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Effect of oxidation in the presence or absence of lipids on hazelnut and soybean protein detectability by commercial ELISA

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Soybean and hazelnut represent major food allergens. Their inadvertent presence in foods might pose a serious threat to allergic consumers. Therefore, reliable detection methods are needed. Commercial enzyme-linked immunosorbent assays (ELISA) are the preferred methods especially by the food industry and enforcement agencies for the detection of contamination levels of food allergens. However, they are susceptible to erroneous results due to the modification of the allergens (proteins) during food processing. In this paper, the impact of protein oxidation on the detectability of soybean and hazelnut was investigated. Lipid-induced oxidation did not have a great impact on the detectability while the more severe hypochlorous acid-induced oxidation led to dramatic decrease in detectability for both hazelnut and soybeans. The observed impact was highly dependent on the type of ELISA kit used.

Keywords: soybean; hazelnut; ELISA; oxidation; detectability

Introduction

Food allergy is an abnormal immunological reaction to usually harmless food components, in this case proteins. It represents an important public health problem which is widespread amongst most countries and affects 2% of the adult population and 6–8% of children (Poms, Klein, & Anklam, 2004). Due to the constant vigilance that is required from the allergic consumers and their surroundings, food allergy has a negative impact on the quality of life (Madsen et al., 2010). There are evidences that the prevalence of food allergies is increasing (Sicherer, Munoz-Furlong, & Sampson, 2003).

Soybean is an important crop largely used in processed food products for the improvement of the nutritional quality and/or the functional properties. Hazelnuts are widely used in the food industry owing to their nutritive value and taste and are considered as an indicator of quality (Koppelman et al., 1999). However, hazelnut and soybean belong to the eight most significant allergenic foods, meaning that their extensive use in food products is leading to an increased chance of

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cross-contamination, representing a threat to allergic consumers. Directive 2007/68/EC specifies that soybean and tree nuts such as hazelnut together with other 12 allergic raw materials (cereals, crustaceans, eggs, fish, peanuts, milk, celery, mustard, sesame, lupine, molluscs, sulphur dioxide and products thereof) have to be labelled when present in the food as an ingredient (European Parliament and Council Directive 2007/68/EC, 2007). Food labelling is requested in order to help allergic consumers to avoid allergen containing foods, therefore the food industry needs reliable analytical methods for allergen detection in order to provide accurate labelling. The access to routine allergen detection methods is rather limited, with mostly enzyme-linked immunosorbent assay (ELISA) being used on a larger scale. However, food processing might modify food allergens leading further to modifications in allergenicity (Chicon, Belloque, Alonso, & Lopez-Fandino, 2008; Frias, Song, Martinez-Villaluen-ga, de Mejia, & Vidal-Valverde, 2008; Gruber, Vieths, Wangorsch, Nerkamp, & Hofmann, 2004; Gruber, Becker, & Hofmann, 2005; Nakamura, Watanabe, Ojima, Ahn, & Saeki, 2005; Nakamura et al., 2008). Remarkably, up until now there is just one study in which the impact of protein oxidation on allergenicity is addressed. It was shown that the incubation of soybean globulins with oxidised oil led to an increase in the allergenicity as determined by ELISA using human sera (Doke, Nakamura, & Torii, 1989). Another observation is that protein digestibility tends to decrease upon oxidation, suggesting that normally labile allergens could reach the intestinal tract in a more intact (non-digested) form (Chiba, Doi, Yoshikawa, & Sugimoto, 1976). It is therefore obvious that food processing might have an impact on allergenicity, however, no clear trends can be established. Considering these variations in allergenicity as result of the food processing, modifications in their detectability cannot be excluded.

Soybean and hazelnuts both have a high (unsaturated) lipid content and are mostly used in food products which are rich in lipids (confectionary, meat, dairy products etc.). Thus, it might be expected that during storage and processing association between proteins and lipids can take place. Lipids are susceptible to oxidation leading to the formation of hydroperoxides and secondary oxidation products. These secondary oxidation products may lead to modification of several amino acids such as cysteine, methionine, histidine, tryptophan, tyrosine and lysine (Refsgaard, Tsai, & Stadtman, 2000; Sanchez-Vioque et al., 1999). Moreover, association of proteins with lipids is known to lead to protein insolubility (Cucu, Devreese, Mestdagh, Kerkaert, & De Meulenaer, 2011a, Liang, 1999; Wu, Zhang, & Hua, 2009). Considering the modifications that are caused on the protein level alterations in the detectability using receptor-based assays, such as ELISA, cannot be excluded. These modifications could modify the epitope that is recognised by the antibodies of the ELISA assay. This could lead to erroneous assay results upon analysis of the processed food product for the presence of the concerning allergens. It is clear that when insoluble aggregates are formed upon protein oxidation, these will not be extracted from the food sample with the commonly used aqueous extraction solutions. Consequently, a distorted quantification will be made, underestimating the actual concentration of the allergenic ingredient present in the food product. This can present a serious risk for allergic consumers in cases where false negative results are obtained by routinely used analytical methods.

