected with immunological methods using peanut specific antiserum. We have raised a rabbit polyclonal antiserum against a protein fraction from ground non-roasted peanut kernels.



Specificity of the antiserum to peanut

The antiserum was tested for specificity in a biosensor-based assay (Biacore®) both in a direct and in a sandwich (enhancer) assay against other legumes as well as against different tree nuts. For the biosensor analysis peanut specific antibodies were immunosorbent purified from the serum by running it over a High trap column to which the peanut fraction was bound. The cross-reactivity of the antiserum against soy was less than 0.5 per mille in the sandwich assay.

Peanut biosensor assay - Direct and sandwich assay



Cross-reactivity comparison [nut analysed at 100% level]

(ppm peanut)	Direct binding assay	Sandwich assay
Hazelnut	17	0.4
Walnut	65	0
Pecan	60	0
Cashew	35	0
Pistage	51	0
Brazil nut	35	0
Nutmeg	>100	0
Almond	58	3.8
Sesame	25	0
Peanut	110000	170000
Coconut	69	1.6
Pine kernel	23	1
Kidney bean	12	0.5
Soja isolat	>100	49
Black eyebean	33	0
Red lentil	53	0
Green pea	72	0
Lupin	64	0
Chick pea	56	0
Soja bean	>100	27

Adapted from H. Jonsson, Biacore AB, Uppsala, 2002.

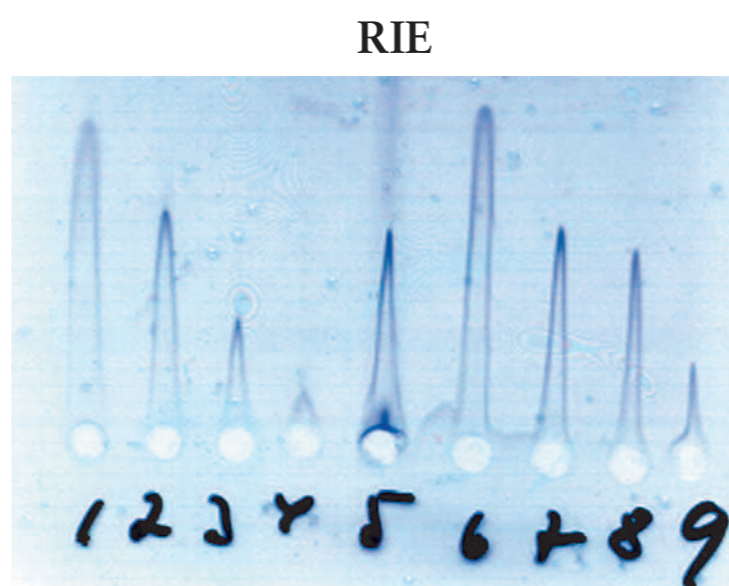
Results

Peanut detection in foods

The detection level of peanut protein in chocolate was determined in model samples, produced by melting different amounts of peanut butter into a milk chocolate matrix at 70°C. Peanut butter contains about 25% of protein and the dilution series were calculated to contain from 1.25% peanut protein down to 0.0000375% peanut protein. The lowest amount corresponds to 0.38 ppm peanut protein or 1.5 ppm peanuts.

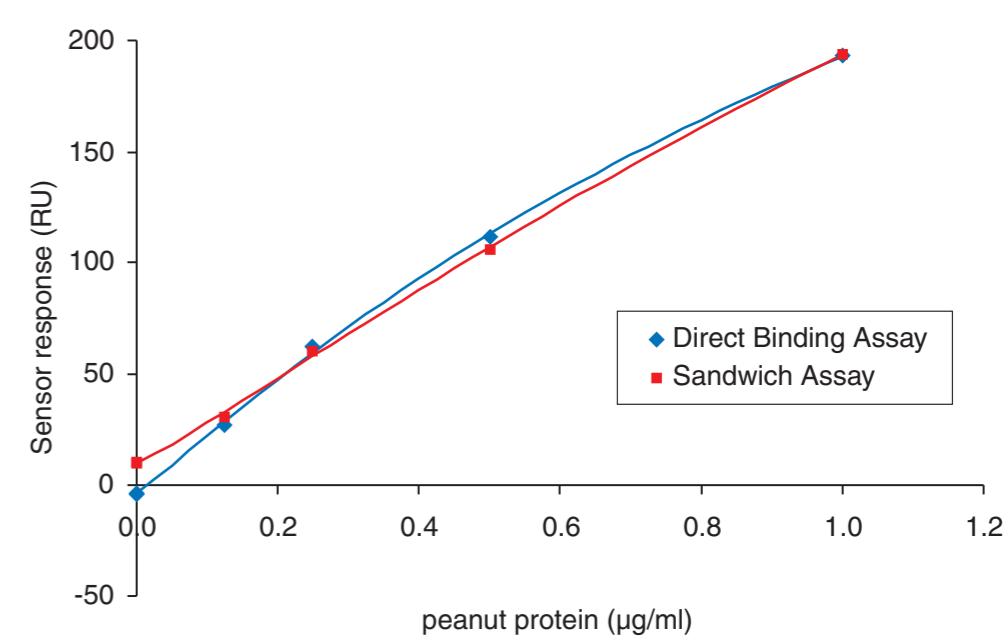
RIE

By rocket immunoelectrophoresis peanut protein down to 70 ppm was detected.



Rocket immunoelectrophoresis. Wells 1 to 4 is the standards 250 ng, 125 ng, 63 ng and 32 ng peanut protein, well 5 contains a reference sample (peanut in chocolate), wells 6 to 9 is dilution of a food sample "Wok" not declaring peanut but containing around 0,3% peanut protein.

Biosensor calibration curve



Biosensor

By using the same antibody in the biosensor assay, levels down to 1 ppm gave a clear signal both in the direct assay as well as in the sandwich assay.

Comparison of methods

The model chocolates were also analysed with two commercially available peanut test kits (R-Biopharm® Ridascreen and Tepnel BioKits®). The results were compared to those obtained with RIE and the biosensor assay. The Biokits assay detected levels of peanut in the same level as the biosensor while the immunoassay from R-Biopharm was somewhat less sensitive.

Comparison of four methods for the measurement of peanut protein in chocolate

Sample	Peanut butter	Chocolate	Calculated amount of peanut protein (ppm)	RIE peanut protein (ppm)	Biosensor peanut protein (ppm)	BioKits EIA peanut protein (ppm)	Ridascreen EIA peanut protein (ppm)
1	0.5 g	9.5 g	12500	13020			
2	0.1 g	9.9 g	2500	3250			
3	0.05 g	9.95 g	1250	1630			
4	0.025 g	9.975 g	625	740			
5	0.0125 g	9.9875 g	310	350		226	
6	0.062 g	9.9938 g	150	70	75	133	80
7	5 g of sample 6	5 g	75		38	53	42
8	5 g of sample 7	5 g	37.5		17	26	19
9	1 g of sample 8	9 g	3.75		1.0	2.35	1.7
10	1 g of sample 9	9 g	0.375		0.07	0.125	n.d.