



Mechanisms behind matrix–protein interactions influencing receptor-based and chromatographic detection of food allergens: A case study with a fruit based snack



Tatiana Cucu, Nathalie De Muer, Stanislav Trashin, Bruno De Meulenaer*

Department of Food Safety and Food Quality, Research Group Food Chemistry and Human Nutrition (Member of Food2Know), Ghent University, Coupure Links 653, B-9000 Gent, Belgium

ARTICLE INFO

Article history:

Received 8 January 2014
Received in revised form
7 July 2014
Accepted 11 August 2014
Available online 20 August 2014

Keywords:

Food allergens
Matrix effect
ELISA
HPLC
False positive
False negative

ABSTRACT

Food allergies represent an important food safety problem. The only effective treatment for food allergies is the complete avoidance of the offending foods or foods containing undeclared traces of allergens. In order to verify whether foods are contaminated with allergens reliable detection methods are needed. Currently enzyme-linked immunosorbent assays (ELISA) especially commercial ones are routinely used by the food industry and enforcement agencies. However, the reliability of such receptor based methods can be affected by the matrix in several ways. In this study we showed that the proteins/allergens can strongly bind to the matrix making them inefficient for the extraction as proven by a high-performance liquid chromatography method. On the other hand, especially in the case of ELISA methods, the matrix can non-specifically bind to the antibodies leading to false positive results. In conclusion, utmost care should be taken when interpreting the results of the methods used for analysis of traces of food allergens especially when important decisions about the acceptance or removal of the products from the market should be taken.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Food allergies represent an important food safety problem especially in industrialized countries affecting about 2% of the adult population and 8% of the children (Poms & Anklam, 2004). The only treatment available for food allergies is the complete avoidance of the offending foods. In order to help the allergic consumers the EU Directive 2007/68/EC specifies a number of foods and products thereof (cereals, crustaceans, eggs, fish, peanuts, soybean, milk, tree nuts, celery, mustard, sesame, lupine and molluscs) which should be declared on the label whenever deliberately added as an ingredient (European Parliament and Council, 2007). Unfortunately due to the growing complexity of food formulations and food processing, foods may be unintentionally contaminated with allergen-containing ingredients. The presence of traces of allergens might be a result of the use of contaminated raw materials, use of shared equipment, inefficient cleaning of the shared equipment,

rework and inappropriate storage conditions. The only way to verify whether foods are free of traces of allergens is by using reliable analytical methods which would allow accurate detection in the raw materials and end products.

Nowadays, the enzyme-linked immunosorbent assay (ELISA) is most frequently used in routine food analysis. The ELISA methods are based on specific molecular interactions between the antibodies used in the assay and the analyte, in this case allergens/proteins. The extensive use of ELISA methods for allergen detection is based on the fact that they are relatively cheap, easy to perform, highly sensitive with a large number of commercial assays available on the market. The commercial assays are used not only by the food industry but enforcement agencies as well. This indicates that important decisions, such as allowance or withdrawal of the products from the market are taken based on results obtained using such methods (Poms & Anklam, 2004). Unfortunately, the robustness of such receptor based analytical methods depends highly on the matrix analyzed and moreover it seems to be severely affected upon processing. Previous data indicate that commercial kits used to detect food allergens in especially processed foods are prone to false negative results (Cucu, De Meulenaer, & Devreese, 2012; Cucu

* Corresponding author. Tel.: +32 92 64 61 66; fax: +32 92 64 62 15.
E-mail address: Bruno.DeMeulenaer@UGent.be (B. De Meulenaer).