In the present study, the impact of lipid and hypochlorous acid induced oxidation on hazelnut and soybean proteins was evaluated. The impact of hypochlorous acid-induced oxidation was evaluated in order to investigate whether association of the

primary and/or secondary lipid oxidation products with the proteins, or the direct protein oxidation is the cause of the modifications of the detectability. The reactions were performed in buffered protein systems in order to overcome the extractability issues induced by protein aggregation.

Materials and methods

Reagents and standards

Chemicals and standards of analytical grade were purchased from Sigma-Aldrich (Bornem, Belgium), VWR (Leuven, Belgium) and Acros Organics (Geel, Belgium). Analytical grade solvents and technical grade hexane were obtained from Chem-Lab (Zedelgem, Belgium). The commercial ELISA kits used for hazelnut proteins determinations were Veratox[®] for hazelnut (Neogen, Michigan Lansing, USA), Ridascreeen[®] FAST Hazelnut (R-Biopharm, Darmstadt, Germany), BioKits Hazelnut Assay (Tepnel, Deeside, Flintshire, UK) and Hazelnut Residue (ELISA Systems, Windsor, Queensland, Australia). The commercial ELISA kits used for soybean protein determinations were Veratox[®] Soy Allergen (Neogen, Michigan, Lansing, USA), BioKits Soya Allergen Assay (Tepnel, Deeside, Flintshire, UK) and Soy Residue (ELISA Systems, Windsor, Queensland, Australia).

Hazelnut and soybean protein extraction

Nine different brands of hazelnuts, among which eight virgin and one roasted, were purchased in Belgian local supermarkets. Soybeans were provided by Alpro (Wevelgem, Belgium) and Cargill (Mechelen, Belgium). Separate mixtures of hazelnuts and soybeans were made by taking equal amounts of each variety. Soybean and hazelnuts were frozen with liquid nitrogen and ground in a two-step process, first with a blender (Moulinex, France) and then with an Ultra Turrax T25 (IKA, Wilmington, NC, USA). The in-house prepared protein extract was obtained following the protocol described earlier (Cucu, Platteau et al., 2011b).

Oxidation with hypochlorous acid

A 200 μ L protein solution (10 mg/mL) in 100 mM phosphate buffer pH 8 or 5.8 was oxidised with 50 μ L hypochlorous acid (0–5 mmol/g protein) at 30°C for 10 min. The initial concentration of the hypochlorous acid was determined by iodometric titration. After treatment, the samples were immediately immersed in ice.

Oxidation in the presence of sunflower oil

The reaction systems were prepared in 50 mM 3-morpholinopropanesulfonic acid pH 7.4 by mixing 1% (w/v) sunflower oil with 2% (w/v) protein isolate by means of a vortex for 2 minutes. The reaction systems in sealed falcon tubes were incubated at 70°C in the presence of 10 μ M copper sulphate solution to initiate the oxidation and 0.2 g/L sodium azide to prevent microbial growth. A reaction system without oils was used as a control. At different periods of time, subsamples were removed for analytical measurements. Following, the samples were treated with 15% TCA (final

concentration) and incubated for 10 min on ice. After centrifugation for 10 min at 10,000 g the pellet was redissolved in H₂O and the pH was adjusted to approximately 10 with 10 M NaOH to facilitate the solubilisation of the pellet. Any undissolved particles were further removed by centrifugation at 10,000 g for 10 min. The clear supernatant was used for analysis.

Protein determination

Protein determination was performed by determining the nitrogen content according to the Kjeldahl procedure (AOAC Official Method 981.10, 1981). A factor of 5.41 was used to convert nitrogen to total hazelnut protein, and 5.71 to convert to total soybean protein. The non-protein nitrogen fraction was determined in the supernatant after a previous protein precipitation with 15% TCA (final concentration).

