# An allergen specific enzyme-linked immunosorbent assay (ELISA) for detection of hidden hazelnut in foods

Stanislav Trashin[a,b], Tatiana Cucu[a,b], Bart Devreese[b] and Bruno De Meulenaer[a]

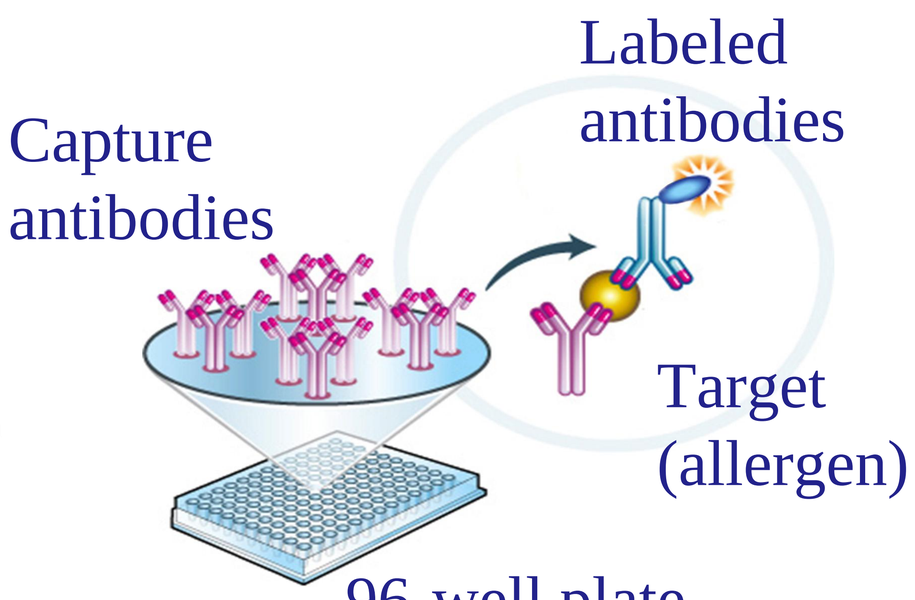
[a]Department of Food Safety and Food Quality, [b]Department of Biochemistry and Microbiology

## Introduction

Tree nut allergy represents a life critical health problem. Sensitized individuals run risk of being killed due to unlabeled allergen contaminations in foods like cookies, chocolate, ice cream, oils *etc*. For this reason, reliable analytical methods for sensitive and accurate detection of the allergens have to be developed. ELISA is widely used in routine analysis and can provide a detection limit less than 1 ppm in complex food matrixes. Meanwhile antibodies with stronger binding constant to its antigen are expected to give a better detection limit (a lower $IC_{50}$ value), sensitivity and specificity.

**Strategy for the development of a new allergen specific ELISA:**

- **Isolation and purification of *Cor a 9*, a major hazelnut allergen**
- **Development and isolation of specific antibodies, labeling**
- **Optimization of ELISA**
- **Test of food samples, verification**




Principle of sandwich ELISA

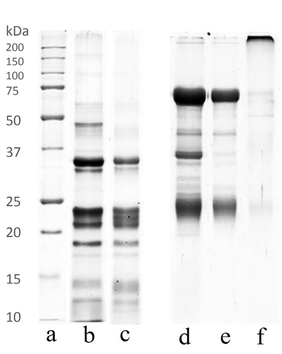



Figure 1. SDS PAGE in reducing conditions of hazelnut crude extract (b), purified *Cor a 9* (c), chicken antibodies IgY isolated (d), purified (e) and coupled with HRP (f). Trek (a) represents molecular weight markers.

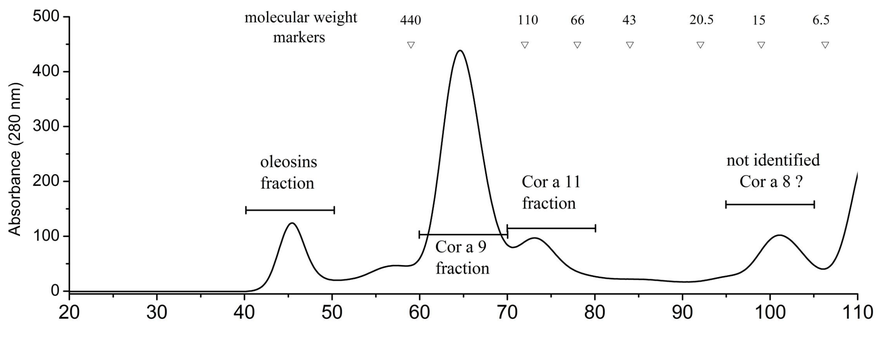



Figure 2. Gel permeation chromatogram of hazelnut crude extract. Peak positions for weight markers are shown on top.