et al., 2011; Downs & Taylor, 2010; Fu, Maks, & Banaszewski, 2010; Platteau et al., 2011; Scaravelli, Brohee, Marchelli, & van Hengel, 2009). The results obtained were moreover kit dependent indicating that one ELISA kit cannot be used for all applications and that careful evaluation of the results obtained should be made. Further, the presence of the matrix can have an important impact on the analytical result as well. On the one hand because of the non-specific binding of the matrix components with the employed antibodies (Cucu, Devreese, Kerkaert, et al., 2012; Cucu, Devreese, Trashin, et al., 2012; Husain, Bretbacher, Nemes, & Cichna-Markl, 2010; Platteau et al., 2011) and on the other hand because of the interaction of the analytical target with the matrix especially during processing which makes their extraction inefficient (Schmitt, Nesbit, Hurlburt, Cheng, & Maleki, 2010). It is therefore of outmost importance that before using receptor based methods for routine analysis, a thorough evaluation of their reliability in different product matrices is performed (Abbott et al., 2010). The aim of this paper was to show how matrix can have an impact on the detection of allergens. This was performed on a fruit based snack which was tested positive for cow's milk using a commercial "RIDASCREEN β -Lactoglobulin" kit from R-Biopharm. The detected concentration of β -lactoglobulin (β -LG) was 0.27 $\mu\text{g/g}$ which was slightly higher than the detection limit of the kit (0.20 $\mu\text{g/g}$ β -LG corresponding to 2 $\mu\text{g/g}$ milk protein). To the authors knowledge, on the whole site of the factory, there was only one milk containing ingredient present (caramel – powder) which, based on the information obtained from the producer, was not used in the production of the snack and of which it is unlikely that cross-contamination would occur.

2. Materials and methods

2.1. Materials

Chemicals and standards of analytical grade were purchased from Sigma-Aldrich (Bornem, Belgium), VWR (Leuven, Belgium) and Acros Organics (Geel, Belgium). Sodium caseinate (Miprodan 30) containing 88% protein, 1.5% fat, 0.3% sugar, 4% ash and 6% moisture were delivered by Acatris Food Belgium (Londerzeel, Belgium). Raspberry "Nature addicts fruit break" snack was obtained directly from the producer (Taura, Olen, Belgium). The fruity snack contained: concentrated apple juice (52%), concentrated apple puree (39%), concentrated raspberry puree (5%), concentrated elderberry juice, fruit fibers, fruit pectin and natural flavorings. Syringe filters with polyether sulfone (PES), polyvinylidene fluoride (PVDF) cellulose acetate (CA) membranes all of 0.45 μm were from Novolab (Belgium).

2.2. HPLC analysis of the casein

Samples were finely ground in a mortar. For extraction, 2 g of sample was incubated with 10 mL of 0.1 M potassium phosphate buffer pH 8 by shaking for 2 h at room temperature. The pH was checked and if necessary adjusted again to 8 with 1 M hydrochloric acid. The extracts were further centrifuged at 9000 g for 10 min at room temperature. The pH of the decanted supernatants was adjusted to 2 with 10 M hydrochloric acid and again centrifuged at 15,000 g for 15 min at 4 °C. The obtained pellets were washed with 0.1 M hydrochloric acid and again centrifuged at 15,000 g for 15 min at 4 °C. Finally the pellets were redissolved in 0.1 M potassium phosphate buffer pH 8. The pH was checked and if necessary adjusted to 8 with 1 M hydrochloric acid. The samples were filtered and analyzed by HPLC. The HPLC (1100 system, Agilent Technologies, Switzerland) was equipped with a reversed-phase polymeric column (PLRP-S 250 $\times$ 4.6 mm, 300 Å pore size, 5 μm particle size)

from Varian Inc. (Belgium). The mobile phase consisted of a gradient of water and acetonitrile both containing 0.1% trifluoroacetic acid (TFA) (v/v). Eluting conditions expressed as proportion of water with 0.1% TFA were as follows: 0–10 min: 69%, 19 min: 49.3%, 20 min: 25%, 20–21 min: 25%, 22 min: 69%, 22–30 min: 69%. The flow rate was 1 mL/min, the column temperature 45 °C and the injected volume 50 μL . Detection was carried out with a fluorescence detector (FLD, G1321, Agilent Technologies, Switzerland) set at the wavelength: 280 nm excitation and 340 nm emission. An emission spectrum was taken between 300 and 500 nm in order to confirm the identity of the peak in combination with a matching eluting time and as such a guarantee of the specificity of the analysis. Several adaptations were applied on this standard HPLC method as discussed in Results and Discussion section; these and their results are further discussed.