ELISA assays

Samples of the model systems in which hazelnut or soybean proteins were oxidised in the presence of sunflower oil or at various concentrations of hypochlorous acid were analysed in the respective different commercial ELISA kits. For the model system in which the proteins were oxidised in the presence of sunflower oil, samples were taken at time zero, after 24 h and 48 h. Control samples to which no lipids were added were also included. Hazelnut and soybean proteins oxidised with 0, 2.5 and 5 mmol/g protein hypochlorous acid at pH 8 or 5.8 were also analyzed. Before the analysis, dilution series were made of the different samples in the extraction buffer or dilution solution of the respective kits. The actual concentrations are the concentrations of hazelnut or soybean proteins based on the absolute protein determination using the Kjeldahl procedure. The dilution series were analyzed in duplicate in a similar manner as kits' sample extracts according to the respective manufacturers' manuals. Absorbance measurements were made in a microtiter plate reader (Multiskan MCC/340, Titertek, Huntsville, Alabama). Each kit was calibrated using the respective standard protein solutions included in each kit. For fitting the absorbance values to the protein concentration of the standard solutions, a four-parameter-logistic dose-response curve was used with the following Equation (1). This equation represents a standard way to express immunoassay data, where a is the maximum of the curve, that is the absorbance value obtained at the highest dose (protein concentration), b is the minimum, that is absorbance value obtained at the lowest concentration, c is the E₅₀-value (concentration at half-maximal saturation), d is the slope of the curve and $conc$ is the concentration of the standard or sample (Englebienne, 2000).

$$\text{Absorbance} = \left[\frac{(b - a)}{\left(1 + \frac{conc}{c}\right)^d} \right] + a. \quad (1)$$

Only the data obtained within the range of the respective standard curves were considered in the results. Data above or below the calibration range are indicated by an asterisk (*) or a 'zero' (0), respectively, in the charts.

Results and discussions

During food processing, oxidation processes can take place which can lead to modification of amino acids, formation of protein bound carbonyls and aggregation (Cucu, Devreese et al., 2011a; Kerkaert et al., 2011). It is clear that these modifications can influence the protein-antibody interaction upon which ELISA assays are based. To investigate this, model systems were prepared in which hazelnut and soybean proteins were oxidised under different conditions.

Conversion of the concentration of the standard protein solutions of the respective ELISA kits to the same dimensions as the analysed samples was done as described elsewhere (Cucu, Platteau et al., 2011b; Platteau et al., 2011). For the evaluation of the kits performance, dilution series of the hazelnut/soybean proteins before and after oxidation with sunflower oil or hypochlorous acid were applied in the appropriate calibration range of each kit. The measured hazelnut/soybean protein concentration was compared with the actual protein concentration present in each diluted solution as initially determined by the Kjeldahl method.

Lipid induced oxidation

Hazelnuts and soybean are frequently used in food products which are rich in lipids. Thus, it might be expected that during storage and processing association between proteins and lipids can take place. Upon oxidation, these lipids can covalently interact with proteins leading to modifications of the amino acids in the epitopes which can eventually hinder their detection by the receptor-based methods as well. Hazelnut and soybean proteins were incubated at 70°C with sunflower oil in the presence of CuSO₄ to initiate the oxidation. Control samples incubated without sunflower oil were tested as well in order to investigate whether the changes in detectability are not due to thermal treatment alone. Unlike the samples incubated with glucose (Cucu, Platteau et al., 2011b), the samples incubated with oils were subjected to additional sample preparation steps, such as precipitation of proteins using TCA and resolubilisation in water with adjustment of pH to 10 to facilitate the solubilisation of the oxidised proteins.

The actual hazelnut protein concentration in the native reference samples (with or without sunflower oil) was underestimated by all hazelnut ELISA kits. The Veratox for Hazelnut kit detected on average 10% of the hazelnut protein concentration across the dilution series while the Hazelnut Residue kit 70% of the actual hazelnut proteins, regardless of the presence or absence of sunflower oil (Figure 1). From these results, it can be concluded that the presence of the sunflower oil without thermal treatment does not interfere with the detection. Interestingly, however, the concentrations detected were slightly higher as compared to the native hazelnut proteins used for the glycation experiments (Cucu, Platteau et al., 2011b). The Ridascreen® FAST Hazelnut kit detected about 25% of the actual hazelnut protein in the sample regardless of the presence or absence of sunflower oil. However, the detected hazelnut proteins in these model systems were about three times lower as compared to the native sample used for the glycation experiment. This indicates that, sample preparation had a critical impact on the protein detection. During precipitation with TCA and resolubilisation, extreme changes in the pH were taking place which could cause changes in protein conformation and consecutively to a

decreased recognition by the antibodies. In addition, using the BioKits Hazelnut assay the reference and oxidised samples returned absorbance values that were below the calibration range (data not shown). Consequently, no conclusion could be made with regard to the influence of protein oxidation on the detection of hazelnut proteins with this kit obviously due to sample preparation steps that strongly interfered with the antibody binding.