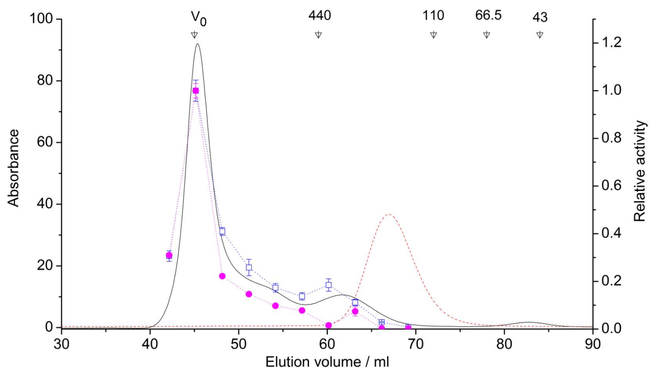



Figure 3. Gel filtration chromatograms of purified IgY (dashed line), IgY-HRP conjugate (solid line), relative enzymatic activity (open squares) and ELISA activity (maximal absorbance multiplied to dilution factor, filled circles) of 1 ml fractions.

✓ Isolation and purification were performed using gel-filtration chromatography (figure 2 and 3). Purity of the products was controlled by SDS-PAGE (figure 1) and MALDI MS. Unfractionated antibodies-enzyme conjugate showed the best results in ELISA comparing with separate fractions collected from gel filtration (figure 3).

✓ Dilution of capture and labeled antibodies were optimized to ensure an appropriate absorbance and background signal. Optimized ELISA showed beneficially low LOD, LOQ and $IC_{50}$ values (table 1). Effect of pH, ionic strength (table 2) and urea has been studied. It has been shown that ELISA can be used in wide range of pH and salt concentrations, however calibration curve should be obtained in the same buffer for more accurate analysis. No impact of 0.1 M urea and only slight impact for 1 and 3 M urea was observed.

✓ ELISA was tested with cookies spiked with hazelnut protein in dough before baking processing (table 3). Additionally the matrix effect of cookies extracts was studied by spiking of the extracts of blank-control cookies (table 4). Spiking before cooking revealed possibility to detect hazelnut in processed food since it is known that processing can effect dramatically on delectability of allergens but not on they allergenicity.

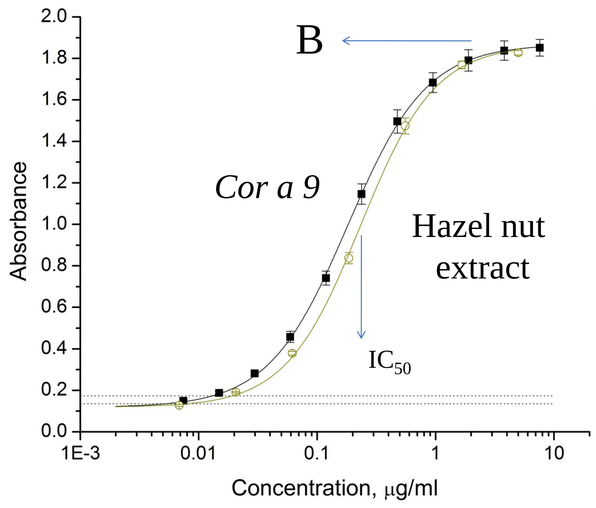



Figure 4. Calibration curves for *Cor a 9* and hazelnut protein extract obtained with the developed *Cor a 9* specific EILSA. Doted lines show absorbance for LOD and LOQ.

Table 1. The ELISA characteristics

| | *Cor a 9* | protein extract |
|---|---|---|
| Coefficient of variance, % | 5-10 | 5- 10 |
| $IC_{50}$, µg/ml | 0.18 | 0.23 |
| Limit of detection, ng/ml | 5 | 8 |
| Limit of quantification, ng/ml | 13 | 21 |

Table 2. Influence of pH and ionic strength on parameters of the developed ELISA. The same upper index marks statistically equal values.