2.3. Sandwich hazelnut ELISA

The grounded samples (1 g) were extracted in duplicate with either 10 mL of 0.1 M potassium phosphate buffer pH 8 or 10 mL water by shaking for 2 h at room temperature. The pH of the extract was checked and if necessary adjusted to 8 with 1 M NaOH. Standards of purified hazelnut protein (*Cor a 9*) in PBS (0.135 M NaCl, 1.5 mM KH_2PO_4 , 8 mM $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 2.7 mM KCl, pH 7.4) of the following concentrations were prepared: 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 $\mu\text{g/mL}$. A Microton 96 K high binding ELISA plate was coated with 100 μL chicken IgY antibodies developed towards *Cor a 9* (10 $\mu\text{g/mL}$) in the coating buffer (0.015 M Na_2CO_3 , 0.035 M NaHCO_3), pH 9.6. After overnight incubation at 4 °C the wells were washed 3 times with 200 μL of 0.05% Tween 20 in 0.9% NaCl solution and this washing step repeated after each incubation step described later, except after the substrate addition. After blocking the remaining binding sites with 200 μL per well of 4.5% sodium caseinate in PBS, 100 μL of standard *Cor a 9* or diluted/non-diluted samples were added to the wells and incubated 75 min at 37 °C in the dark. Following washing, the plates were incubated with 100 μL of HRP-anti-hazelnut IgY (diluted 1/15,000 in PBS) for 1 h at 37 °C in the dark. Finally, the wells were incubated with 100 μL of TMB substrate, containing 0.45% hydrogen peroxide for 1 h at 37 °C. To stop the color reaction 25 μL of 4 M sulfuric acid was added to each well and the absorbance was read at 450 nm using a micro plate reader (Bio-Rad, Belgium, Nazareth Eke). The obtained experimental points for the calibration curve were fitted by the standard four parameters theoretical curve:

$$\text{Absorbance} = \frac{B_{\max} - B_0}{1 + \left(\frac{C}{EC_{50}}\right)^d} + B_0,$$

where: $B_{\max}$ and B_0 – maximal and minimal value of absorbance, C – protein concentration, EC_{50} – concentration at which response is a half of the amplitude ($B_{\max} - B_0$) (Trashin, Cucu, Devreese, Adriaens, & De Meulenaer, 2011).

2.4. Phenol content determination

The Folin Ciocalteu method was used for the total polyphenol content determination as previously described (Vandekinderen et al., 2008).

3. Results and discussion

3.1. HPLC analysis of casein

Because caseins represent 80% of the total proteins in the milk, they were used as analytical target in this study (Belitz, Grosch, &

Schieberle, 2009) and their presence in the fruit based snack was analyzed by an HPLC method with fluorescent detection. The samples used for the HPLC analysis have to be filtered before injection in order to prevent damages to the column caused by impurities in the samples. However, losses due to binding of proteins on the filter are likely to occur. Therefore, initially the recovery of the casein after filtration was evaluated. For this purpose, three different types of filters were used and tested in especially the lower concentration ranges of casein: type 1: PES, type 2: PVDF and type 3: CA. Standard solutions of casein were filtered and injected on HPLC. The same non filtered standards were injected to be able to quantify the losses. For low casein concentrations (below 10 $\mu\text{g/mL}$) both PES and CA filters showed almost complete losses of casein due to filtration while the PVDF filters gave a recovery of approximately 55–60%. For casein concentration of 50 $\mu\text{g/mL}$ the PES filter gave a recovery of 28% while the CA and PVDF filters of 58% and 57% respectively. In general the PVDF filters proved to be the most suitable filters for the HPLC sample preparations therefore they were further used in the sample preparation procedure. A typical chromatogram of a casein proteins solution filtered over a PVDF filter is shown in Fig. 1 (red line). From the chromatogram it can be seen that there are 3 peaks eluting at 17 min representing the κ -casein, a peak eluting at 19 min representing the α -casein and finally at 20 min the β -casein which is in line with their hydrophobicity (Veloso, Teixeira, & Ferreira, 2002). Application of the standard extraction procedure on the snack samples resulted in chromatograms in which no clear casein peaks could be detected (Fig. 1, green line).