After 48 h incubation at 70°C, the Veratox for Hazelnut kit only detected about 7% of the actual hazelnut proteins concentration present in the model systems regardless of the presence or absence of the sunflower oil (Figure 1A), which was comparable with the hazelnut protein incubated with glucose (Cucu, Platteau et al., 2011b). With the Ridascreen® FAST Hazelnut kit, after 24 h a further decrease in detectability was observed with on average 10% of the actual hazelnut being detected for the samples without or with lipids, respectively (Figure 1B). The observed decrease was not due to the modification of proteins on molecular level since no significant difference between the incubation with or without lipids was observed. Most probably, the observed decrease was due to aggregation of proteins which led to the blocking of the epitopes involved in the antibody-antigen binding. However, further incubation up to 48 h resulted in a slight concentration-dependent increase in detection for the two model systems. Similarly as for the native hazelnut protein, the detection of the hazelnut proteins after incubation with oils was about five times lower compared to the untreated sample used for the glycation experiment. Similar changes were seen with the Hazelnut Residue kit: after 24 h incubation a decrease in detectability was observed with about 50% of the actual hazelnut protein concentration being detected in the absence or presence of sunflower oil, respectively, compared to the 70% detected in the untreated sample (Figure 1C). The detection then slightly increased after 48 h incubation with about 55% of the actual hazelnut protein being detected for both model systems, however, this increase was only observed for the highest hazelnut protein concentrations used (400 ng/mL). Interestingly, the loss in detectability observed after incubation with the sunflower oil were significantly less severe as compared to incubation with glucose, which was in line with the degree of induced modifications on molecular level of proteins. During the Maillard reaction higher amounts of protein bound carbonyls and losses of especially lysine were observed as compared to the incubation with sunflower oil (Cucu, Platteau et al., 2011b).

Veratox and BioKits Soy Allergen assays highly overestimated the actual soybean protein content with on average across the dilution series of 308% and 212% in the model systems prepared in the absence of lipids and with 242% and 308% in the presence of lipids (Figure 2A, C). These observations were similar to the reference samples used during the assessment of the impact of glycation (Platteau et al., 2011). Using the Soy Residue kit the actual soybean protein measured was slightly underestimated with about 70–90% of the actual protein being detected in the untreated samples (Figure 2B). Heat treatment (almost) completely prevented the detection of the soybean proteins with the Veratox Soy Allergen and Soy Residue kit, regardless of presence or absence of sunflower oil during the incubation indicating that the observed decrease was not due to the induced modifications of soybean proteins by the sunflower oil. The observed decrease was probably caused by thermal denaturation of the proteins, especially the trypsin inhibitors and the 7S conglycinin. On the other hand, an increased detection was registered for the BioKits Soy