| parameter | Citrate buffer pH 3 | 4 | 5 | 6 | Phosphate buffer pH 6.5 | 7.4 | 8.5 | Borate buffer pH 9 | 10 | 11 | $Na_2HPO_4$ pH 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B / a.u. | <0.2 | $1.33^a$ | $1.46^b$ | $1.73^c$ | $1.67^c$ | $1.90^d$ | $1.94^d$ | $1.90^d$ | $1.87^d$ | $1.73^e$ | $1.70^e$ | $1.43^f$ |
| $IC_{50}$ / µg $ml^{-1}$ | - | $0.54^a$ | $0.11^b$ | $0.10^b$ | $0.09^b$ | $0.10^b$ | $0.11^b$ | $0.15^c$ | $0.25^d$ | $0.79^e$ | $0.31^f$ | $3.26^g$ |

| parameter | Ionic strength / M 3 | 1.5 | 0.5 | 0.15 | 0.05 | 0.015 |
|---|---|---|---|---|---|---|
| B / a.u. | $1.71^a$ | $1.76^{a,b}$ | $1.94^b$ | $1.88^b$ | $1.78^{a,b}$ | $1.45^c$ |
| $IC_{50}$ / µg $ml^{-1}$ | $0.40^a$ | $0.24^b$ | $0.21^{b,c}$ | $0.15^{c,d}$ | $0.14^d$ | $0.20^e$ |

**Series of nuts and possible food ingredients did not show noticeable cross-reactivity:** almond, brazil nut cacao, cashew, chickpea, coconut, flour, macadamia, ovalbumin (eggs), peanut, pecan, pine nut, pistach, poppy seeds, pumpkin seed, red bean, sesame seeds, sunflower seeds, walnut, wheat protein, whey protein (milk), white bean

Table 3. Recovery of *Cor a 9* from cookies spiked before baking with protein extract, defatted powder and with protein extract when 10 % of sucrose in dough was replaced with lactose.

| mg protein spiked for kg of dough (ppm) | | Dilution for ELISA | Found concentration of *Cor a 9* ± error (n=3, p=0.9), ng/ml | Recovery for dry matter ± error (n=3, p=0.9), % |
|---|---|---|---|---|
| 0 | | 1 | < LOD | - |
| 0 | | 3 | < LOD | - |
| 1 | protein extract | 3 | 23.9 ± 3.2 | 65.3 ± 8.6 |
| 10 | | 10 | 65.2 ± 3.7 | 58.8 ± 3.3 |
| 50 | | 50 | 68.3 ± 4.8 | 61.9 ± 4.4 |
| 100 | | 100 | 75.5 ± 5.9 | 67.8 ± 5.3 |
| 1 | defatted powder | 3 | 20.8 ± 2.6 | 56.0 ± 7.2 |
| 10 | | 10 | 65.1 ± 5.6 | 58.4 ± 5.1 |
| 50 | | 50 | 87.2 ± 15.1 | 77.1 ± 13.4 |
| 100 | | 100 | 80.8 ± 19.5 | 71.1 ± 17.2 |
| 10 | protein extract + lactose | 10 | 45.2 ± 5.2 | 39.2 ± 4.5 |
| 100 | | 100 | 53.9 ± 4.5 | 48.0 ± 4.0 |

Table 4. Recovery of *Cor a 9* from control cookies extracts spiked with *Cor a 9* solutions, effect of dilutions and buffer composition.

| µg protein spiked for ml of extracts | ppm equivalent | Dilution for ELISA | Found Cor a 9 in the solutions, µg/ml | Recovery, % |
|---|---|---|---|---|
| Extracts are prepared with 20 mM Tris-HCl buffer pH 8.2 and diluted like for cookies | | | | |
| 0 | 0 | 1 | 0 | - |
| 0.1 | 1 | 3 | 0.032 | 95.7 |
| 1.0 | 10 | 10 | 0.112 | 112.4 |
| 5.0 | 50 | 50 | 0.127 | 126.8 |
| 10.0 | 100 | 100 | 0.137 | 136.7 |
| Extract are prepared with 20 mM Tris-HCl buffer pH 8.2 | | | | |
| 0.11 | 1.1 | 1 | 0.067 | 60.3 |
| 0.037 | 0.37 | 1 | 0.023 | 62.4 |
| 0.012 | 0.12 | 1 | 0.007 | 53.1 |
| Extract prepared with phosphate buffered saline pH 7.4 + 5 mM urea | | | | |
| 0.1 | 1 | 1 | 0.087 | 86.9 |
| 0.033 | 0.33 | 1 | 0.038 | 115.0 |
| 0.011 | 0.11 | 1 | 0.016 | 146.0 |

Conclusion: A new allergen specific ELISA for detection of hazelnut traces has been developed and tested. The limits of detection and quantification were at least several times lower than these for available commercial kits (E.Garber, Anal Bioanal Chem, 2010). Absence of cross reactivity with large series of food components and good recovery from cookies after baking demonstrated beneficial proprieties of the ELISA developed.

Financial support from Belgian Science Policy within post-doc fellowship and ALLERRISK project is gratefully acknowledged