Application of the standard procedure on the snack sample resulted in chromatograms in which no clear casein peaks could be detected even if the sample amount for extraction was increased from 2 to 5 g. In order to investigate further the lack of clearly detectable peaks, the sample was spiked with casein proteins and extracted according to the standard procedure with up-concentration which would result in a final concentration in the filtrate of 37 μg casein proteins/mL. However, as shown in Fig. 2 again no casein peaks could be detected although a similar concentration in standard solutions resulted in clearly distinguishable peaks.

One of the possible causes for the fact that no peaks were observed might be because of the extraction buffer which was not able to extract the caseins from the spiked samples. In order to investigate this, the standard extraction procedure was performed

on 5 g sample but using a 1 M potassium phosphate buffer pH 8 instead of 0.1 M potassium phosphate buffer. Samples were spiked with up to 20 μg casein/g sample. In parallel standard casein solutions corresponding to a 10 and 20 μg casein/g sample level on sample basis were analyzed according to the complete adjusted procedure. For the standard solutions, a recovery of about 60% was obtained which was in agreement with the expected losses due to filtration of the sample. However, when analyzing the spiked samples, again no casein peak could be detected. Thus it seemed that despite using a more powerful extraction buffer, it was still not possible to extract casein proteins added to the samples, even if, in comparison with the earlier reported milk protein traces detected in the sample, extremely high spiking levels were applied. Most probably the (casein)proteins added to the sample interacted strongly with the matrix hindering therefore their extraction. Considering the composition of the sample analyzed it is very likely that on one hand the polyphenols present (7.47 mg/g sample) can interact strongly with the proteins hampering their extractability (Siebert, Troukhanova, & Lynn, 1996) and on the other hand the pectin which was added to the product as such or through the apple puree and juice which is also known to interact with proteins (Jaramillo, Roberts, & Coupland, 2011; Marozienka & de Kruif, 2000).

In order to prove this hypothesis, samples were spiked using three different methods and analyzed using the standard HPLC procedure. Spiking was performed at an unrealistically high 748 $\mu\text{g/g}$ sample level on sample basis. In the first case the sample was spiked on the original sample and after extraction with 1 M potassium phosphate buffer pH 8 the redissolved pellet in same buffer was filtered and injected on the HPLC. In the second case, the sample was mixed with the 1 M potassium phosphate buffer pH 8, shaken for 2 h and after centrifugation the supernatant was spiked with casein and after precipitation the redissolved pellet was filtered and injected on the HPLC. In brief, the spiking was performed on the unfiltered sample extract. In the third case, the sample was extracted with the 1 M phosphate buffer pH 8 with shaking for 2 h and the redissolved pellet after filtration was spiked and further injected on the HPLC. In this case the spiking was performed on the filtered sample extract. Finally, as control a non-spiked sample was also extracted and analyzed according to the standard procedure. In the non-spiked sample, no casein peak could be observed (Fig. 3). The sample spiked according to method one, returned a peak corresponding to a concentration of 14 $\mu\text{g/mL}$

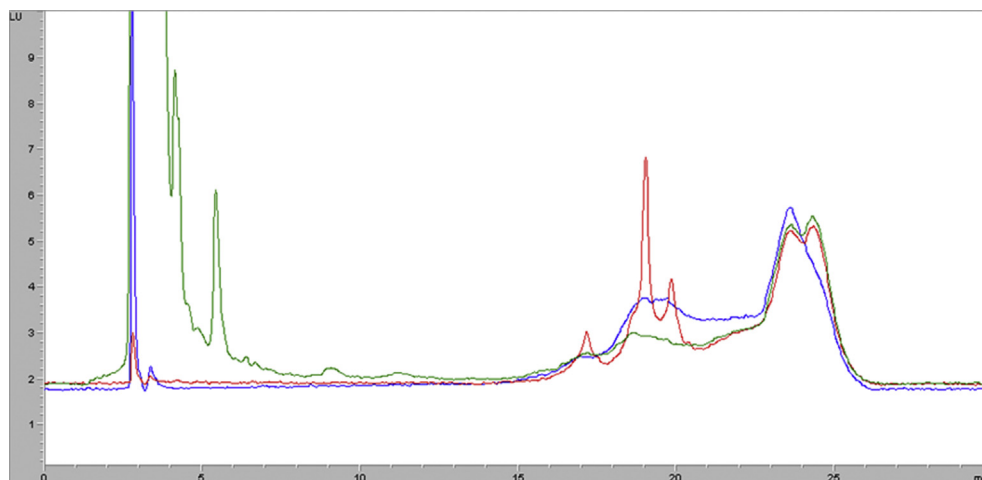


Fig. 1. Chromatographic profile of the: (green line) blank solution; (blue line) sample after standard extraction procedure and (red line) 40 $\mu\text{g/mL}$ casein solution filtered over the PVDF filter. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