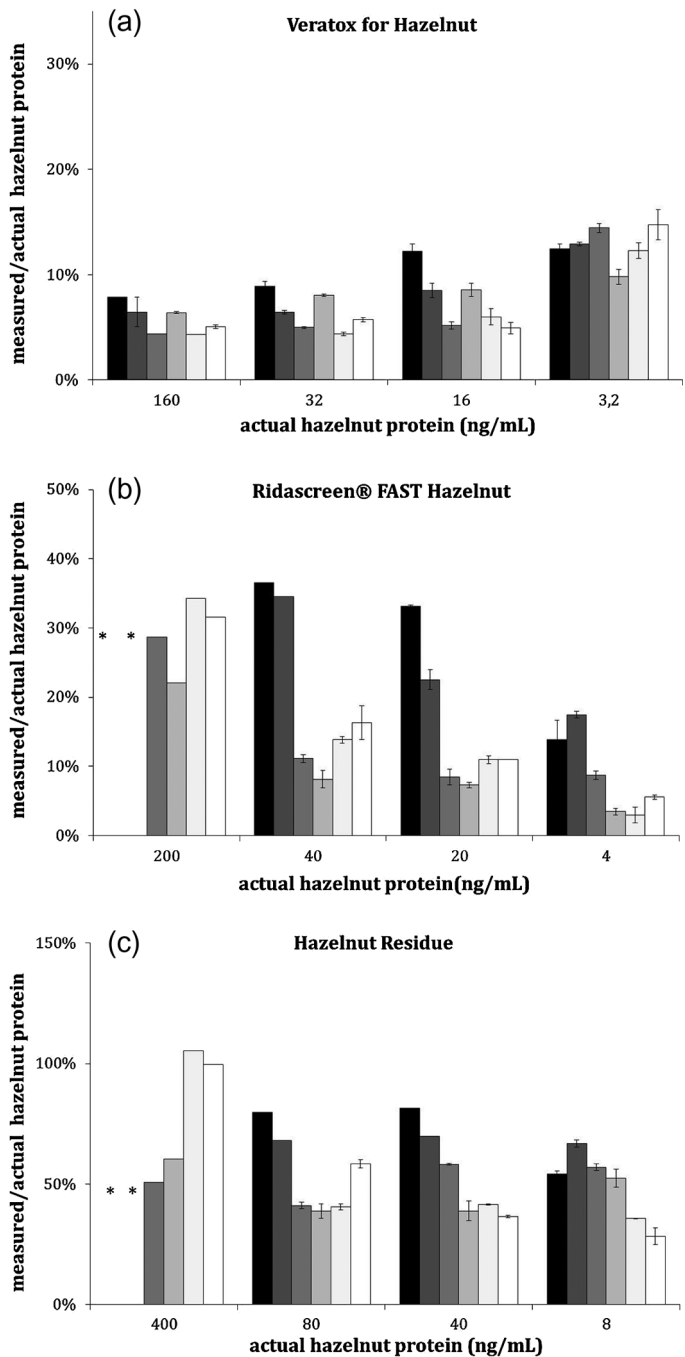


Figure 1. Ratio of measured over actual hazelnut protein concentration (y-axis) in different ELISA kits after duplicate analysis of dilution series (x-axis) of a reference hazelnut sample and after heat treatment in the presence or absence of sunflower oil: ■-0 h, without oil, ■-0 h, with oil, ■-24 h, without oil, ■-24 h, with oil, ■-48 h, without oil, ■-48 h with oil (* = absorbance value of the sample above the calibration range).

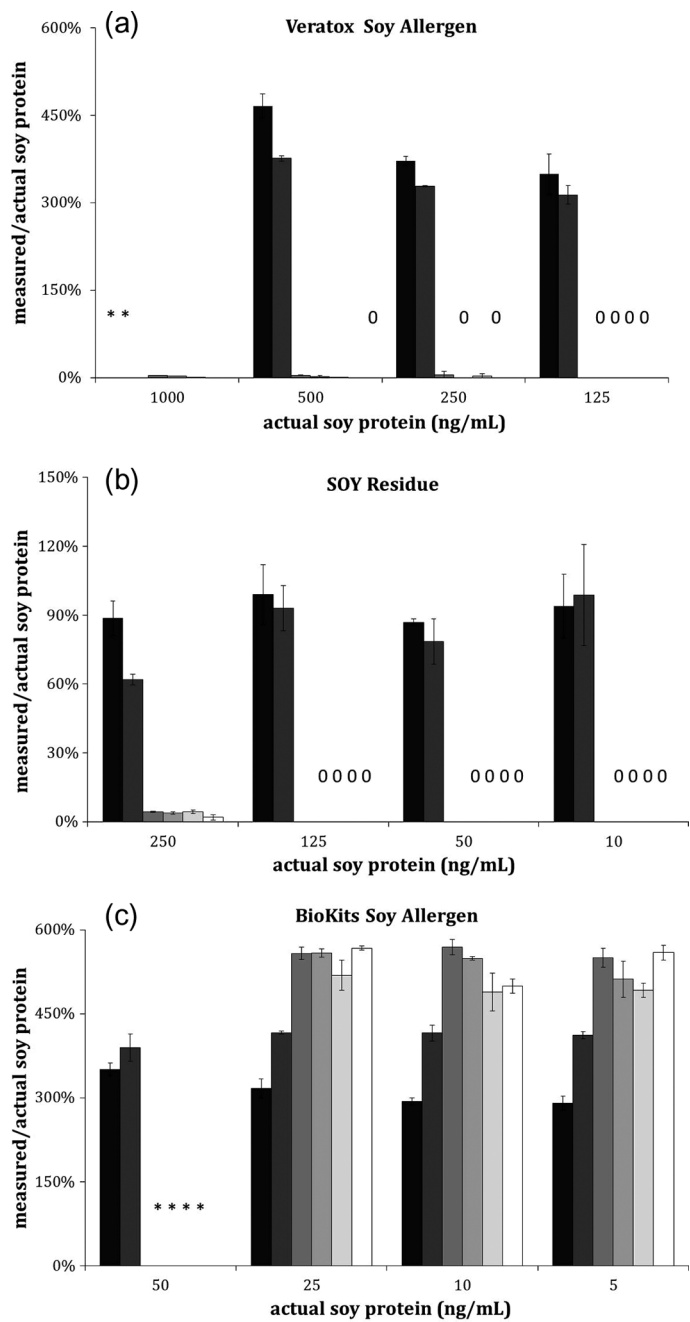


Figure 2. Ratio of measured over actual soybean protein concentration (y-axis) in different ELISA kits after duplicate analysis of dilution series (x-axis) of a reference soybean sample and after heat incubation in the presence or absence of sunflower oil: ■-0 h without oil, ■-0 h with oil, ■-24 h without oil, ■-24 h with oil, ■-48 h without oil, ■-48 h with oil (* = absorbance value of the sample above the calibration range, 0 = absorbance value of sample below the calibration range).

Allergen kit, again regardless of the presence or absence of sunflower oil during incubation. This was somehow unexpected, considering that changes on the molecular level were occurring and were expected to lead to a decrease in detectability.

It can be concluded, therefore, that lipid-induced oxidation does not lead to severe changes in the detectability of hazelnut and soybean proteins.

Hypochlorous acid induced oxidation

To investigate to what extent direct protein oxidation can influence the detection of hazelnut proteins with commercial ELISA kits, protein extracts from hazelnuts and soybean were oxidised with hypochlorous acid. Changes occurring on protein level as result of protein oxidation will highly depend on the oxidative mechanism involved. Unlike lipid induced oxidation, hypochlorous acid-induced oxidation can lead to severe increases in protein bound carbonyls and losses of tryptophan, methionine, cysteine and tyrosine residues (Kerkaert et al., 2011). Hypochlorous acid-induced oxidation is reported to be pH dependent (Hawkins, Pattison, & Davies, 2003; Kerkaert et al., 2011), therefore, extreme pHs were used to investigate the impact on detectability, namely pH 5.8 and 8.

Analysis of the untreated reference hazelnut protein sample in the different kits showed that the actual hazelnut protein concentration was underestimated with the Veratox for Hazelnut and the Ridascreen® FAST Hazelnut kits (Figure 3A, B). With the former only on average 15% of the actual hazelnut protein concentration was detected in the samples regardless of the pH used. With the latter, this was about 80% and underestimation was especially observed at the highest hazelnut protein concentration (200 ng/mL). Similarly as for the model systems incubated with glucose or sunflower oil, the detected hazelnut proteins were comparable for these two ELISA kits. In contrast, the other two kits (Hazelnut Residues and Biokits Hazelnut kits) overestimated the actual hazelnut protein concentration in the reference sample with about 20% regardless of the pH used (Figure 3C, D). Unlike the previous model systems, the detected hazelnut proteins in phosphate buffer pH 5.8 and 8 were slightly higher which was probably due to changes in protein conformation which might have exposed some of the buried epitopes. Using the Biokits Hazelnut assay the measurements were concentration dependent and overestimation was only observed up to 40 ng/mL hazelnut protein while at 200 ng/mL the hazelnut protein concentration was underestimated with about 10%. Moreover, the detected hazelnut protein was comparable with the model systems which were containing glucose incubated in phosphate buffer at pH 7.4.

As soon as oxidation was induced almost no hazelnut proteins were detected anymore, regardless of the pH used. This effect was especially observed in the case of the Veratox for Hazelnut kit. For the Biokits Hazelnut assay, about 20% of the actual hazelnut protein could be still detected after oxidation with 2.5 mmol hypochlorous acid/g protein while oxidation with higher concentrations led to complete loss of detectability. The Hazelnut Residue kit was still able to detect about 5% of the actual hazelnut protein concentration after oxidation with either 2.5 or 5 mmol hypochlorous acid/g protein. The observed severe decrease in the hazelnut protein detected was obviously related to the severe changes occurring at molecular level (data not shown). Moreover, the observed impact on the detectability was solely due