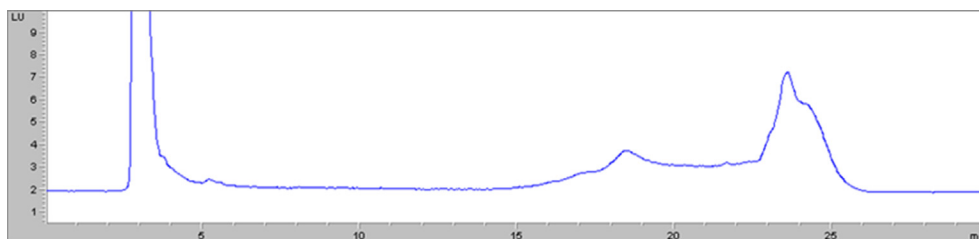


Fig. 2. Chromatographic profile of the 20 µg/g spiked sample.

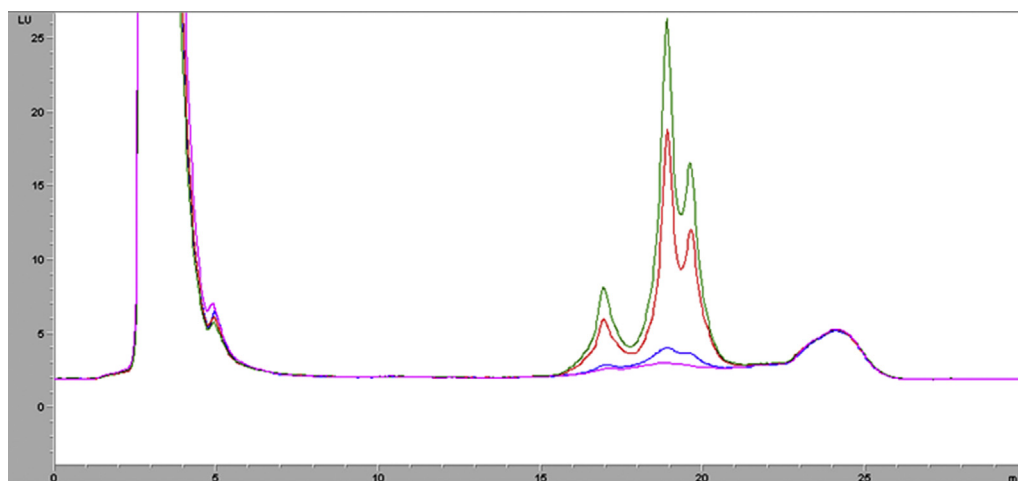


Fig. 3. Overlay of the chromatograms of samples obtained with different spiking methods: spiking method 1 (blue line), spiking method 2 (red line), spiking method 3 (green line) no spiking (pink line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

or to a recovery of about 5% of the casein proteins. The sample spiked according to method three returned a peak corresponding to a casein concentration of 277 µg/mL, or to a recovery of 97%. The sample spiked according to method two returned a clear peak but smaller than the one obtained using spiking method three with a recovery of only 70%. This experiment showed therefore that upon spiking in the presence of the sample matrix major interferences occurred (Table 1). When spiking was performed after extraction, interference was still present, but to a lower extent. This confirmed our previous hypothesis that the sample matrix was able to bind significant amounts of (casein) proteins. Consequently, these experiments showed as well that for this particular sample and probably samples with similar composition, allergen extraction is almost impossible due to strong protein binding to the matrix. This means that what is not extracted cannot be detected by any method employed, be it receptor based or chromatographic. Considering that a rather mild extraction buffer is applied in the previously used commercial RIDASCREEN ELISA kit, it cannot be excluded that a similar problem with respect to the extractability of proteins presumed to be present in the samples (on basis of the ELISA results) occurred. This might therefore imply that the results obtained using the kit were false positive. The origin of these apparently contradictory observations was further investigated using an in-house developed hazelnut sandwich ELISA.