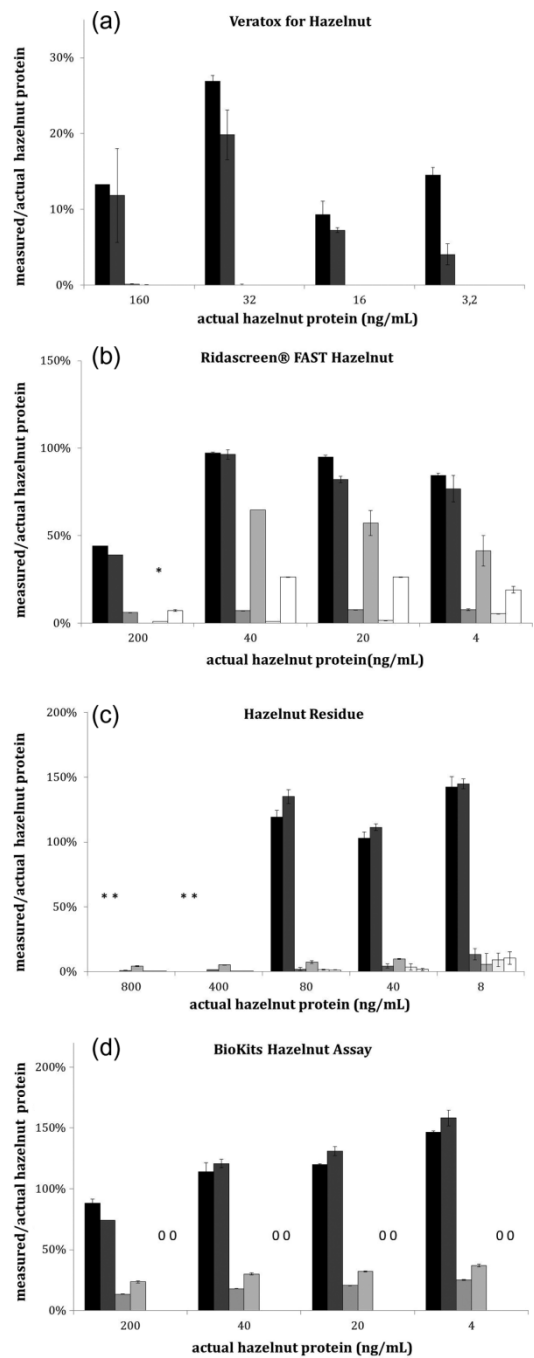


Figure 3. Ratio of measured over actual hazelnut protein concentration (y-axis) in different ELISA kits after duplicate analysis of dilution series (x-axis) of an untreated hazelnut protein sample and after oxidation with hypochlorous acid: [■]-0 mM/g protein, pH 8, [▒]-0 mM/g protein, pH 5.8, [■]-2.5 mM/g protein, pH 8, [▒]-2.5 mM/g protein, pH 5.8, [■]-5 mM/g protein pH 8, [▒]-5 mM/g protein, pH 5.8 (* = absorbance value of the sample above the calibration range, 0 = absorbance value of the sample below the calibration range).

to the changes induced by the direct oxidation since model systems were incubated at 30°C for 10 min implying that no other factors (such as heat treatment) could affect the detectability. Interestingly, no pH impact was observed for the untreated samples, indicating that no severe changes in protein conformation which could affect the recognition of the epitopes by the antibodies took place. Remarkably, using the Ridascreen® FAST Hazelnut kit about 50% of the oxidised proteins were still detected at pH 5.8 and only about 10% of the oxidised proteins at pH 8 when 2.5 mmol hypochlorous acid/g protein was used. Furthermore, upon oxidation with 5mM hypochlorous acid only about 20% of the proteins oxidised at pH 5.8 and less than 5% of the ones oxidised at pH 8 could be still detected (Figure 3B). The pH difference could be due to the different changes induced at pH 5.8 and 8.

Furthermore, the impact of the direct oxidation of soybean proteins, by incubation with hypochlorous acid, was investigated. The actual soybean protein concentration in the reference sample was seriously overestimated with the Veratox Soy Allergen and BioKits Soy Allergen kits, with an average across the dilution series of 220% and 160% for the former and 540% and 470% for the latter at pH 8 and 5.8, respectively (Figure 4A, C). Using the Soy Residue kit the detection of the native soybean proteins was relatively accurate (Figure 4B). Unlike the Veratox for Hazelnut, the Veratox for Soy Allergen could still detect about 50% of the actual protein concentration when oxidised with 2.5 mmol hypochlorous acid/g protein and below 10% when highly oxidised with 5 mmol hypochlorous acid/g proteins. Nevertheless, no pH impact could be established. For the Soy Residue kit, however, the detectability was almost completely hindered by the induced oxidation. The detectability using these Veratox for Soy and the BioKits for Soy Allergen kits was therefore, better for the soybean proteins oxidised with hypochlorous acid where no heat treatment was applied. This confirmed, once again, that the losses in detectability observed for the samples incubated with glucose and sunflower oil were solely due to the thermal denaturation of soybean proteins. Interestingly, for the BioKits for Soy Allergen, after oxidation with hypochlorous acid, the detected soybean protein concentration was almost accurate, with slight underestimations being established for the highly oxidised proteins with 5 mmol hypochlorous acid/g protein. Nevertheless, the observed decrease in detectability, was in line with the previously reported severe modifications of proteins, which probably led to the modification of the epitopes involved in the antibody-antigen binding.

In conclusion, for both hazelnut and soy proteins, a clear impact of the hypochlorous-induced oxidation was observed. Only one of the four evaluated hazelnut ELISA kits (Ridascreen FAST) was able to detect hazelnut proteins after hypochlorous acid treatment. On the contrary, no major impact of the incubation in the presence or absence of lipids on the detectability of hazelnut proteins could be observed. This indicates that the altered detection that was observed is mainly caused by protein denaturation and/or aggregation. In the case of lipid-induced oxidation of soybean proteins, with two out of three ELISA kits the detection was completely knocked down upon incubation at 70°C either in the absence or presence of lipids. It can be concluded that the proteins were denatured during the incubation resulting in disruption of some of the epitopes identical to the data obtained upon studying the impact of glycation (Platteau et al., 2011). Consequently, no further conclusions could be made with regard to the impact of the lipid-induced oxidation on soybean protein detection for these kits. BioKits for soy was able to detect the proteins, regardless the chemical modifications