Table 1
Recovery of casein using different spiking procedures (spiking with 748 µg casein/g sample).

Spiking procedure	Recovery, %
Spiking the sample (before extraction)	4.93 ± 0.24
Spiking the unfiltered extract of the sample	69.82 ± 3.37
Spiking the filtered extract of the sample (before injection)	97.05 ± 4.62

3.2. Sandwich Cor a 9 – ELISA assay

ELISA methods are based on specific molecular interactions between the analyte which in the case of the competitive RIDASCREEN ELISA kit were milk proteins and the antibodies (proteins by nature as well). When using a competitive ELISA, food allergens immobilized on the plate are competing with the allergens from the sample to bind with the antibodies which are labeled with an enzyme. In this case, the more antigen is present in the sample the more it binds to the antibody which makes them not available for interaction with the antigen bound to the well and as a result the weaker the signal. Considering this basic principle of a competitive ELISA method we can imply that the compounds present in the sample extract (e.g. polyphenols, pectin) can strongly bind to the added antibodies leading to a decrease in the analytical signal and subsequently to a positive analytical result (Fig. 4A and B). Of course it cannot be excluded that part of the matrix compounds can interact with the coated antigen as well, but it is more plausible that the interaction of the matrix with the free antibodies added with the sample is favored. In order to verify this, a highly specific in-house developed sandwich ELISA for hazelnut was used. Unlike competitive ELISA, in the case of sandwich ELISA the primary antibody immobilized on the plate captures the analytical target (proteins) which are further detected by a secondary allergen specific antibody labeled with enzyme (Fig. 4C and D). In case of a false positive result in sandwich ELISA, this would be solely due to the interaction between the matrix components and the antibodies. Additionally, based on the information received from the producer no hazelnut is used in the production facilities in which the snack samples were produced.

The sandwich hazelnut ELISA which uses antibodies raised against Cor a 9, had a limit of detection of 4 ng Cor a 9/mL and a limit

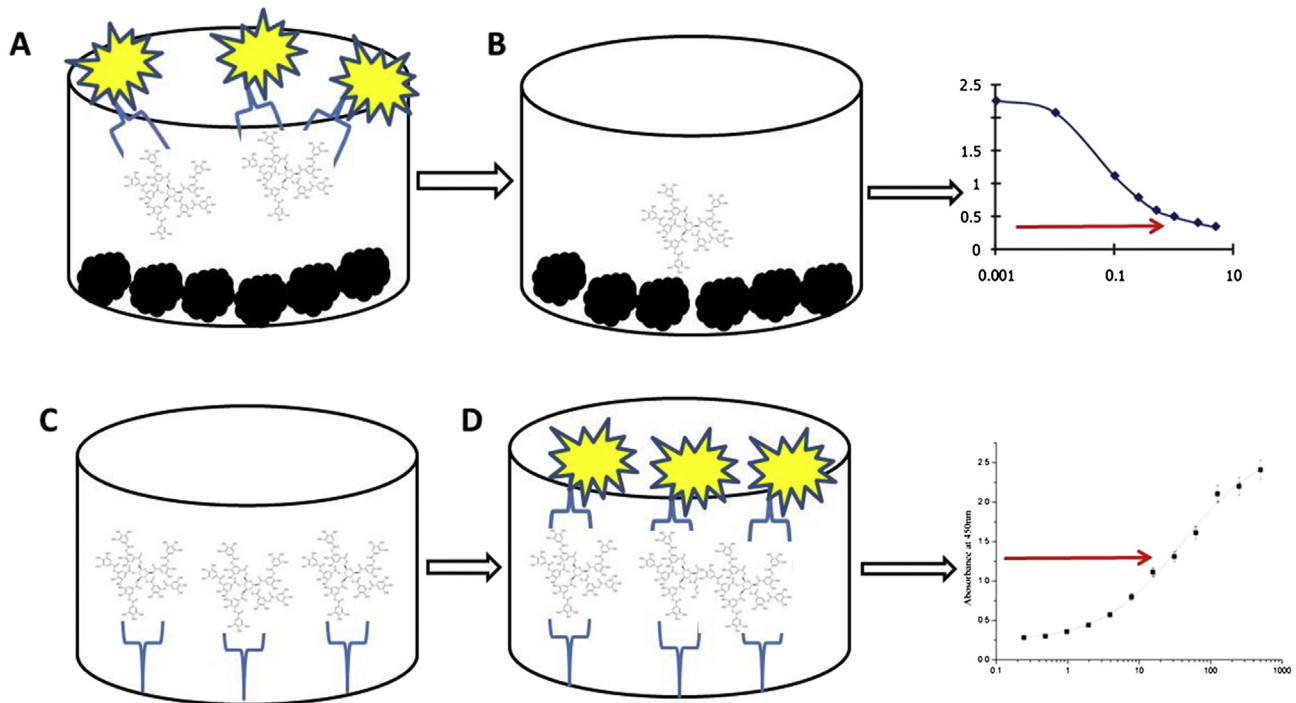


Fig. 4. Schematic representation of the competitive ELISA (A and B) and sandwich ELISA (C and D) and the impact of the matrix (i.e. polyphenols) on detectability.

of quantification of 10 ng *Cor a 9*/mL (Trashin et al., 2011). To evaluate whether non-specific binding of the antibodies to the matrix components was taking place, samples were extracted with water and with 0.1 M potassium phosphate buffer pH 8. When analyzing the sample extracts obtained with water a concentration of 1.32 µg hazelnut proteins/g sample was detected. In the samples extracted with 0.1 M potassium phosphate buffer pH 8 a concentration of 0.43 µg hazelnut/g sample was detected. Thus, according to the hazelnut ELISA results the sample would contain between 0.4 and 1.3 µg hazelnut/g sample protein depending on the extraction buffer used regardless of the fact that the samples are almost certainly not containing any hazelnut proteins.

This experiment confirmed the earlier hypothesis that due to non-specific binding between sample constituents (most likely polyphenolic or related compounds and pectin in view of the nature of the sample) and the antibodies used in the ELISA, the results of this receptor-based method can be biased and false positive results are likely to be obtained. As a consequence, outmost care with respect to the interpretation of the results obtained via ELISA methods should be taken especially when sample matrix is likely to interfere due to for example high amounts of polyphenols and/or polysaccharides.

4. Conclusions

It was long known that detection of food allergens is hampered by the presence of matrix components. Food allergens can be masked by the food matrix in different ways. First of all the matrix can bind the analytical target (proteins/allergens) therefore hampering their extraction. This was proven in this study where cow's milk proteins, namely caseins, bound to the matrix making their extraction inefficient especially in low concentration ranges. This indicated that detection of especially trace amounts of allergens would be severely affected regardless of the type of analytical method used. Further, we have also shown that depending on the matrix, non-specific binding of the matrix components with the

antibodies might occur which can bias the ELISA results regardless of the format used.

Acknowledgments

The authors would like to thank Taura NV for kindly providing us the snack fruit samples.