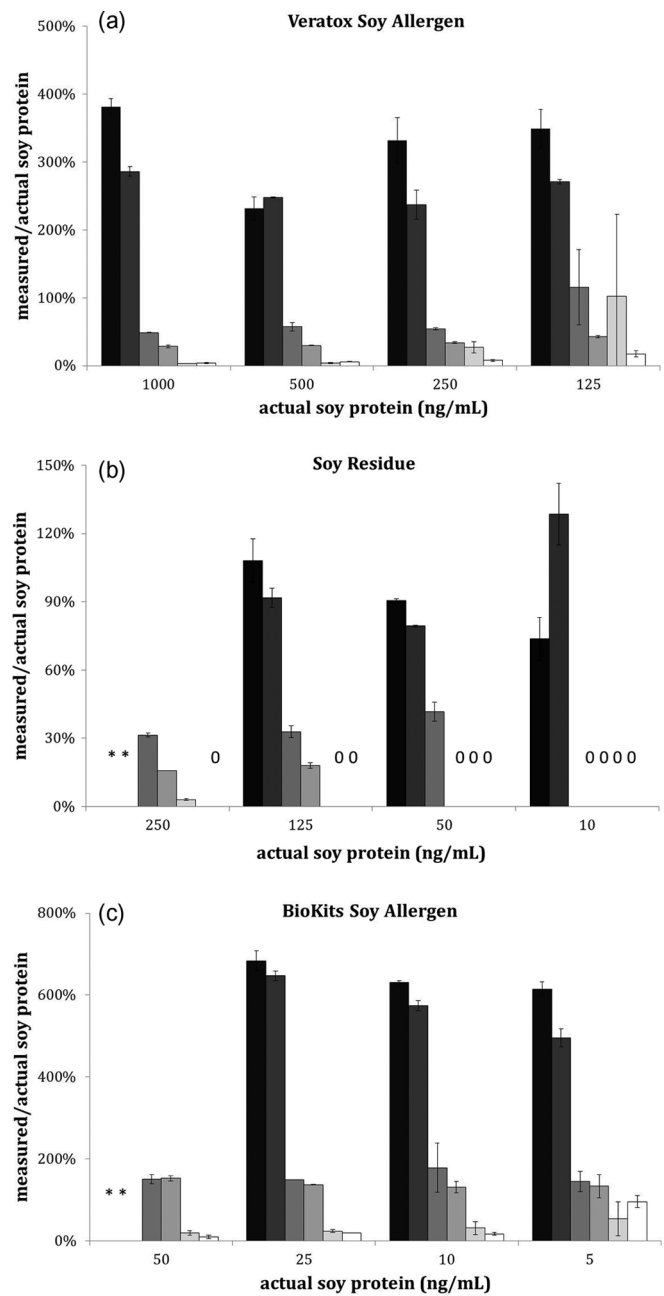


Figure 4. Ratio of measured over actual soybean protein concentration (y-axis) in different ELISA kits after duplicate analysis of dilution series (x-axis) of an untreated soybean protein sample and after oxidation with hypochlorous acid: [■]-0 mmol/g protein, pH 8, [▒]-0 mmol/g protein, pH 5.8, [░]-2.5 mmol/g protein, pH 8, [░░]-2.5 mmol/g protein, pH 5.8, [░░░]-5 mmol/g protein, pH 8, [░░░░]-5 mmol/g protein, pH 5.8 (* = absorbance value of the sample above the calibration range, 0 = absorbance value of the sample below the calibration range).

induced by the incubation at 70°C with or without lipids, which resulted even in an increased detection. This suggests that the antibodies used in this kit have been probably raised against partially modified soy proteins.

To date, ELISA is the most preferred method used by the industry and food safety control agencies to check products for the presence or absence of food allergens. ELISA's are relatively cheap, easy to use and non-specialised personnel is demanded. However, the results presented clearly demonstrate that the outcome of the test is dependent on the manufacturer, pointing out the need for reference materials to develop validated allergen test kits. Although oxidising lipids are known to interact with proteins, this study showed that this interaction does not seem to affect the receptor-based detection with the commercial ELISA kits. In contrast, hypochlorite-induced protein oxidation has a discernible impact on the detection of hazelnut and soybean proteins with commercial ELISA kits, which in most cases lead to a severely reduced detection. This is worrisome, considering that risk management decisions can be based on the results obtained with these kits. Hypochlorous acid might not be part of a food recipe, but this chemical reagent is being applied as a disinfectant in cleaning and disinfection programmes. If traces of the product are left on the production line due to inadequate rinsing, this might severely affect the detection of allergen contamination in the food products run on this line. The results presented illustrate that the performance of an analytical tool is strongly affected by chemical modification of the target proteins induced by food processing, in general lacking robustness with regard to the latter to detect or correctly quantify the amount of allergenic food ingredient present. Although under certain circumstances the kits are still able to provide qualitative information, quantification of the allergenic ingredient is essential in terms of risk assessment. Finally, it must be noted that to elucidate whether possible mislabelling of food products due to altered detection of allergens caused by food processing poses a threat to the allergic consumer, additional research is necessary to determine the allergic potential of chemically modified allergenic proteins.

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