References

- Abbott, M., Hayward, S., Ross, W., Godefroy-Benrejeb, S., Ulberth, F., Van Hoeyveld, E. M., et al. (2010). Validation procedures for quantitative food allergen ELISA methods: community guidance and best practices. *Journal of AOAC International*, 93, 442–450.
- Belitz, H., Grosch, W., & Schieberle, P. (2009). Milk and dairy products. In *Food chemistry* (4 ed.). (p. 498). Springer.
- Cucu, T., De Meulenaer, B., & Devreese, B. (2012). MALDI-based identification of stable hazelnut protein derived tryptic marker peptides. *Food Additives and Contaminants Part A – Chemistry Analysis Control Exposure & Risk Assessment*, 29, 1821–1831.
- Cucu, T., Devreese, B., Kerkaert, B., Rogge, M., Vercruysse, L., & De Meulenaer, B. (2012). ELISA-based detection of soybean proteins: a comparative study using antibodies against modified and native proteins. *Food Analytical Methods*, 5, 1121–1130.
- Cucu, T., Devreese, B., Trashin, S., Kerkaert, B., Rogge, M., & De Meulenaer, B. (2012). Detection of hazelnut in foods using ELISA: challenges related to the detectability in processed foodstuffs. *Journal of AOAC International*, 95, 149–156.
- Cucu, T., Platteau, C., Taverniers, I., Devreese, B., De Loose, M., & De Meulenaer, B. (2011). ELISA detection of hazelnut proteins: effect of protein glycation in the presence or absence of wheat proteins. *Food Additives and Contaminants Part A – Chemistry Analysis Control Exposure & Risk Assessment*, 28, 1–10.
- Downs, M. L., & Taylor, S. L. (2010). Effects of thermal processing on the enzyme-linked immunosorbent assay (ELISA) detection of milk residues in a model food matrix. *Journal of Agricultural and Food Chemistry*, 58, 10085–10091.
- European Parliament and Council. (2007). Directive 2007/68/EC of 27 November 2007 amending Directive 2000/13/EC as regards certain food ingredients. *Official Journal of the European Communities*, L310/11.
- Fu, T. J., Maks, N., & Banaszewski, K. (2010). Effect of heat treatment on the quantitative detection of egg protein residues by commercial enzyme-linked immunosorbent assay test kits. *Journal of Agricultural and Food Chemistry*, 58, 4831–4838.
- Husain, F. T., Bretbacher, I. E., Nemes, A., & Cichna-Markl, M. (2010). Development and validation of an indirect competitive enzyme linked-immunosorbent assay

- for the determination of potentially allergenic sesame (*Sesamum indicum*) in food. *Journal of Agricultural and Food Chemistry*, 58, 1434–1441.
- Jaramillo, D. P., Roberts, R. F., & Coupland, J. N. (2011). Effect of the pH on the properties of soy protein–pectin complexes. *Food Research International*, 44, 911–916.
- Marozienė, & de Kruif, C. G. (2000). Interaction of pectin and casein micelles. *Food Hydrocolloids*, 14, 391–394.
- Platteau, C., Cucu, T., De Meulenaer, B., Devreese, B., De Loose, M., & Taverniers, I. (2011). Effect of protein glycation in the presence or absence of wheat proteins on detection of soybean proteins by commercial ELISA. *Food Additives and Contaminants Part A – Chemistry Analysis Control Exposure & Risk Assessment*, 28, 127–135.
- Poms, R. E., & Anklam, E. (2004). Effects of chemical, physical, and technological processes on the nature of food allergens. *Journal of AOAC International*, 87, 1466–1474.
- Scaravelli, E., Brohee, M., Marchelli, R., & van Hengel, A. J. (2009). The effect of heat treatment on the detection of peanut allergens as determined by ELISA and real-time PCR. *Analytical and Bioanalytical Chemistry*, 395, 127–137.
- Schmitt, D. A., Nesbit, J. B., Hurlburt, B. K., Cheng, H. P., & Maleki, S. J. (2010). Processing can alter the properties of peanut extract preparations. *Journal of Agricultural and Food Chemistry*, 58, 1138–1143.
- Siebert, K. J., Troukhanova, N. V., & Lynn, P. Y. (1996). Nature of polyphenol–protein interactions. *Journal of Agricultural and Food Chemistry*, 44, 80–85.
- Trashin, S., Cucu, T., Devreese, B., Adriaens, A., & De Meulenaer, B. (2011). Development of a highly sensitive and robust Cor a 9 specific enzyme-linked immunosorbent assay for the detection of hazelnut traces. *Analytica Chimica Acta*, 708, 116–122.
- Vandekinderen, I., Van Camp, J., Devreese, B., Veramme, K., Denon, Q., Ragaert, P., et al. (2008). Effect of decontamination agents on the microbial population, sensorial quality, and nutrient content of grated carrots (*Daucus carota* L.). *Journal of Agricultural and Food Chemistry*, 56, 5723–5731.
- Veloso, A. C. A., Teixeira, N., & Ferreira, I. M. P. L. (2002). Separation and quantification of the major casein fractions by reverse-phase high-performance liquid chromatography and urea-polyacrylamide gel electrophoresis – detection of milk adulterations. *Journal of Chromatography A*, 967, 209–218.